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CHARACTERIZATION OF A MOUSE/HUMAN CHIMERIC MONOCLONAL ANTIBODY (17-1A) TO A COLON CANCER TUMOR-ASSOCIATED ANTIGEN[1]

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Mouse monoclonal antibody 17-1A is specific for an antigen expressed on cells of human gastrointestinal malignancies and has been used in radioimmune imaging and therapy trials for patients with colon and pancreatic cancer. The cell line SG3/5 was generated by transfection of a nonproducing mouse myeloma line (SP2/0) with a chimeric gene construct composed of variable regions from the mouse 17-1A immunoglobulin (γ2a,κ) and constant regions of human κ and γ3 immunoglobulin genes. The secreted immunoglobulin was bound by mouse monoclonal antibodies to human IgG(Fc) and IgG3 but not by staphylococcal protein A. Gel filtration HPLC profiles of purified chimeric antibody were similar to normal human IgG3 but quite different from native 17-1A and normal human IgG1, 2, and 4. Native and chimeric 17-1A had similar patterns of reactivity with colon cancer, other adenocarcinoma, and leukemic cell lines. Competitive inhibition documented that native and chimeric 17-1A had identical capacities to inhibit radiolabeled native 17-1A binding to colon cancer cell lines. Thus, the chimeric 17-1A exhibits molecular characteristics of normal human IgG3 but retains the specificity and binding affinity of the native 17-1A murine monoclonal antibody. The native and chimeric 17-1A mediated similar modest degrees of human lymphocyte and monocyte ADCC in a 4-hr ^{51}Cr release assay, and both failed to mediate complement lysis of colon carcinoma cell lines in the presence of human complement. This human/mouse chimeric monoclonal antibody may be a good candidate for use in clinical trials because it retains the tumor antigen specificity and human effector cell recognition of the native 17-1A, would presumably have a fivefold to 10-fold longer circulating half-life in man, and should be considerably less immunogenic as compared with native murine immunoglobulins.

The recent construction of chimeric immunoglobulin genes composed of cloned variable region exons from mouse hybridoma cell lines ligated to human constant region exons and their successful transfer into lymphoid cells provides the opportunity to generate chimeric monoclonal antibodies (mouse/human) that may be useful for human immunodiagnosis and therapy (1–5). The mouse monoclonal antibody 17-1A (γ2a,κ), produced and initially characterized at the Wistar Institute (6), has a relative specificity for cells of human gastrointestinal malignancies (colon, gastric, and pancreatic adenocarcinoma). It has been used successfully in radioimmune imaging studies in man (7) as well as in phase I and phase II therapy trials in patients with colon and pancreatic cancer (8–10). Sun et al. (4, 5) recently described a genetic construct composed of the variable regions of 17-1A murine monoclonal antibody and the constant regions of human IgG (human γ3,κ). Protoplasts carrying the chimeric gene construct were fused with a nonproducing mouse myeloma line (SP2/0) to produce a cell line secreting a protein containing human immunoglobulin antigens composed of two heavy and two light chains. This study presents initial characterization of the putative mouse/human chimeric monoclonal antibody to a colon cancer-associated surface antigen.

MATERIALS AND METHODS

Cell lines and culture. The transfected cell line (4, 5) was subcloned with selection of a cell lineage capable of secreting 10 to 15 μg antibody/ml/10^6 cultured cells. The line, designated SG3/5, was grown in Dulbecco's modified Eagle's medium containing antibiotic G418 at 0.8 mg/ml; xanthine, 50 μg/ml; hypoxanthine, 4 μg/ml; mycophenolic acid, 0.8 μg/ml; and 15% fetal calf serum in T-150 flasks (5). Supernatants were harvested and cultures were passaged every 3 to 4 days. Immunoglobulin concentration in the SG3/5 supernatants was quantitated by a particle concentration fluorescence immunoassay (11) with the use of polystyrene beads coated with a goat anti-human IgG(Fc), fluorescein-conjugated goat anti-human IgG(Fc), and standard curves obtained with purified IgG.

Human tumor cell lines were obtained from the following sources and were cultured as described. Unless otherwise noted, all media components were from Flow Laboratories: SW1116 and HT-29 colon cancer lines (American Type Culture Collection) in McCoy's 5A medium supplemented with 15% fetal bovine serum (FBS), 2 mM glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 50 IU/ml penicillin, 50 μg/ml streptomycin, and 100 μg/ml gentamicin sulfate (Elkins-Sinn, Inc., Cherry Hill, NJ), subcultured with trypsin-EDTA every 10 days; PC3, GP6F2, and DU145 prostate cancer lines (Dr. M. Moore, Grady Memorial Hospital, Atlanta, GA) and MCF-7 breast cancer line (Dr. M. Lipman, National Cancer Institute) in a 50-50 mixture of Dulbecco's minimal essential and Ham's F-12 media (D/H medium) with 5% FBS and other supplements as above, subcultured with trypsin-EDTA weekly; HL-60 promyelocytic leukemic line (Dr. D. Miller, Birmingham, AL) in D/H medium with 10% FBS and other supplements as above, grown as suspension cultures and passaged biweekly. PLB-985 myeloblast line cells were cultured and provided by Dr. Thomas Rado (Birmingham, AL).

Immunoglobulin preparations. The native 17-1A mouse monoclonal antibody was obtained from Centocor as a chromatographically purified preparation of 10 mg/ml.

Chimeric 17-1A antibody was initially purified by direct passage

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of SG3/5 cell culture supernatant media over an anti-human IgG3 affinity column, prepared by CNBr-activated Sepharose (Pharmacia) coupling to the mouse monoclonal antibody C3-8-80 ($\gamma 2a,\kappa$) (from Dr. Mary Ellen Conley, Philadelphia, PA), which is specific for human $\gamma 3$ heavy chains. Bound antibody was eluted with 0.58% acetic acid, 0.15 M NaCl, pH 2.6, and the eluted fractions were neutralized immediately by collection into 0.2 M borate buffer, pH 9.5. The eluate was then dialyzed against PBS and concentrated on Amicon PM 30 membranes in stirred cells under nitrogen to 500 to 1000 μg/ml.

Alternatively, tissue culture supernatant from the SG3/5 cell line was concentrated 30-fold on a Minitan concentrator (100,000 M_r cutoff), dialyzed against 35 mM Tris/HCl, pH 8.5, and loaded onto a DEAE-Sepharose 6B column (Pharmacia) equilibrated with the same buffer. SG3/5 antibody was collected from the column filtrate, concentrated fivefold (Amicon concentrator, 50,000 M_r cutoff), and dialyzed against 50 mM sodium acetate, pH 5.0. Antibody solution was then loaded on a Pharmacia fast protein liquid chromatography system with a Mono S column equilibrated in the same buffer and eluted with a NaCl gradient. The final purified preparation was dialyzed against PBS and sterilized with a 0.2-μm filter.

Normal human immunoglobulin fractions were prepared essentially as described by Bird et al. (12) with some modifications. For isolation of normal human IgG3, healthy donor plasma was diluted with an equal volume of PBS, filtered, and applied to a staphylococcal protein A (SPA[3]-Sepharose column (Pharmacia). Most IgG3 molecules do not bind to SPA, whereas IgG1, 2, and 4 subclasses bind efficiently under these conditions. Human IgG3 was then purified from the nonbinding plasma fraction by subsequent immunoaffinity chromatography on the anti-IgG3 column as described above.

Normal human IgG1 + 2 + 4 was prepared by acid elution of bound plasma proteins from the SPA-Sepharose column, using the same procedure as for elution of the anti-IgG3 immunoaffinity column. Eluted fractions were then dialyzed against PBS and depleted of any contaminating IgG3 by passage over the anti-IgG3 column. Both the IgG1 + 2 + 4 and IgG3 fractions of human plasma were dialyzed and concentrated as described above for the 17-1A chimera preparation.

High-titer polyclonal rabbit anti-human cell antibody, raised against the CEM lymphoblast cell line as described (13), was used as a positive control antibody in antibody-dependent cell-mediated cytotoxicity (ADCC) assays.

High-pressure liquid chromatography (HPLC) analyses. Immunoglobulin preparations were analyzed for purity and IgG concentration by using a Bio-Rad Quick Check Analyser system with a Bio-Sil TSK 250 column (300 x 7.5 mm) and a 10-mM phosphate, pH 6.8, 0.3 M NaCl, 10% dimethyl sulfoxide (v/v) buffer system, with pump speed of 1 ml/min. When necessary, monomeric fractions of immunoglobulin preparations were obtained on the same system with the use of a larger preparative column (TSK 250 600 x 21.5 mm).

Antibody binding assays. To compare the binding of native and chimeric 17-1A to tumor cells, 1×10^6 tumor cells were incubated with identical amounts (33 μg) of each antibody in 150 μl RPMI 1640 for 60 min at 37°C, washed thrice with PBS, and resuspended in RPMI 1640, and the cell number was determined. The amount of murine 17-1A bound to the surface was determined by using a quantitative ^{125}I-SPA assay (14), and the amount of chimera bound was determined by quantitative ^{125}I-monoclonal anti-human IgG(Fc) assay (15, 16). The results were expressed as molecules of antibody bound per tumor cell.

To examine the epitope specificity and to compare binding affinities of native and chimeric 17-1A, competitive inhibition experiments were carried out. Native 17-1A was radiolabeled with ^{125}I by the chloramine-T method (15). Fractions of radiolabeled 17-1A (10^7 cpm) were incubated with 5×10^5 tumor cells alone or with varying concentrations of competing native or chimeric 17-1A. The percentage of inhibition of binding of ^{125}I-17-1A was plotted against concentration of competing native or chimeric 17-1A added, and the concentration required to produce 50% inhibition (I_{50}) of binding was determined.

ADCC assays. Monocyte and lymphocyte lysis of antibody-coated tumor cells was assayed essentially as described (13). Suspensions of lymphocytes and monocytes purified from peripheral blood (13) were washed twice in RPMI 1640 (Gibco) + 10% FBS. Tumor cells were harvested with trypsin-EDTA and labeled with ^{51}Cr-Na_2-CrO_4 (Amersham). ^{51}Cr-labeled SW1116 or HT-29 colon cancer cell lines were either unsensitized or coated with optimal amounts of the various antibody preparations (40 μg/10^6 cells for 17-1A and chimera) to give >100,000 molecules of IgG antibody per cell, and were added to duplicate round-bottom microwells at 10^4 cells/well. Lymphocyte and monocyte suspensions were then added to give the indicated ratios of effector to target (E:T) cells in a final volume of 300 μl RPMI-10% FBS per well. Microwell trays were centrifuged at 600 × G for 3 min and then incubated for 4 hr at 37°C in a humidified atmosphere of 8% CO_2-air. After incubation, trays were centrifuged at 600 × G for 10 min, and 150 μl (half volume) of supernatant from each well were transferred to counting vials. The percentage of lysis was calculated by the formula

$$\% \text{ Lysis} = \frac{A - B}{C - B} \times 100$$

A = 2 × mean cpm in 150 μl supernatant from wells containing effector cells plus target cells; B = 2 × mean cpm in 150 μl supernatant from wells containing target cells alone; C = mean total ^{51}Cr cpm of added target cells.

Monocyte ADCC of ^{51}Cr-labeled O^+ human red cells was performed in a similar manner and as previously described (17), using a high-titer anti-D alloantiserum. For inhibition studies, the ^{51}Cr-labeled and anti-D-coated red cells were added to monocytes at an E:T ratio of 1:1 in the presence of competing soluble antibody preparations at final concentrations from 0.15 to 3.0 μg/ml.

Complement lysis. ^{51}Cr-labeled tumor cells, sensitized with antibody as above and washed, were added to round-bottom microwell plates at 10^4 cells/well and mixed with fresh-frozen human serum diluted in gelatin-Veronal-buffered saline as a complement source (18). After incubation at 37°C for 45 min, plates were mixed and centrifuged, and half-volumes of supernatant were removed for measurement of released ^{51}Cr. Positive controls for complement activity were ^{51}Cr-labeled sheep red cells sensitized with rabbit anti-sheep red cell hemolysin. Human sera were routinely absorbed on ice with sheep red cells to remove anti-sheep antibodies (18).

RESULTS

Gel filtration analysis of the chimeric antibody. HPLC analysis on a molecular sizing column demonstrated that the chimeric antibody had a highly reproducible retention time of 7.7 min (Fig. 1*B*), whereas the native 17-1A retention time was 8.5 min (Fig. 1*A*). Normal human IgG3 (Fig. 1*C*), which is known to be of higher M_r than the other human IgG subclasses (19, 20), eluted identically to the chimeric 17-1A; normal human IgG depleted of IgG3 (Fig. 1*D*) was similar to native 17-1A. These analyses also indicated that our antibody preparations were of high purity. Some immunoglobulin preparations contained small amounts of higher M_r material, which may reflect aggregation resulting from the immunoaffinity preparative technique or from storage, and which were removed by subsequent preparative HPLC fractionation. Five other murine monoclonal antibodies (two IgG2a and three IgG1) eluted identically to native 17-1A (data not shown).

Binding of chimeric 17-1A to tumor cells. Native 17-1A bound to colon cancer cells was readily detected with ^{125}I-SPA but not by ^{125}I-monoclonal anti-human IgG(Fc). Conversely, chimeric 17-1A bound to colon cancer cells was readily detected with ^{125}I-monoclonal anti-human IgG(Fc) but not by ^{125}I-SPA (data not shown). We examined the binding of native and chimeric 17-1A with colon cancer cell lines, other adenocarcinoma cell lines (prostate and breast), and non-adenocarcinoma cell types. As seen in Figure 2, similar patterns of reactivity were observed with the native and chimeric 17-1A preparations. Both bound well to two colon cancer cell lines and less well to other target cell populations. We then compared the ability of various doses of native and chimeric antibody to bind to malignant cell lines. Both native and chimeric 17-1A demonstrated high binding to the colon adenocarcinoma cell line HT-29 and modest binding to the prostate adenocarcinoma cell line DU145, in a dose-dependent manner (Table I). These results suggest that

[3] Abbreviations used in this paper: SPA, staphylococcal protein A; ADCC, antibody-dependent cell-mediated cytotoxicity; E:T, effector cell to target cell; RAH, rabbit anti-human cell polyclonal antibody.

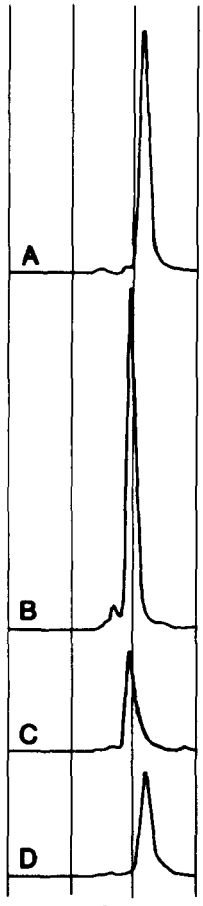

Figure 1. HPLC profiles of purified immunoglobulin preparations. Samples diluted to 50 to 500 μg/ml were analyzed as described in the text. Vertical lines on the absorbance 280 nm profiles represent 4 min, with sample injection at left. (*A*) Native 17-1A, retention time 8.5 min; (*B*) chimeric 17-1A, 7.7 min; (*C*) normal human IgG3, 7.8 min; (*D*) normal human IgG1 + 2 + 4, 8.7 min.

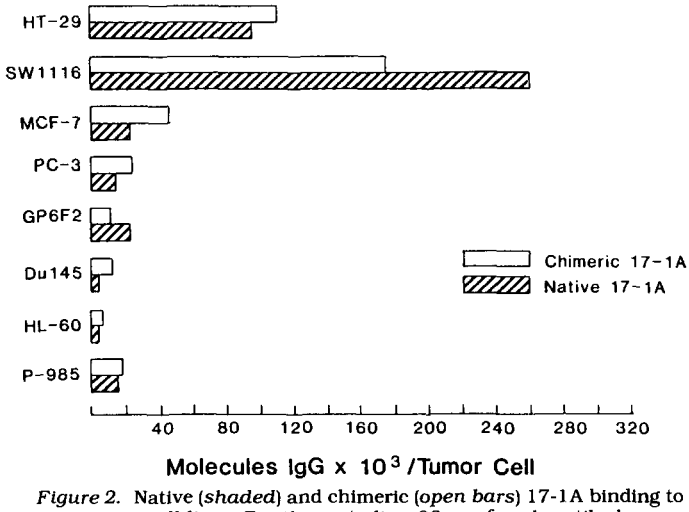

Figure 2. Native (*shaded*) and chimeric (*open bars*) 17-1A binding to human tumor cell lines. For these studies, 33 μg of each antibody were incubated with 1 × 10^6 tumor cells at 37°C for 1 hr and then assayed for native 17-1A binding with ^{125}I-SPA and chimera binding with ^{125}I-monoclonal anti-human IgG(Fc) as described in the text. Cell lines tested were: HT-29 and SW1116, colon cancer; MCF-7, breast cancer; PC3, GP6F2 and DU145, prostate cancer; HL-60 and P985, promyelocytic and myeloblastic leukemia, respectively.

the presence of human heavy and light chain constant regions in the chimeric antibody molecule did not alter the antigen specificity or reactivity of the murine variable region (antigen combining site).

TABLE I

Native and chimeric 17-1A binding to colon (HT-29) and prostate (DU145) cancer cells

Antibody[a]	Molecules of IgG/Cell[b]			
	HT-29 cells		DU-145 cells	
	17-1A	Chimera	17-1A	Chimera
33 μg	108,532	95,922	12,045	10,212
16.5 μg	66,288	71,073	11,944	9,890
5 μg	31,944	36,136	3,777	4,807

[a] The amount of purified native or chimeric 17-1A incubated with 1 × 10^6 tumor cells at 37°C for 60 min.

[b] Results are expressed as molecules of immunoglobulin/cell (mean of duplicate values). Native 17-1A was quantitated with ^{125}I-SPA and chimera with ^{125}I-monoclonal anti-human IgG.

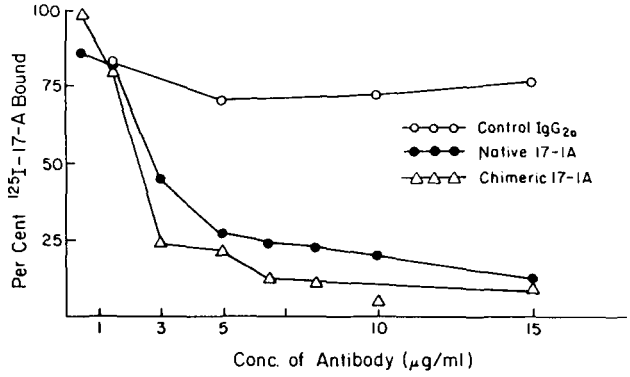

Figure 3. Competitive inhibition of ^{125}I-labeled 17-1A binding to HT-29 colon cancer cell line by native 17-1A (●), chimeric 17-1A (△), and control mouse IgG2a (○).

To examine this in a more rigorous fashion, competitive inhibition studies were carried out to compare the ability of nonradioactive native and chimeric 17-1A to inhibit the binding of ^{125}I-labeled native 17-1A to target cells. A typical experiment is depicted in Figure 3. The native and chimeric 17-1A preparations were equally effective at inhibiting the binding of ^{125}I-17-1A to HT-29 colon cancer cells, whereas a control murine monoclonal IgG2a directed to an irrelevant antigen did not inhibit binding. The results of three independent competitive inhibition experiments demonstrated that the mean (±SE) I_{50} value for native 17-1A was 2.6 ± 0.2 μg/ml as compared with 2.4 ± 0.2 μg/ml for the chimeric 17-1A. A similar set of experiments was done with SW1116 colon cancer cells; the native and chimeric 17-1A produced comparable inhibition of binding of ^{125}I-17-1A to this cell line as well (data not shown).

Biologic activity. The ability of native and chimeric 17-1A to mediate tumor cell lysis by human lymphocytes and monocytes was analyzed by using two colon cancer lines, SW1116 (Table II) and HT-29 (Table III). In some experiments, tumor cells were also coated with polyclonal rabbit anti-human antibody (RAH) as a known positive control. Both native and chimeric 17-1A promoted moderate levels of SW1116 tumor cell lysis by lymphocyte effector cells but were never observed to be as efficient as RAH (Table II). Lymphocyte ADCC against the HT-29 targets is more difficult to assess because of relatively high levels of antibody-independent destruction of these cells by most donor lymphocytes (Table III); this is presumably due to NK activity in the lymphocyte preparations, because monocyte effectors did not lyse the identical uncoated HT-29 targets in the same experiments. Monocytes were competent in ADCC against tumor cells coated with RAH, but monocyte lysis of 17-1A or chi-

TABLE II
ADCC against SW1116 cells

Expt.	Antibody	Lymphocytes[a]			Monocytes[a]		
		100:1	30:1	10:1	25:1	10:1	3:1
1	None	12	5	2			
	17-1A	34	8	2			
	Chim. 17-1A	29	12	4			
2	None	7	3	3	3	5	5
	17-1A	43	13	5	6	5	4
	RAH	59	42	23	17	11	3
3	None	5	1	0	1	0	2
	Chim. 17-1A	33	10	3	10	2	0
	RAH	65	54	28	35	15	7
4	None	4	1	0	2	0	2
	Chim. 17-1A	8	3	1	4	2	3
	RAH	64	55	32	30	23	13

[a] Mean percent lysis at the indicated E:T ratios.

TABLE III
ADCC against HT-29 cells

Expt.	Antibody	Lymphocytes[a]			Monocytes[a]		
		100:1	30:1	10:1	25:1	10:1	3:1
1	None	30	12	6		8	2
	17-1A	40	12	6		9	4
	Chim. 17-1A	33	13	6		7	2
	RAH	73	59	36		27	11
2	None	12	1	2	2	0	2
	17-1A	24	8	5	5	2	3
	Chim. 17-1A	22	3	4	2	3	3
3	None	38	12	3	9	5	1
	Chim. 17-1A	45	14	3	12	1	1
	RAH	67	56	29	23	11	5
4	None	39	14	5	12	6	4
	Chim. 17-1A	43	16	5	9	2	1
	RAH	66	51	27	32	18	7
5	None	4	4	0	1	0	0
	17-1A	19	13	1	8	6	3
	Chim. 17-1A	16	9	7	10	6	2
	RAH	44	32	14	23	11	4

[a] Mean percent lysis at the indicated E:T ratios.

mera-sensitized tumor cells was rarely significant at any E:T ratio tested (Tables II and III). Identical results were obtained with either immunoaffinity- or ion-exchange chromatography-purified preparations of the chimeric 17-1A.

We considered the possibility that the genetically engineered chimeric antibody might bind to human monocyte Fc receptors with a different efficiency than native human IgG. To examine this, we compared the abilities of whole human IgG (Cappel Laboratories, affinity-chromatographically purified) and the 17-1A/human $\gamma 3$ chimera to inhibit monocyte ADCC against red cells coated with human IgG anti-D. Both preparations were highly effective at inhibiting ADCC. The average I_{50} value for the chimera was 2.2 μg/ml vs 1.8 μg/ml for whole human IgG, and both preparations inhibited monocyte ADCC by >90% at concentrations of approximately 3 μg/ml.

We have also investigated the susceptibility of tumor cell lines sensitized with various antibody preparations to undergo human complement-mediated lysis. The human colon cancer cell lines used, SW1116 and HT-29, are apparently resistant to human complement lysis; cells highly sensitized with the polyclonal rabbit antibody (250,000 to 400,000 IgG molecules/tumor cell) demonstrated less than 10% specific lysis in the presence of human complement at concentrations 10-fold higher than required to produce >95% lysis of rabbit antibody-coated sheep red cells. Native and chimeric 17-1A, at doses of 100,000 to 200,000 molecules/cell, also did not mediate complement lysis of HT-29 or SW1116 cells.

DISCUSSION

The cell line SG3/5 was transfected with a chimeric gene construct encoding the variable regions (antigen combining site) of the murine monoclonal 17-1A antibody linked to the constant regions of human $\gamma 3$ heavy chain and κ light chain (5). The immunoglobulin secreted by this cell line was further characterized in this study by a variety of physicochemical, immunologic, and functional assays, which indicate that it behaves as a true chimeric antibody.

Native 17-1A (mouse $\gamma 2a,\kappa$) was readily bound by ^{125}I-SPA, whereas the chimeric antibody was not, a characteristic of normal human IgG3. Conversely, the chimeric protein reacted well with murine monoclonal antibody specific for the Fc portion of human immunoglobulin (16) and was bound to an immunoaffinity column made with murine monoclonal antibody specific for human $\gamma 3$ heavy chains. The isolated chimeric antibody demonstrated a larger molecular size by gel filtration chromatography than either the native 17-1A monoclonal antibody or normal human immunoglobulin of subclasses 1, 2, and 4, but was identical to affinity-purified normal human IgG3 (Fig. 1). Previous studies have shown that human IgG3 has repeated sequences in the hinge region that are not present in IgG1, 2, or 4 subclasses and that account for its unique chromatographic behavior (19, 20).

The chimeric antibody reacted with human tumor cell lines in a manner paralleling the activity of native 17-1A. In competitive inhibition studies, native and chimeric 17-1A had nearly identical I_{50} values, confirming that chimeric antibody binds the same antigen as native 17-1A and demonstrating that the presence of human constant regions in the molecule had not alterated the binding affinity. It is therefore clear that the cell line SG3/5 secretes a functional chimeric antibody with the antigen combining site (variable region) of murine 17-1A and the constant regions of human IgG3.

Clinical studies to date with mouse monoclonal antibodies specific for human antigens (including 17-1A) have documented several shortcomings of these reagents in terms of successful therapeutic applications. The murine antibodies are intensely immunogenic in man, resulting in brisk immune responses, sometimes including anaphylaxis (8, 10, 21, 22), and appear to have relatively short circulating half-lives of 15 to 30 hr (10). Infused human IgG3 has been reported to have a circulating half-life in man of about 7 days (23); so it is presumed that the human/mouse chimeric antibody described here would demonstrate a fivefold to 10-fold longer half-life in vivo than has been observed in human trials with the native 17-1A (10). It might also be expected that the human chimeric 17-1A would obviate the humoral anti-mouse immunoglobulin response, although researchers of several studies have reported that a significant proportion of such human anti-mouse antibodies are apparently idiotype specific (21, 22). Whether human/mouse chimeric antibodies will elicit human anti-idiotypic responses with the same efficiency as mouse monoclonal antibodies remains to be determined.

Another potential advantage of human/mouse chimeric antibodies in the treatment of human diseases is that homologous immunoglobulin protein might be more efficient in mediating the desired immune effector func-

tions than heterologous antibodies. Our in vitro studies of tumor cell ADCC by human lymphocytes and monocytes and of complement lysis have not revealed any advantage of the human γ3/mouse chimeric antibody as compared with the native 17-1A (mouse γ2a) monoclonal antibody. It was previously reported that human IgG3 and mouse IgG2a compete equally well with the binding of mouse 17-1A to Fc receptors on activated U937 human monocytic line cells (24), so our ADCC results are perhaps not surprising. The human γ3 chimera did not mediate complement lysis of target tumor cell lines, even though human IgG3 is regarded as an efficient complement-fixing antibody (25). However, this may reflect the observation that many nucleated cells are resistant to human complement-mediated lysis (26, 27).

Human γ1 predominates among the IgG subclasses in the circulation as well as in most antigen-specific antibody responses studied to date (12, 25). Additionally, infused human IgG1 myeloma proteins demonstrate significantly longer circulating half-lives in man compared with IgG3 myelomas (23). It might therefore be argued that we should have first studied human γ1/mouse 17-1A chimeras. However, for technical reasons, the γ3-expressing chimera was the first to be successfully constructed and transfected. (The analogous human γ1 chimera has only recently been produced and is currently being characterized.) Because human IgG1 and IgG3 are reported to have identical activities in Fc receptor binding and complement-fixation assays (24, 25), we believe that the results reported here are probably indicative of what can be expected from the analogous γ1 chimera in in vitro assays.

In summary, the mouse/human γ3 chimeric 17-1A antibody appears to have the antigen combining specificity and affinity as well as the biologic behavior of native murine 17-1A. Human constant/mouse variable region chimeric monoclonal antibodies of the type reported here may represent an attractive alternative to the use of murine heterologous monoclonal antibodies in immunotherapy of human diseases.

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