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Biological characterization of a chimeric mouse-human IgM antibody directed against the 17-1A antigen

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Summary. A chimeric antibody was constructed in which the murine H- and L-chain variable regions of mAb 17-1A, raised against human colorectal cancer cells, were joined with the human constant μ and κ regions. Transfection of these constructs into the murine myeloma Sp2/0 resulted in the expression and secretion of a pentameric Ig, designated chimeric 17-1A IgM. The chimeric 17-1A IgM was subsequently compared to a previously described chimeric 17-1A IgG1 for biological activities. Both chimeric mAbs were equally effective (weight basis) in competing against the binding of murine ^{125}I-17-1A to cultures of HT-29 colon carcinoma cells. The calculated association constants for the chimeric 17-1A IgM and IgG1 were 1.63×10^8 l/mol and 3.41×10^7 l/mol, respectively. Unlike chimeric 17-1A IgG1, the chimeric 17-1A IgM was able to render colon carcinoma target cells susceptible to lysis by both xenogeneic (rabbit) and human complement. The extent of complement-mediated lysis dependent upon chimeric 17-1A IgM was correlated to 17-1A antigen expression on target cells. HT-29 colon carcinoma cells treated with chimeric 17-1A IgM did not directly result in antibody-dependent cellular cytotoxicity by human peripheral blood monocytes. However, chimeric 17-1A IgM greatly enhanced the deposition of C3 on complement-treated HT-29 cells, and concomitant incubation with monocytes resulted in heightened lysis of the tumor cells. The feasibility of enhancing host defense against gastrointestinal malignancies by the administration of this chimeric 17-1A IgM may have certain clinical advantages.

Introduction

The murine mAb 17-1A (IgG2aκ) has been demonstrated to have a relative specificity for cells of human gastrointestinal adenocarcinomas [12, 17]. The mAb 17-1A mediates antibody-dependent cellular cytotoxicity (ADCC) of colon carcinoma cell lines in vitro [11, 33], and suppresses tumor growth in nude mice transplanted with human colorectal carcinomas [13]. Moreover, mAb 17-1A has been used successfully in radioimaging studies in man [22] as well as in phase I and phase II therapy trials in patients with colon and pancreatic cancer [28, 29]. However, the clinical use of murine Ig is hampered by their short half-life and potential to elicit an anti-(mouse Ig) response, which may interfere with therapy [4, 19, 28, 32].

Advances in recombinant DNA technology have made possible the construction of chimeric immunoglobulin genes in which DNA segments encoding the variable regions from the heavy and light chains of murine hybridomas are ligated to the DNA segments encoding human constant regions [24, 25]. Transfection of expression vectors containing these chimeric immunoglobulin genes into myeloma cells have resulted in the production of functional chimeric antibodies that may be useful for human diagnosis and therapy [2, 18, 27, 35].

Recently, we have constructed mouse/human IgG1, IgG2, IgG3 and IgG4 chimeric 17-1A mAbs. These chimeric mAbs retained their specificity for the 17-1A antigen and their antitumor effects have been reported [30, 31, 34]. However, like the native murine 17-1A mAb, the chimeric IgG mAbs were unable to mediate complement lysis of tumor cells in vitro [30, 31, 34]. Because antibody-dependent complement-mediated cytotoxicity (ADCMC) may represent an important host response for the in vivo destruction of malignant cells [7], we sought to construct a chimeric 17-1A Ig capable of mediating this response. Classically, this could be accomplished by engineering an IgM reactive to the 17-1A antigen. In the present paper, we report on the biological characterization of a pentameric chimeric 17-1A mAb composed of the murine H and L variable regions of mAb 17-1A and the human μ and κ constant regions.

Materials and methods

Reagents. All media and additives were purchased from Hazleton Research Products (Denver, Pa). Fetal bovine serum was purchased from HyClone (Logan, Utah). Rabbit complement was purchased from Pel-Freez Biological (Rogers, Ariz). Human complement was purchased from Cordis Laboratories (Miami, Fla). The hemolytic titer in CH_{50} units/ml was 128 for both complement sources. Affinity-purified goat anti-(human C3) was purchased from Chemicon (El Segundo, Calif). Sodium [^{51}Cr]chromate (sp. act. 250–500 mCi/mg), [^{111}In]indium oxyquinoline (1 mCi/ml) and carrier-free sodium [^{125}I]iodide (100 mCi/ml) were purchased from Amersham Corporation (Arlington Heights, Ill).

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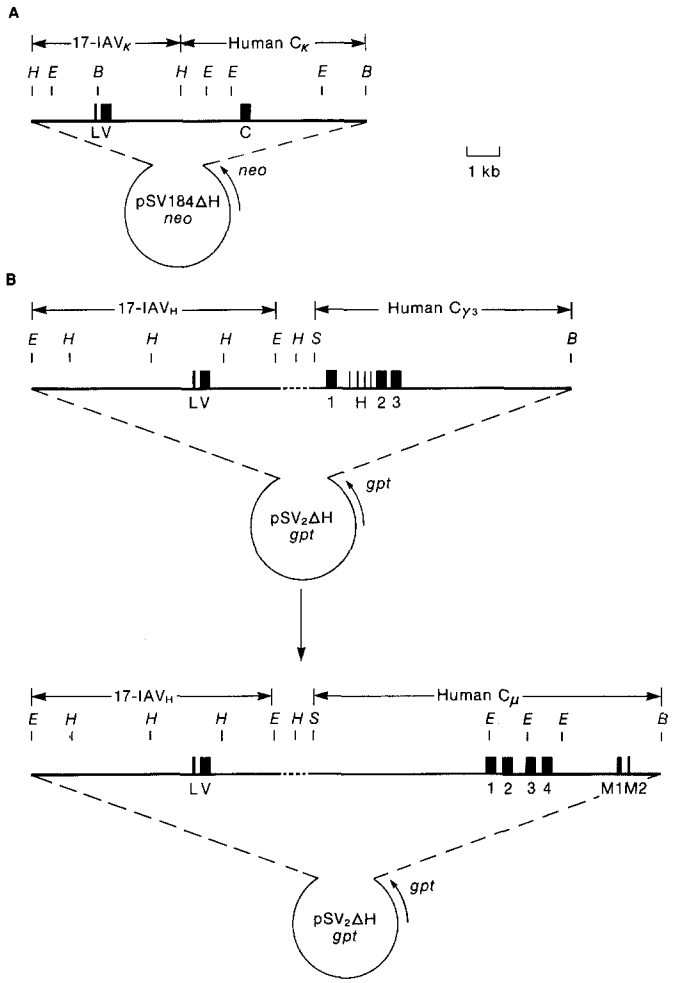

Fig. 1. A Structure of the chimeric 17-1A light-chain vector. **B** Construction of expression vector containing the 17-1A V_H and human Cμ genes. The human γ3 constant region of (pSV$_2$ΔH*gpt* 17-1A V_H)-(human Cγ3) was replaced with the 10-kb *SalI*/*Bam*HI DNA fragment containing the human μ constant region gene to give (pSV$_2$ΔH*gpt* 17-1A V_H)-(human Cμ). The ranges of the DNA sequences are indicated with two-way horizontal arrows. The transcriptional directions of the *gpt* and *neo* genes are as indicated. Ig exons are represented by boxes. *L*, leader exon; *V*, *VJ* or *VDJ* exon; *C*, constant region exon; *H*, hinge exon; *1*, *2*, *3*, and *4* other exons of the heavy-chain region; *M1* and *M2*, membrane exon. *Broken line* between 17-1A V_H and the constant-region genes represents residual S107 intervening sequences carried over during the derivation of this plasmid. Restriction endonuclease abbreviations = *B*, *Bam*HI; *E*, *Eco*RI; *H*, *Hin*dIII; S, *Sal*I

*Construction of expression vector (pSV$_2$ΔH*gpt* 17-1A V_H)-(human Cμ).* Chimeric Ig genes were constructed by ligation of cloned mouse variable (V) genes with human κ or μ constant (C) genes. The cloning of V genes of 17-1A and the construction of expression vectors containing chimeric κ and Cγ3 genes were as described [35]. The 10-kb *Hind*III/*Bam*HI human Cμ genomic DNA (a generous gift from Dr. Louise Showe, Wistar Institute) was used in the derivation of the chimeric μ vector, as shown in Fig. 1. The 5′ *Hind*III site of Cμ was modified to a *Sal*I site using commercial linkers. The resulting *Sal*I/*Bam*HI DNA fragment, containing the human Cμ gene, was used to replace the human Cγ3 gene in the chimeric γ3 vector to yield (pSV$_2$ΔH*gpt* 17-1A V_H)-(human Cμ).

Gene transfer by electroporation. The light and heavy-chain vectors (pSV-184Δ*Hneo* 17-1A V_H)-(human Cκ) (Fig. 1 A) and (pSV$_2$Δ*Hgpt* 17-1A V_H)-(human Cμ) (Fig. 1 B), were used to transfect the nonproducing mouse myeloma Sp2/0 cells sequentially. Stable light-chain-producing cell lines were established first. The heavy-chain construct was then introduced into the light-chain producers. The efficiency of electroporation was approximately 10^{-5} at each step. DNA was introduced into SP2/0 cells by electroporation using a Gene Pulser transfection apparatus (Bio-Rad Laboratories, Richmond, Calif). Cells were subjected to an electric field of 0.2 kV with the capacitor set at 960 μF in the presence of 20 μg plasmid DNA in 0.8 ml Dulbecco's phosphate-buffered saline (PBS). After incubation at 37° C for 48 h, the transformants were selected in growth medium containing G418 at 1 mg/ml for light-chain plasmid uptake, and mycophenolic acid at 1 μg/ml, xanthine at 50 μg/ml and hypoxanthine at 2.5 μg/ml for the heavy-chain uptake.

Cell lines producing chimeric antibody. The stable transformant lines were subsequently carried in medium containing G418, mycophenolic acid, xanthine, and hypoxanthine. Tissue-culture supernatant was analyzed for IgM protein content by particle concentration fluorescence immunoassay [15] using standard curves generated with human IgM (Jackson ImmunoResearch Lab, West Grove, Pa). The concentration of chimeric 17-1A IgM was determined by using polystyrene beads coated with goat antibody to human IgM Fc5μ (Jackson ImmunoResearch Lab, West Grove, Pa) and fluorescein-conjugated goat antibody to human IgM. The assays were carried out with an automated instrument (Pandex Laboratories, Mundelein, Ill).

Preparation of monoclonal antibodies. The clone designated MK was chosen and expanded for further analysis. The IgM concentration in the culture supernatant of the MK cell line was 5 μg/ml. The chimeric 17-1A IgM mAb was purified by passing the tissue-culture supernatant over an immune-affinity column, which was prepared by coupling goat anti-(human IgM) antibody to CnBr-activated Sepharose (Pharmacia, Piscataway, NJ). The bound antibody was eluted with 0.1 M glycine at pH 2.2 and fractions were neutralized with 1.0 M TRIS, pH 8.5 to a final concentration of 0.1 M. The purified IgM was then dialyzed against PBS and concentrated using Centricon 30 micro-concentrators (Amicon, Danvers, Mass). Affinity chromatography of MK supernatant on a goat anti-(human IgM) matrix resulted in a 35% recovery of applied chimeric IgM. HPLC of the eluted peak demonstrated a retention time comparable to that of a standard pentameric IgM.

The mouse/human chimeric mAb 17-1A (IgG1) was obtained and purified as described [31]. The murine mAb 17-1A (IgG2a) and the human mAb HA-1A (IgM) were obtained in purified form from Dr. Jeffrey Mattis at Centocor (Malvern, Pa).

Target cell cultures. Human tumor and embryonic fibroblast cell lines were obtained from the following sources: HT-29 colon carcinoma line (Dr. Isaiah J. Fidler, M. D. Anderson Hospital and Tumor Institute, Houston, Tex); SW1116 colon carcinoma line (Dr. A. Leibovitz, Scott White Clinic, Temple, Tex); SW948 colon carcinoma line (American Type Culture Collection, Rockville, Md);

ZR75-1 and BT-20 breast carcinoma lines (E. G. & G. Mason Research Institute, Worchester, Mass); OVCAR3 ovarian carcinoma line (Dr. David Segal, NCI, Bethesda, Md); human embryonic kidney (HEK) and intestine fibroblasts (HEI, Earl Clay Laboratories, Palo Alto, Calif). All cultures were maintained on plastic in Eagle's minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum and 2 mM L-glutamine at 37° C in a humidified atmosphere containing 5% CO_2.

Radioimmunoassay for binding of monoclonal antibodies to human colon carcinoma cells. Radioiodinated murine mAb 17-1A for use in competitive binding studies was prepared using the iodogen (1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril) technique [20]. The resulting specific activity was 8.47 μCi/ μg. Carcinoma cells in exponential growth phase were harvested by a 4-min trypsinization with 0.25% Difco trypsin and 0.2% EDTA. The cells were washed by centrifugation at 250 *g* for 10 min, resuspended in medium and 2×10^5 were plated into each culture well of a 96-well flat-bottom Microtest II plate (Falcon Plastics, Oxnard, Calif). Eighteen hours after initial plating, the cultures were washed three times for 5 min each with PBS containing 0.3% gelatin, hereafter referred to as binding medium. Fifty microliters of mAb diluted in binding medium (0.5–100 μg/ml) containing 10^6 cpm ^{125}I-labeled murine 17-1A mAb were added to the wells. Following a 60-min incubation at 20° C, the cultures were washed three times with binding medium and the cell monolayer was lysed with 0.1 ml 0.1 M NaOH. The radioactivity of the lysate was measured in a gamma counter and specific cell-bound radioactivity was determined by subtracting values in control wells containing cells incubated with 500 μg/ml unlabeled mAb and ^{125}I-labeled murine 17-1A.

Antibody-dependent complement-mediated cytotoxicity assays. Target cells in exponential growth phase were harvested as described above and 1×10^7 cells in 1 ml RPMI medium with 5% heat-inactivated fetal bovine serum were labeled with 100 μCi [^{111}In]indium oxyquinoline [37] or 500 μCi sodium [^{51}Cr]chromate [36]. The target cells were washed three times in medium and incubated with the mAbs for 60 min at 4° C. The target cells were washed and added in triplicate to 96-well U-bottomed plates (Falcon Plastics, Oxnard, Calif) at 1×10^4 cells/well. Complement was then added at predefined dilutions. The total volume in the wells was 0.2 ml. The plates were incubated for 45 min (^{51}Cr-labeled target cells) or up to 24 h (^{111}In-labeled target cells) at 37° C in a humidified atmosphere of 5% CO_2. After this period the plates were centrifuged at 100 *g* for 5 min and 0.1 ml supernatant was monitored for radioactivity. For all experiments, the percentage of cytotoxicity was calculated according to the formula:

$$\text{ADCMC generated (\%)} = (A - B/C - B) \times 100$$

where A = radioactivity (cpm) in supernatants from target cells incubated with complement and mAb, B = radioactivity (cpm) in supernatants from target cells incubated alone (spontaneous release), C = radioactivity (cpm) in supernatants from target cells incubated with 1% NP-40 (maximum release).

Isolation and culture of human monocytes from peripheral blood. Mononuclear blood leukocytes were collected from the peripheral blood of normal donors by separation on Ficoll-Paque (Pharmacia, Piscataway, NJ), and washed twice in Hanks' balanced salt solution (HBSS). Peripheral blood monocytes were isolated from the lymphocytes by adherence selection on gelatin/fibronectin-coated flasks as described elsewhere [9]. Briefly, 75-cm^2 tissue-culture flasks, which had been precoated with a 1% gelatin solution, were incubated with 10 ml autologous plasma for 60 min at 37° C. The flasks were then rinsed twice with HBSS and $(20–40) \times 10^6$ mononuclear cells in RPMI 1640 were added. The cultures were incubated for 60 min at 37° C, after which the nonadherent cells were removed. The flasks were washed twice with HBSS and the adherent cells (monocyte-enriched) were harvested by a 1-min exposure to HBSS containing 0.2% EDTA and 0.1% bovine serum albumin. The adherent cell population was washed twice and resuspended in RPMI medium with 10% heat-inactivated fetal bovine serum and 2 mM glutamine. At this point, the purity of the monocyte population was between 93%–96% as assessed by morphology and myeloperoxidase staining.

Antibody-dependent cellular cytotoxicity assays. Colon carcinoma cells were labeled with [^{111}In]indium oxyquinoline as described above. The target cells were washed three times in medium and incubated with the mAbs for 60 min at 37° C. The target cells were washed and added in triplicate to 96-well U-bottomed plates (Falcon Plastics, Oxnard, Calif) at 1×10^4 cells/well. Monocytes were then added to obtain a 50:1 E/T ratio. The total volume in the wells was 0.2 ml. The plates were incubated for 18 h at 37° C in a humidified atmosphere of 5% CO_2. After this period the plates were centrifuged at 100 *g* for 5 min and 0.1 ml supernatant was monitored for radioactivity. The percentage of cytotoxicity was calculated according to the formula described above.

Determination of the third component of complement (C3) deposition on tumor cells. Affinity-purified goat anti-(human C3) (0.5 mg) was absorbed three times against 2×10^7 HT-29 carcinoma cells in 2 ml PBS by sequential incubations for 60 min at 4° C followed by centrifugation at 250 *g* for 10 min. The resulting antibody was labeled with ^{125}I as described above. Cultures of HT-29 cells were established as described above and incubated at 4° C with 6 μg chimeric 17-1A IgM to achieve equilibrium binding. The cultures were washed three times and sequentially incubated for 30 min each with a 1/4 dilution of human complement followed by ^{125}I-labeled goat anti-(human C3). The cultures were assessed for cell-bound radioactivity as described above.

Results

Binding specificity of chimeric 17-1A IgM to the HT-29 human colon carcinoma cell line

Previous results have demonstrated that the presence of human H- and L-chain constant regions in chimeric 17-1A IgG does not alter the antigen specificity or reactivity of the murine V region [30, 31, 35]. To examine this for the newly constructed chimeric 17-1A IgM, competitive inhibition studies were carried out comparing the ability of non-radioactive chimeric 17-1A IgG1 or chimeric 17-1A IgM to inhibit the binding of ^{125}I-labeled native murine 17-1A mAb to cultures of HT-29 colon carcinoma cells.

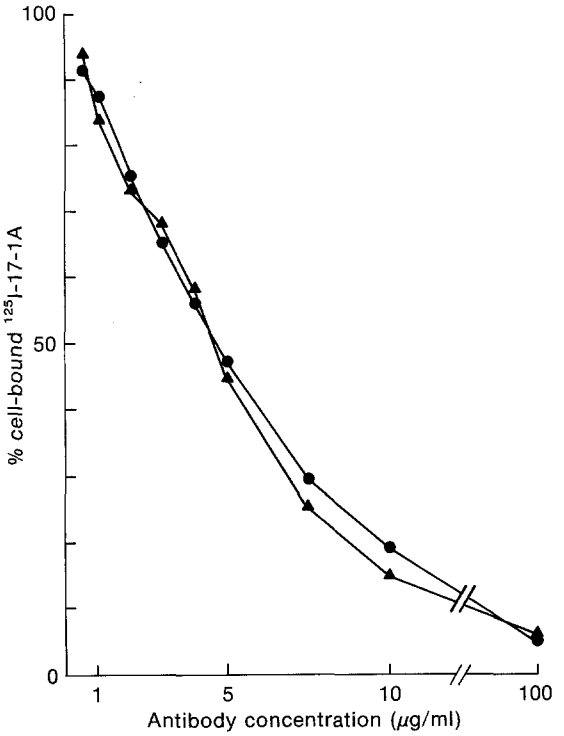

Fig. 2. Competitive inhibition of ^{125}I-labeled murine 17-1A mAb to HT-29 colon carcinoma cells with the chimeric 17-1A IgG1 or IgM. Triplicate sets of HT-29 cells (2×10^5) were incubated at 37° C with ^{125}I-labeled 17-1A murine mAb admixed with appropriate concentrations of unlabeled chimeric 17-1A IgG1 (●) or IgM (▲) to achieve equilibrium binding. Specific cell-associated radioactivity was determined and expressed as a percentage of total ^{125}I-labeled murine 17-1A mAb bound. Specific cell-associated radioactivity in the absence of competing mAb equaled 16273 cpm. Individual variations from the mean did not exceed 10%

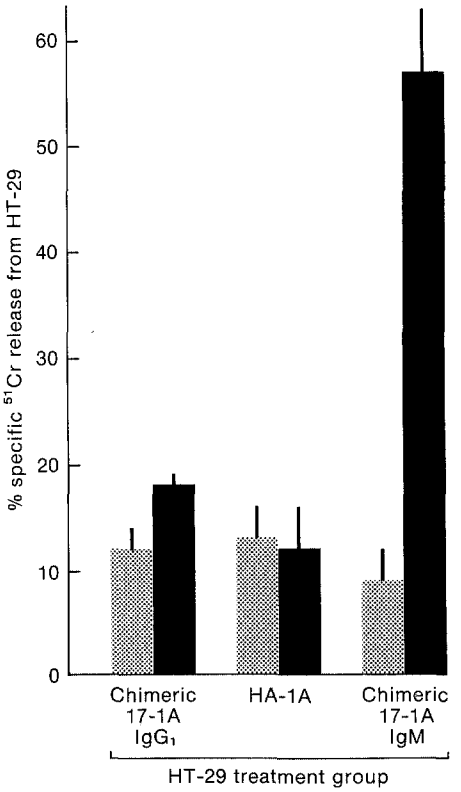

Fig. 3. ADCMC against HT-29 cells incubated with chimeric 17-1A IgM and rabbit complement. HT-29 cells (2×10^4) prelabeled with ^{51}Cr were incubated at 37° C with a 1/4 dilution of rabbit complement alone (*open bars*) or with complement and 30 pg mAb/cell (*closed bars*). Radioactivity released into the media was determined after 45 min of cultivation. HT-29 input totaled 9866 cpm. Spontaneous release of radiolabel at 45 min of cultivation was 3%. The means ± SE of triplicate cultures are indicated.

The chimeric 17-1A IgG1 and chimeric 17-1A IgM were equally effective (weight basis) at inhibiting the binding of murine ^{125}I-17-1A to the target cells (Fig. 2). The results of three separate competitive inhibition experiments demonstrated that the mean (± SE) concentration of competing (unlabeled) antibody required to produce a 50% reduction in tracer antibody binding was 4.4 ± 0.3 μg/ml and 4.6 ± 0.2 μg/ml for chimeric 17-1A IgG1 and IgM, respectively. These data are in agreement with previously reported results obtained with native murine 17-1A mAb [8]. Comparison of chimeric 17-1A IgG1 and chimeric 17-1A IgM on a mole basis revealed association constants of 3.41×10^7 l/mol and 1.63×10^8 l/mol, respectively (data not shown).

ADCMC with chimeric 17-1A IgM

The ability of chimeric 17-1A IgG1 or IgM to mediate complement lysis of ^{51}Cr-labeled HT-29 human colon carcinoma cells is shown in Fig. 3. In agreement with previous studies [31], target cells preincubated with saturating concentrations of chimeric 17-1A IgG1 were only minimally lysed by xenogeneic (rabbit) complement (18% cytotoxicity). Under identical culture conditions, the chimeric 17-1A IgM rendered ^{51}Cr-labeled HT-29 colon carcinoma cells susceptible to complement destruction (57% cytotoxicity). Control cultures of HT-29 cells incubated with complement alone or an irrelevant human IgM antibody (HA-1A against the lipopolysaccharide of the J5 strain of *E. coli*) and complement were only marginally lysed (range 9%–13% cytotoxicity). Under identical assay conditions, target cells incubated with chimeric 17-1A IgM were not rendered susceptible to lysis by human complement.

The ability of chimeric 17-1A IgM to render cell lines expressing differing levels of 17-1A antigen susceptible to xenogeneic complement lysis is shown in Fig. 4. Of eight cell lines assayed, the human colon carcinoma lines SW948, SW1116, and HT-29 incubated with chimeric 17-1A IgM were most susceptible to complement lysis. Expression of the 17-1A antigen on these cell lines ranged from 7.68×10^5 to 9.79×10^5 sites/cell (data not shown). Chimeric 17-1A IgM incubated with the human breast carcinoma lines BT-20 and ZR75-1 and the ovarian carcinoma line OVCAR3 (17-1A antigen expression 8.42×10^4, 8.65×10^4, and 4.58×10^4 sites/cell) resulted in moderate levels of lysis at the highest dilutions of complement. HEI and HEK cell lines (17-1A antigen expression $< 5 \times 10^3$ sites/cell) were not lysed by chimeric 17-1A IgM and complement.

In the above experiments, the ability of chimeric 17-1A IgM to mediate ADCMC was demonstrated with xenogeneic (rabbit) complement utilizing a 45-min ^{51}Cr-release assay. To investigate the kinetics of ADCMC as observed

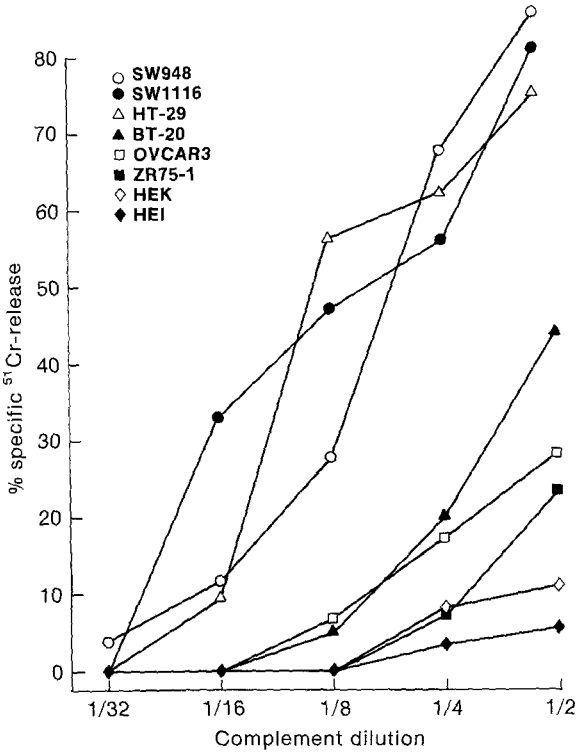

Fig. 4. Specificity of ADCMC mediated by chimeric 17-1A IgM, with rabbit complement. Cells (2×10^4) prelabeled with ^{51}Cr were incubated at 37° C with 30 pg chimeric 17-1A IgM/cell and serial dilutions of rabbit complement. Radioactivity released into the media was determined after 45 min of cultivation. Cell input ranged from 6335 cpm to 11747 cpm. Spontaneous release of radiolabel at 45 min of cultivation ranged from 3% to 4%. Individual variations from the mean did not exceed 5%

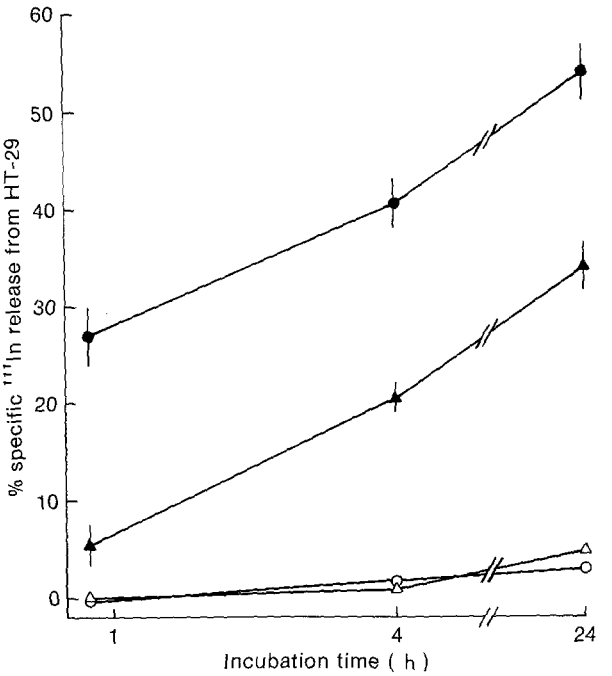

Fig. 5. Kinetics of ADCMC against HT-29 cells mediated by chimeric 17-1A IgM, with rabbit or human complement. HT-29 cells (2×10^4) prelabeled with ^{111}In were incubated at 37° C with a 1/4 dilution of rabbit (○) or human (△) complement alone or with 30 pg mAb/cell and a 1/4 dilution of rabbit (●) or human (▲) complement. Radioactivity released into the media was determined at the indicated time points. HT-29 input totaled 65129 cpm. Spontaneous release of radiolabel over the time sequence studies was 4%. The means ± SE of triplicate cultures are indicated.

with the chimeric 17-1A IgM we utilized ^{111}In-labeled HT-29 cells. This assay allowed for extended incubation times without high levels of spontaneous release [37]. The results of chimeric 17-1A-IgM-mediated lysis of ^{111}In-labeled HT-29 cells in the presence of rabbit or human complement over a 24-h period is shown in Fig. 5. Under these assay conditions the chimeric 17-1A IgM mediated rabbit complement lysis of HT-29, reaching a maximum of 54% cytotoxicity at 24 h. Incubations for longer than 24 h did not result in increased ADCMC (data not shown). Under identical culture conditions chimeric 17-1A IgM mediated lysis of HT-29 cells with human complement (albeit to a lower extent) reaching a 24-h maximum value of 33% cytotoxicity.

ADCC with chimeric 17-1A IgM

In accordance with previous studies [31, 34], incubation of human blood monocytes with HT-29 cells and saturating concentrations of chimeric 17-1A IgG1 resulted in a significant level of target cell lysis (47% cytotoxicity, Table 1). This experiment was carried out four times. The data were very similar and thus a detailed representative experiment is shown in Table 1. Under identical culture conditions, chimeric 17-1A IgM did not appreciably effect ADCC activity of monocytes (15% cytotoxicity). Control cultures of HT-29 cells incubated with monocytes or mAbs alone were not significantly lysed.

The incubation of monocytes and HT-29 colon carcinoma cells in the presence of human complement resulted in a 22% release of radiolabel from the target cells (Table 1). HT-29 cells incubated with complement alone were not lysed. Enhanced monocyte-mediated tumor cell destruction by chimeric 17-1A IgG1 was observed in the presence

Table 1. Enhanced HT-29 cell cytotoxicity with chimeric 17-1A IgM and human complement in the presence of peripheral blood monocytes[a]

HT-29 treatment group	Complement	Specific ^{111}In release from HT-29 at 18 h[b] (%)	
		− Monocytes	+ Monocytes
None, media alone	−	4±3	9±3
	+	5±2	22±6
Chimeric 17-1A IgG1[c]	−	2±1	47±4
	+	4±2	58±4
Chimeric 17-1A IgM[c]	−	6±1	15±3
	+	32±4	70±6

[a] HT-29 cells prelabeled with ^{111}In were incubated at 37° C for 60 min with the indicated mAbs. The cells were washed by centrifugation and 10^4 were added to cultures of human monocytes at a 50/1 E/T ratio in the presence or absence of a 1/4 dilution of human complement. Radioactivity released into the media was determined after 18 h of cultivation. HT-29 input totaled 28642 cpm. Spontaneous release of radiolabel at 18 h of cultivation was 4%

[b] Mean ± SE of triplicate cultures

[c] 30 pg mAb/cell

Table 2. Deposition of human C3 on HT-29 cells mediated by chimeric 17-1A IgM[a]

HT-29 treatment group	Complement	Cell-associated radioactivity (cpm)[b]
None, media alone	−	219 ± 81
	+	3699 ± 108
Chimeric 17-1A IgM	−	274 ± 63
	+	24788 ± 1395

[a] Triplicate sets of HT-29 cells (2×10^5) were incubated at 37°C with 6 μg chimeric 17-1A IgM to achieve equilibrium binding. The cultures were washed three times and sequentially incubated for 30 min each with a 1/4 dilution of human complement followed by ^{125}I-labeled goat anti-(human C3). Specific cell-bound radioactivity was determined as described in Materials and methods

[b] Mean ± SE of triplicate cultures

of human complement (58% cytotoxicity). This level of target cell lysis was approximately additive to monocyte-mediated cytotoxicity observed with complement alone and ADCC obtained with chimeric 17-1A IgG1 alone. Of particular interest was the observation that incubation of monocytes with HT-29 cells treated with saturating concentrations of chimeric 17-1A IgM in the presence of human complement resulted in 70% cytotoxicity. This resulting level of target cell lysis was nonadditive to ADCMC obtained with chimeric 17-1A IgM (32% cytotoxicity) and monocytes incubated in complement alone (22%).

Chimeric-17-1A-mediated deposition of C3 on target cell membranes

Previous studies have indicated that the complement protein C3 can mediate monocyte destruction of tumor cells [1]. We therefore investigated the potential of chimeric 17-1A IgM to fix C3 intermediates on the target cell surface (Table 2). HT-29 cells incubated with complement alone and probed with ^{125}I-labeled goat anti-(human C3) demonstrated the adsorption of this protein onto the tumor cell surface. Incubation of HT-29 cells with chimeric 17-1A IgM and human complement resulted in an approximately sevenfold increase in C3 deposition.

Discussion

In the present report chimeric gene constructs, composed of the V regions of the murine mAb 17-1A linked to the constant regions of human μ H chain and κ L chain, were transfected into the nonproducing murine myeloma SP2/0. The resulting pentameric immunoglobulin secreted by this cell line is the first description of a mouse-human chimeric IgM that is directed against a tumor-associated antigen.

The immunoreactivity of the chimeric 17-1A IgM on HT-29 human colon carcinoma cells paralleled the activity of chimeric 17-1A IgG1. Previous studies have demonstrated that chimeric 17-1A IgG mAbs bind the same antigen as native murine 17-1A mAb and that the presence of human constant regions in the molecule does not alter the binding affinity [30, 31, 35]. The present study, employing the chimeric 17-1A IgM and IgG1, resulted in calculated relative association constants of 1.63×10^8 1/mol and 3.41×10^7 1/mol, respectively. In comparison to chimeric 17-1A IgG1, the fivefold increase in the association constant of chimeric 17-1A IgM is consistent with an antigen-combining valency of five, observed for IgM antibodies [7].

Unlike the native murine 17-1A mAb [14] or chimeric 17-1A IgG mAbs with human Cγ1, Cγ2, Cγ3, or Cγ4 regions [30, 31, 34], the chimeric 17-1A IgM was able to render human cell lines expressing the 17-1A antigen susceptible to the lytic activity of complement (Figs. 3 and 4). The ability of chimeric 17-1A IgM to render human colon carcinoma cells susceptible to ADCMC was greater for xenogeneic (rabbit) complement than with human complement (Fig. 5). The mechanism whereby this difference in complement sensitivity occurs is unclear at this time. Several groups have provided evidence that the membrane attack complex, formed by the complement cascade, can be eliminated from the membranes of nucleated cells [3, 23]; however, a direct comparison between heterologous and autologous complement has not been made. A recent report has described that decay-accelerating factor, a membrane regulatory protein that protects blood cells from autologous complement attack, can account for variations in sensitivity of tumor cells to complement-mediated lysis [5].

The ability of chimeric 17-1A IgM to fix complement proteins on the cell surface of colon carcinomas could have further consequences for immunological destruction. In this regard, it has been shown that activated human lymphocytes express membrane-associated proteases capable of cleaving C3, resulting in C3b binding to receptors on lymphocytes and leading ultimately to enhancement of ADCC, possibly by augmenting contact between the effector cell and the target cell [26, 38]. Binding and ADCC activity of human pulmonary alveolar macrophages and blood monocytes are increased when targets bear C activated via the classical pathway [10, 16], and a role for C3 has been proposed in the recognition of target cells by mononuclear phagocytes [1]. The present results extend these findings, demonstrating that HT-29 cells are rendered susceptible to monocyte destruction in the presence of complement (Table 1). The chimeric 17-1A IgG1, although not capable of mediating ADCMC, showed enhanced monocyte ADCC activity in the presence of complement. The heightened monocyte antitumor effect appeared to be additive to the effects of chimeric 17-1A IgG1 and complement alone. The chimeric 17-1A IgM, although not capable of mediating monocyte ADCC directly, fixed C3 on the surface of HT-29 cells and yielded synergistic levels of tumor cell lysis in the presence of human complement and monocytes.

The clinical utility of IgM monoclonal antibodies directed against tumor antigens has been considered to be limited by molecular size constraints on pharmacokinetics and the ability to localize to tumor. However, recent preclinical [21] and clinical [6] diagnostic studies of systemically administered radiolabeled human IgM antitumor monoclonal antibodies have illustrated successful tumor images. The 17-1A antigen of human gastrointestinal adenocarcinomas has been exploited for therapeutic intervention utilizing murine monoclonal antibodies [19, 22, 28, 29]. However, the clinical utility of individual murine monoclonal antibodies specific for human antigens (including 17-1A) used alone may be limited. The murine mAbs are immunogenic in man and appear to have rela-

tively short circulating half-lives of 15–30 h [4, 19, 29, 32]. The construction of human mouse chimeric mAbs may obviate these shortcomings. Another potential advantage of chimeric mAbs is that homologous Ig might be more efficient in mediating the desired host responses than heterologous antibodies. Both ADCC and ADCMC are felt to be important components in the host response to neoplasia [7], therefore the inability of native murine 17-1A or chimeric 17-1A IgG to render antigen-positive target cells susceptible to ADCMC is disappointing. The present study detailing the biological activity of chimeric 17-1A IgM demonstrates that appropriate targeting of this antigen can result in complement fixation and lysis. The chimeric 17-1A IgM may have a clinical advantage in treating gastrointestinal malignancies. Moreover, data presented here suggest that a combination of chimeric 17-1A IgM and IgG mAbs may enhance therapeutic intervention.

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