

Growth inhibition of murine mammary carcinoma by monoclonal IgE antibodies specific for the mammary tumor virus

Eva Nagy, Istvan Berczi, and Alec H. Schon

MRC Group for Allergy Research, Department of Immunology, Faculty of Medicine, The University of Manitoba Winnipeg, Manitoba, Canada

Received 14 February 1991/Accepted 16 May 1991

Summary. Two IgE-producing hybridomas were established from spleen cells of Balb/c mice, which had been immunized with mouse mammary tumor virus (MMTV). These IgE monoclonal antibodies (mAbs) reacted specifically with the major envelope glycoprotein (gp36) of MMTV, as established by the immunoblot assay and by passive cutaneous anaphylaxis. The effect of the IgE mAbs (produced by clone A8) on the growth of the MMTV-secreting mammary adenocarcinoma H2712 was investigated in syngeneic C3H/HeJ mice. The mice were inoculated s.c. with either 10^5 ($\approx 100 \times \text{LD}_{50}$) or 10^6 ($\approx 1000 \times \text{LD}_{50}$) tumor cells and received repeated i.p. injections of 25 μg anti-gp36 IgE mAbs at 4-day intervals for 8 weeks. This treatment prevented the development of subcutaneous tumors in 50% of the animals. Similar protection was observed when the tumor cells ($10^5/\text{animal}$) were injected i.p. 4 days prior to the beginning of the i.p. treatment consisting of injections of 25 μg mAbs at 4-day intervals for 6 weeks. However, these mAbs did not protect C3H/HeJ mice against the MMTV-negative MA16/c carcinoma cells. Hence, these results support the view that IgE-mediated cytotoxic mechanisms may play an immunologically specific antitumor surveillance role and that laboratory-induced antitumor IgE mAbs have the potential of specific therapeutic agents for in vivo destruction of tumor cells.

Key words: Murine mammary tumor virus – IgE, Protective antibodies – Tumor immunity

Introduction

A number of epidemiological studies suggest that cancer is less frequent in individuals suffering from allergies and

that, conversely, the frequency of allergic diseases in cancer patients is lower than in the general population [1, 19, 39, 40]. However, other studies failed to support this inverse relationship between atopy and cancer [9, 35]. Using the skin window technique, it was shown that cancer patients reacted to their autologous tumors by infiltration of basophils [6, 7]. Lower blood histamine levels were found in cancer patients than in healthy controls, and a sudden decrease was observed during cancer progression or metastasis [13].

Several laboratories reported a depressed IgE serum level in cancer patients [2, 3, 26]; this finding was, however, not supported in another laboratory [49]. In 8 cancer patients a wheel-and-flare reaction was elicited 15–20 min after the intradermal injection of the extract of each patient's own tumor, which did not occur on injection of the patient's normal tissue extracts. Moreover, the wheal-and-flare reaction could be transferred to healthy volunteers by the Prausnitz-Küstner test, in which the patient's serum was first injected intradermally and the tumor extract was injected into the same site 24 h later [22]. Tumor-reactive IgE antibodies were also demonstrated in this laboratory by an appropriate "double-antibody" test in approximately one-third of patients with colon cancer [5]; these IgE antibodies decreased or disappeared 8–13 days after surgical removal of the tumor.

The tissue histamine level was found to be higher in tumor-bearing mice and rats than in normal animals and it was shown to depend on the presence of the tumor and not on the number of mast cells within the tumor tissue [10, 43].

There was a wide variation in the mast cell content of chemically induced syngeneic fibrosarcomas in rats; the primary tumors were found to be heavily infiltrated with mast cells, whereas transplanted tumors showed little or no infiltration. Burtin et al. [14] showed that the tissue histamine level was low in genetically selected, mast-cell-deficient W/W^v mice, and that all these mice developed full-blown tumors on receiving the transplantable fibrosarcoma MC-B6-1 or the 3LL Lewis lung carcinoma. By contrast, in normal littermates of the same mouse strain

Offprint requests to: A. H. Schon, Department of Immunology, The University of Manitoba, 730 William Avenue, Winnipeg, Manitoba, Canada R3E 0W3

(+/+ genotype) having a normal tissue histamine level, the incidence of development of these tumors was markedly lower, i.e., 1 out of 13 animals succumbed to the transplanted sarcoma. The related, partially mast-cell-deficient $W^{v/+}$ mice had an intermediate tissue histamine level, and the incidence of tumors was 50%. Moreover, Burtin et al. [11, 12] showed that a significant inhibition of methylcholanthrene-induced fibrosarcomas was achieved in both C3H and C57/B1 mice, i.e., in strains having a normal mast cell level.

The production of tumor-reactive IgE antibodies has been described in several animal systems [4, 8, 47]. Likhite et al. [33] showed that mammary adenocarcinomas underwent a rapid and permanent rejection, if the tumor cells were injected s.c. into DBA2 mice in the form of a mixture with killed *B. pertussis* organisms, which are known to stimulate IgE production. The histological sections of regressing tumors were shown to be infiltrated by lymphocytes, mast cells and macrophages. Lynch et al. [37] induced local anaphylactic reactions in MCC-3 fibrosarcomas of C3H mice, which resulted in accelerated rejection of the tumors.

Although these observations may be interpreted to indicate that IgE-mediated immune mechanisms may be involved in the host's resistance to tumors, the evidence in support of this hypothesis is by no means conclusive. We believe that this strategy for tumor destruction has not been fully explored because of technical difficulties encountered in the past, i.e. (a) identification and synthesis of tumor-specific antigens in sufficient amounts, and (b) production of IgE antibodies against these antigens in adequate quantities. Both these technical hurdles may now be overcome by the use of the recombinant DNA and hybridoma technologies. One may even visualize the use of "humanized" anti-tumor IgE antibodies and other similar Fc ϵ -containing constructs, which can be produced by genetic engineering [29, 30].

With a view to testing the above hypothesis, we have produced in this exploratory study murine IgE mAbs specific for one of the major envelope glycoproteins (gp36) of the murine mammary tumor virus (MMTV). The results to be reported below demonstrate that these antibodies were able to protect in an immunologically specific manner a significant number of mice against a lethal dose of H2712 carcinoma cells carrying the MMTV. Hence, it is suggested that the possibility of arresting tumor growth by cytotoxic mechanism(s) mediated by IgE mAbs to markers of tumor cells deserves further experimental exploration.

Materials and methods

Animals. Balb/c mice, 6–8 weeks old, were obtained from the animal breeding facility of the University of Manitoba. Inbred C3H/HeJ female mice weighing 20–25 g were purchased from the Jackson Laboratory, Bar Harbor, Me. All animals were housed in plastic cages in a temperature- and humidity-controlled room, provided with 12 h light/12 h dark cycles; their food consisted of Wayne's Laboratory blocks, with a 6% fat content (Chicago, Ill.) and water ad libitum.

Tumors. The two mammary adenocarcinomas, H2712 and MA16/c, transplantable in C3H/HeJ mice, were obtained from the National Cancer

Institute, Frederick Cancer Research Facility, Frederick, Md. The H2712 carcinoma is MMTV-positive, whereas the MA16/c tumor is virus-free. For the preparation of cell suspensions, tumor chunks were digested in Spinner Eagle salt solution (Gibco, 310–4230) containing 2.5% trypsin (Gibco, 610-5095). The cells were then suspended in RPMI-1640 tissue-culture medium, supplemented with sodium bicarbonate (2.0 g/l), non-essential amino acids, glutamine, vitamins (Gibco, each at 10 ml/l) and 10% fetal calf serum. Cell viability was determined by a trypan blue exclusion test. Graded numbers of tumor cells were injected into syngeneic recipients either s.c. or i.p., with or without additional treatment.

Virus and myeloma cells. The MMTV (lot 7.245) was obtained from the Biological Carcinogenesis Branch, National Cancer Institute, Bethesda, Md. USA. The P3 $\times$ 20 myeloma cells used for the generation of the hybridomas were originally donated by Dr. M. Leon, Wayne State University, Detroit, Mich.; these cells were kept in liquid N₂ and were revived for each fusion.

Immunization of mice. The virus was disrupted by freezing (in a –20°C freezer) and thawing (in a 37°C incubator) three times [18]. For the production of IgE antibodies Balb/c mice were injected i.p. repeatedly at monthly intervals with 1–10 μ g killed MMTV adsorbed onto 1 mg Al(OH)₃ gel in 0.5 ml phosphate-buffered saline (PBS). The mice were bled 7 days after each injection and their sera were tested for the production of IgE antibodies by the enzyme-linked immunoassay (ELISA), as described below. After the third bleeding two mice were selected, boosted 10 days after testing and their spleen cells were used 5 days later for fusion with P3 $\times$ 20 myeloma cells.

Cell hybridization. Fusions were performed according to a modification of the procedure originally described by Galfre and Milstein [20]. For each fusion 10⁷ P3 $\times$ 20 myeloma cells were mixed with 10⁷ spleen lymphocytes and pelleted by centrifugation at 1000 rpm for 5 min. The pellet was then suspended in 1 ml prewarmed RPMI-1640 containing 50% (w/v) of polyethyleneglycol (M 4000; Merck, Darmstadt, FRG). The suspension was shaken continuously for 1 min at 37°C, diluted thereafter by dropwise addition of 1 ml RPMI-1640 medium during 1 min, and finally 10 ml RPMI-1640 was added with continuous shaking over a period of 5 min. The suspension was then centrifuged at 1000 rpm for 5 min at room temperature. The cell pellet was resuspended to a final concentration of 2 $\times$ 10⁵ cells/ml in complete medium (CM-HAT) consisting of RPMI-1640, 20% fetal bovine serum, 5 μ g/ml insulin, 500 μ M sodium pyruvate, 50 IU/ml penicillin, 50 μ g/ml streptomycin, 10 mM hypoxanthine (Sigma), 40 μ M aminopterin (Sigma), 1.6 mM thymidine (Sigma). To each well of 24-well tissue-culture Costar plates, containing a feeder layer of 1 $\times$ 10⁵ mouse peritoneal cells in 1 ml CM-HAT, was added 1 ml cell suspension. The supernatants were screened for MMTV-specific antibody after 14 days by ELISA.

Enzyme-linked immunoassay. The ELISA was performed according to Shoenfeld et al. [45]; volumes of 100 μ l of the different reagents were used in all the steps and three washings of PBS containing 0.5% Tween-20 were performed between successive steps. Immulon² micro-ELISA plates, with 96 flat-bottom wells (Gibco) were incubated overnight at 4°C with 5 μ g/ml killed MMTV in 0.05 M bicarbonate buffer (pH 9.6). To block uncoated sites of the wells, the wells were treated with a 1% solution of gelatine in PBS at 37°C for 2 h. The sera of mice immunized with MMTV, or the supernatants of cell fusions expected to contain anti-MMTV antibodies, were added to the plates at different dilutions. After 1 h of incubation at 37°C and washings, peroxidase conjugates of goat antibodies to mouse IgE (Game, Nordic), or of rabbit antibodies to mouse IgG F(ab')₂ (Bio/Can, Toronto, Ontario), were added at dilutions of 1:5000 or 1:2000 to the wells, and incubated for 1 h at 37°C. Freshly prepared substrate 0.4 mg/ml *o*-phenylenediamine, 0.3 μ l/ml hydrogen peroxidase, in citrate buffer pH 5.0) was added and the plates were incubated for 45 min in the dark at room temperature; finally, the absorbance was determined at 490 nm for each well (micro-ELISA Auto-reader, Dynatech). The murine IgE mAbs 26.82 to dinitrophenol, in the form of ascites, served as a positive control; (the corresponding hybridoma was originally provided by Dr. David H. Katz [34]; the wells were

Table 1. Stabilization of clones producing IgE antibody to murine mammary tumor virus (MMTV)

Primary clone	No. of subcloning	IgE MMTV (%)	ELISA ^a
A8	1st	50	0.567
	2nd	80	0.822
	3rd	100	0.726
H11	1st	30	0.417
	2nd	60	0.427
	3rd	100	0.375

^a Enzyme-linked immunosorbent assay

coated with dinitrophenylated bovine immunoglobulin (DNP₂₆BGG) donated by Dr. E. Rector of this Department. A goat antiserum to gp52 of MMTV (which is the other major envelope glycoprotein of MMTV), purchased from the Biological Carcinogenesis Branch, National Cancer Institute, Bethesda, Md., served as a further control.

Passive cutaneous anaphylaxis (PCA). For the demonstration of MMTV-specific IgE antibodies in the culture supernatants of hybridomas, volumes of 200 µl serially diluted culture supernatant were injected intradermally (i.d.) into the backs of hooded rats. After 24 h the same sites were injected i.d. with 50 µl (50 µg) MMTV and immediately thereafter 1 ml Evans blue dye (1% in PBS) was injected i.v. for visualization of the PCA reaction by extravasation of the dye in the positive sites. The titer of antibodies was defined as the reciprocal of the highest dilution of the supernatant that caused a dye exudation reaction of at least 5 mm in diameter. This adaptation of the Prausnitz-Küstner test was used instead of the conventional PCA reaction requiring the i.v. injection of the challenging antigen so as to economize on the use of the MMTV.

Ascites production. Balb/c mice received i.p. 0.5 ml pristane (Aldrich Chemical Co., Milwaukee, Wis.) and 10 days later 5×10^6 monoclonal hybridoma cells [25]. Tumorous ascites appeared about 10 days later and it was drained every 2–3 days until the death of the mice.

Enzyme-linked immunoelectrotransfer blot technique. The enzyme-linked immunoelectrotransfer blot technique is a combination of sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) and ELISA [21, 32, 48]. Electrophoresis in SDS-PAGE was performed using 10% resolving gel and 5% stacking gel. The gel was loaded with 25 µl containing 250 ng each of the two protein standards (bovine serum albumin, $M = 68\,200$; ovalbumin ($M = 42\,699$) and 50 µl (25 µg) MMTV. The samples were maintained for 2–3 min in boiling water before application onto the gel. After absorption of the samples into the stacking gel, electrophoresis was performed at 125 V for 45–50 min. One of the gels was then stained with 0.2% Coomassie brilliant blue R-250 and the other gel was transferred to nitrocellulose paper (0.45 µm, Bio-Rad). The nitrocellulose paper, fiber pads (Bio-Rad), and filter papers (Whatman) were soaked in transfer buffer for about 30 min before preparing the multi-layer sandwich (i.e., fiber pad, two filter papers, gel, nitrocellulose paper, two filter papers, fiber pad). All air bubbles were removed from the interface between the gel and the nitrocellulose sheet, and the "sandwich" was locked inside a plastic support frame and placed into the trans-blot cell filled with transfer buffer, with the nitrocellulose paper facing the positive electrode. For transfer, the voltage of 100 V was applied at 250 mA for 1 h and the blots were developed by ELISA.

Purification of monoclonal dinitrophenol-specific IgE antibodies. The antidinitrophenol IgE mAbs 26.82 were isolated from ascites by affinity chromatography according to the method of Liu et al. [34], as modified by Kelly et al. [28]. Briefly, bovine serum albumin (BSA) (fraction, Sigma) was coupled to cyanogen-bromide-activated Sepharose-4B (Pharmacia) at a concentration of 1 mg BSA/ml Sepharose 4B [46]. The BSA-Sepharose complex was treated for 16 h at 22°C with 2 g 2,4-dinitrobenzenesulfonic acid, sodium salt (Aldrich), dissolved in Na₂CO₃ (10 mg/ml) solution. The immunoadsorbent was washed with Na₂CO₃ solution (10 mg/ml), H₂O, 4 M guanidine/HCl in 0.25 M

Table 2. Levels of IgE antibody to MMTV

Fraction	ELISA (Δ)	PCA titer ^a	IgE content (µg/ml)
H11 supernatant ^b	0.137	16	0.039
A8 supernatant	0.224	32	0.066
H11 ascites ^c	0.332	64	32
A8 ascites	0.471	128	250

^a PCA, passive cutaneous anaphylaxis

^b The supernatants of in vitro tissue cultures were tested after ten-fold concentration by precipitation with ammonium sulfate

^c Ascites fluids were diluted 1 : 100 for ELISA

sodium acetate buffer (pH 4.0), and finally with PBS containing 0.5% Tween 20. An IgE-enriched fraction was isolated from the ascites fluid in two steps: first, the bulk of immunoglobulins other than IgE were precipitated with ammonium sulfate [(NH₄)₂SO₄] at 40% saturation, pelleted and discarded; next, the IgE-enriched fraction was precipitated from the supernatant with (NH₄)₂SO₄ at 50% saturation. This IgE-enriched fraction was dissolved in PBS, dialyzed overnight against PBS containing 0.5% Tween 20, and applied to the dinitrophenol-BSA-Sepharose 4B immunosorbent column. The column was washed with PBS/0.5% Tween 20, 0.05 M *N*-carbobenzoxycysteine (pH 8.0) (Sigma). Finally, the IgE antibodies were displaced by 0.02 M dinitrophenylglycine (pH 8.0) (Sigma) and the dinitrophenyl glycine was removed by dialysis against PBS. The protein content of the IgE antibodies was measured by the Lowry method [36]. The purified IgE was then used for establishing the standard curve for the measurement of the IgE content of culture supernatants and ascitic fluids by the ELISA test.

Results

Production and characterization of monoclonal IgE anti-MMTV antibodies

Twenty Balb/c female mice were immunized with killed MMTV adsorbed onto Al(OH)₃ gel, as described under Materials and methods. No anti-MMTV antibodies were detected after the first immunizing injection. A strong anti-MMTV IgG antibody response was induced by the second immunizing injection, but again no IgE antibodies could be detected. However, after the third injection, two mice had detectable IgE antibody levels ($A = 0.150$) and all animals continued to show a strong anti-IgG response. These two mice were boosted and their spleens were used for the generation of anti-(MMTV)-IgE-antibody-producing hybridomas. Several fusions were done with P3 × 20 myeloma cells, and the supernatants were tested 14 days after each fusion, and at weekly intervals thereafter. On the third screening, four primary clones producing supernatants corresponding to absorbance readings in the range of 0.128–0.324 were detected. Three of these clones were subcloned by the limiting-dilution technique and two of them (A8 and H11) were stabilized (Table 1).

The supernatants of both clones, as well as the ascitic fluids of these hybridomas, contained MMTV-specific IgE antibodies as revealed by ELISA and PCA (Table 2 and Figs. 1 and 2). For the PCA assay, the IgE antibodies in the supernatants of the ascites, obtained by precipitation with (NH₄)₂SO₄ at 50% saturation and dialysis, were concentrated tenfold. The IgE concentrations in the A8 and H11 ascitic fluids were 250 µg/ml and 32 µg/ml, respectively, and the culture supernatants of A8 and H11 contained

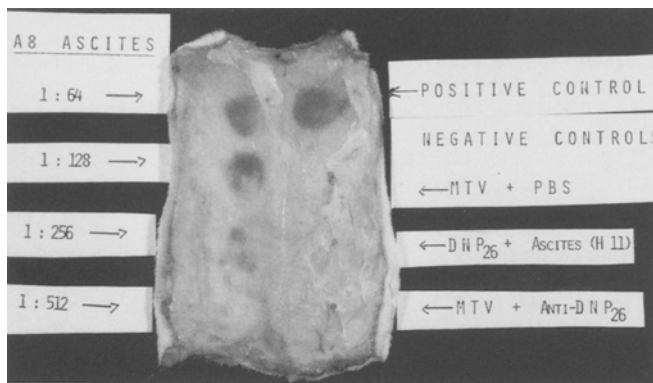


Fig. 1. Passive cutaneous anaphylaxis (PCA) with A8 ascites fluid. Samples of 200 μ l of twofold serial dilutions of the A8 ascites containing IgE antibodies to murine mammary tumor virus (MMTV) were injected i.d. into the backs of hooded rats. After 24 h the PCA reaction was elicited by the i.d. injection of 50 μ g MMTV into the same sites accompanied by the i.v. injection of 1% Evans blue. The positive control consisted in sensitizing the skin with anti-DNP IgE antibodies and subsequent challenge with DNP₂₅BGG. For negative controls, the skin was injected with the reagents listed above

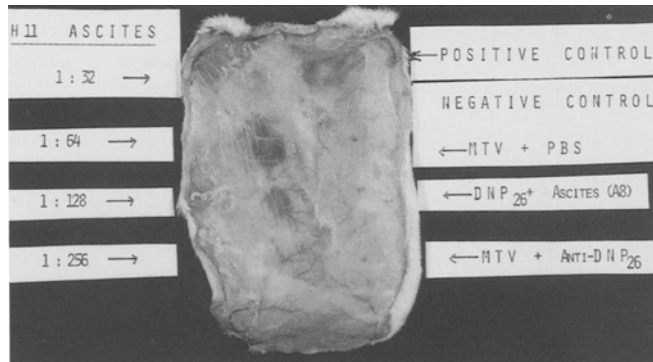


Fig. 2. Passive cutaneous anaphylaxis reaction with H11 ascites fluid. H11 ascites containing IgE antibodies of MMTV was used. Please see legend to Fig. 1 for more details

66 ng/ml and 39 ng/ml IgE mAbs respectively (Table 2). In view of the higher levels of IgE mAbs produced by the A8 hybridoma, only these mAbs were used in subsequent experiments designed to test the *in vivo* effect of anti-MMTV mAbs on the growth of H2712 carcinoma.

As illustrated in Fig. 3, the mAbs A8 and H11 bound to gp36 of MMTV, whereas the goat antiserum to MMTV recognized gp52.

In vivo inhibition of tumor growth by anti-MMTV IgE A8 mAbs

For the determination of the minimum lethal dose of H2712 and MA16/C tumor cells, groups of six mice received s.c. injections of 0.2-ml volumes containing 10^3 – 10^6 cells of each tumor per animal. One thousand H2712 tumor cells killed five of the six recipients within 160 days; by contrast, only three of six animals injected with the same dose of MA16/C tumor cells died within the

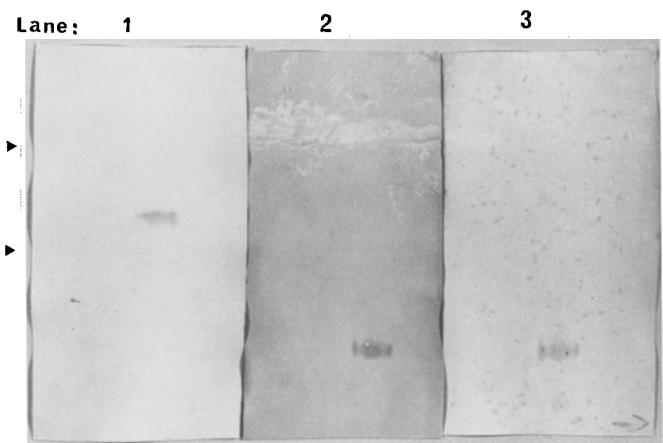


Fig. 3. Enzyme-linked immunoelectrotransfer blot of MMTV by mouse mAbs A8 and H11 and by goat anti-MMTV. Lane 1, goat antiserum to MMTV; lane 2, A8 ascites; lane 3, H11 ascites. Molecular mass markers are indicated on the left. Ascites fluid was used as first Ab in 1:10 dilution; goat antiserum to MMTV was used in 1:100 dilution; goat anti-(mouse horseradish peroxidase) (HRP) was applied in 1:5000 dilution; rabbit anti-(goat-HRP) in 1:2000 dilution. Horseradish peroxidase was detected using 10 mg 4-chloronaphthol dissolved in 100 ml phosphate-buffered saline to which 70 μ l H₂O₂ was added

same period. At a dose higher than 10^4 cells of either tumor, all animals succumbed within 45 days.

In the initial experiment designed to test the *in vivo* effect of the A8 mAbs on the H2712 tumor, three groups of five mice each received s.c. 10^5 tumor cells suspended in 250 μ l Hank's balanced salt solution (HBSS) that contained (20% by volume) either normal serum, murine ascitic fluid containing IgM antibodies to bacterial endotoxin, or ascitic fluid containing a total of 12.5 μ g A8 mAbs. A slight, but not significant, retardation of tumor growth was observed in the third group, which had received the IgE mAbs.

In the next experiment, the control and test groups of six mice each received s.c. 10^5 tumor cells in HBSS containing 20% normal mouse serum or 20% A8 ascites as above. Moreover, the control and test groups, respectively, received in addition repeated i.p. doses of 0.1 ml normal serum and ascites containing 25 μ g A8 antibodies, at 4-day intervals for 8 weeks. All the control mice died by day 44, whereas four of the six mice treated with A8 mAbs were still alive. After cessation of antibody treatment, one mouse developed a palpable tumor starting on day 58 and died on day 72, but the three remaining animals were alive and free of detectable tumor 175 days after injection of tumor cells (Fig. 4). In an analogous experiment, the same experimental conditions were maintained except that the tumor dose was increased to 10^6 cells/mouse. By day 32 all of the control animals had developed tumors and died, whereas at that time only two of the mice in the test group had succumbed to the tumor, and the other four mice had no apparent tumors; however, all of the latter mice died by day 161 (Fig. 5).

In another experiment to be reported here, 10^5 H2712 tumor cells were suspended in HBSS containing either normal mouse serum or A8 mAbs as described previously, except that in this case both control and test groups of six

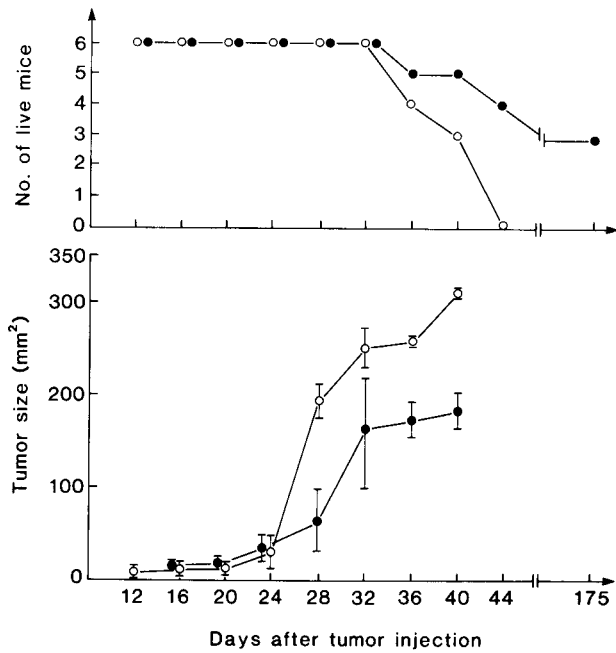


Fig. 4. Inhibition of the H2712 mammary carcinoma by monoclonal anti-MMTV IgE antibodies. Six control (○—○) mice were injected with 10^5 tumor cells s.c. in Hank's balanced salt-solution (HBSS) that contained 20% normal mouse serum. The second group (●—●) received 10^5 tumor cells s.c. in HBSS with 20% ascites (containing 12.5 μ g IgE anti-MTV antibody). The latter group also received 25 μ g IgE anti-MMTV antibodies i.p. every 4th day for 8 weeks. Bars represent SE

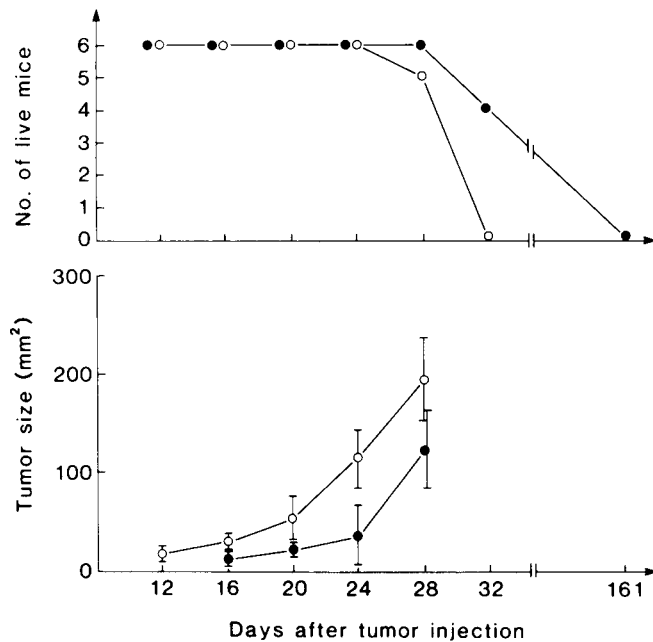


Fig. 5. Inhibition of H2712 tumor growth by anti-MMTV IgE antibody treatment. See Fig. 1, except that the mice were injected with 10^6 tumor cells. (○—○), Control; (●—●), treated with IgE anti-MMTV

animals each received the tumor cells by the i.p. route. As in the earlier experiments, the test group continued to receive i.p. doses of 0.1 ml A8 ascites (containing 25 μ g IgE anti-MMTV IgE mAbs) every 4 days for 6 weeks. All the control mice had developed tumors and died by day 34,

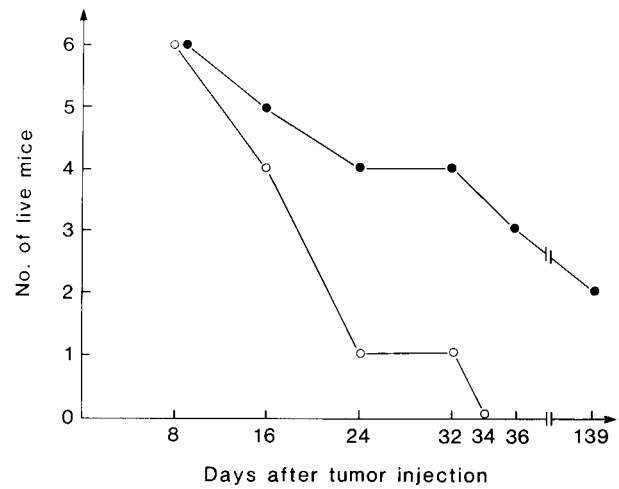


Fig. 6. Protection of mice injected with both H2712 tumor cells and anti-MMTV IgE antibodies intraperitoneally. Controls (○—○) received 10^5 tumor cells i.p. in HBSS +20% normal mouse serum. The treated group (●—●) received the same dose of tumor cells i.p. in HBSS +20% ascites fluid that contained 12.5 μ g IgE anti-MMTV antibodies. This group was injected with 0.1 ml ascites fluid (25 μ g IgE anti-MMTV) every 4 days for 6 weeks

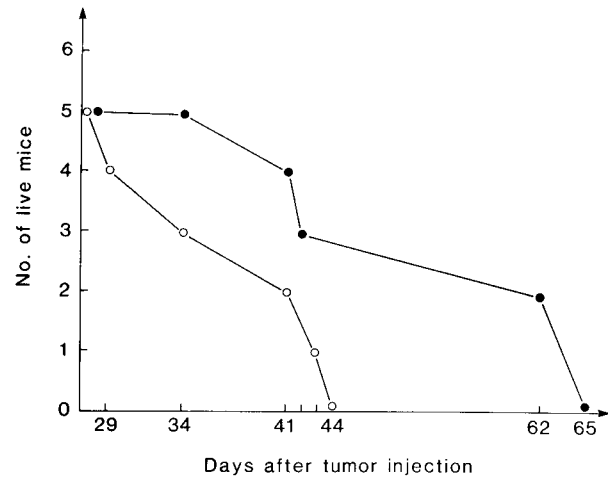


Fig. 7. The effect of anti-MMTV IgE antibodies on the growth of virus-free MA-16/c mammary carcinoma. Groups of 5 C3H/HeJ mice were injected with 10^5 MA16/c mammary carcinoma cells i.p. and one of the groups was treated with anti-MMTV IgE antibodies as described for Fig. 6. (○—○), Control; (●—●), IgE-treated

whereas two of the six mice treated with antibodies were still alive 139 days after the initiation of the experiment (Fig. 6).

To test the specificity of the retardation effect of A8 mAbs on tumor growth, each mouse of two additional control groups received i.p. 10^5 MA16/C mammary carcinoma cells, which are MMTV-negative; all the other conditions were identical to those used for the test and control groups in the earlier experiments. It was interesting to note that the A8 mAbs did not protect these mice, which had all died by day 65; however, the animals treated with normal mouse serum in lieu of the mAbs died somewhat earlier (Fig. 7).

Discussion

The results of this exploratory study, conducted with a relatively small number of inbred mice, on the possible protective antitumor effect of IgE antibodies to tumor-specific markers, such as the MMTV, demonstrate that (a) it is feasible to generate murine hybridomas producing specific monoclonal IgE antibodies to the gp36 of MMTV, and (b) these mAbs were able to protect syngeneic mice in an immunologically specific manner against a lethal dose of the H2712 mammary carcinoma secreting the MMTV virus; this conclusion is supported by the finding that these mAbs did not inhibit significantly the growth of the MMTV-negative mammary carcinoma MA16/C.

These promising results are deemed to justify further efforts for the generation of additional hybridomas producing larger amounts of IgE anti-MMTV mAbs, and for an in-depth investigation of the effector mechanisms responsible for the antitumor protective effects of the anti-gp36 IgE mAbs utilizing a larger number of animals.

Considering that the half-life of murine IgE in circulation is only 6–8 h as contrasted to its half-life of 8 days when attached to mast cells [23, 24], it is highly probable that most of the A8 mAbs injected into tumor-bearing mice disappeared rapidly from circulation and became attached to IgE receptors, which have been shown to be present on a wide spectrum of cells, i.e., mast cells, eosinophils, basophils, platelets, macrophages, B and T cells [15, 17, 41].

Clearly, additional experiments are required for the definition of the effector cells and of their cytotoxic factors that lead to the observed inhibition of tumor growth. The observation that the i.p. injection of IgE antibodies led to the suppression of the growth of s.c. tumors provides indirect support for the view that some of the injected IgE-binding cells had migrated from the peritoneum to the subcutaneous tumor sites, where they exerted their cytotoxic effects on the carcinoma cells.

IgE-mediated cytotoxic mechanisms are known to play a major role in immunity to parasites. Thus, IgE-armed eosinophils are able to kill specifically helminth larvae, and IgE-armed platelets and monocytes have been shown to be effective in destroying parasites [15]. It is also worth noting that Keller et al. [27] demonstrated that the growth of a syngeneic mammary adenocarcinoma was suppressed in mice infected with *Nippostrongylus brasiliensis*, which were producing a strong anti-helminth IgE antibody response, and suggested that the inhibition of tumor growth in these animals was due to reaction of these antibodies with cross-reacting antigens of the tumor and parasite. A similar mechanism may have contributed to the decreased incidence of spontaneous mammary tumors in mice infected with *Trichinella spiralis* [50] as well as their longer survival.

The results of this study support the view that antitumor IgE mAbs produced by xenogeneic hybridomas or by recombinant DNA technology (i.e., "humanized" or chimeric Abs) may prove to be useful therapeutic agents for inhibition of tumor growth in man if one could overcome their immunogenicity [41]. In this connection it is appropriate to mention that there is a large body of evidence demonstrating that covalent conjugates of xeno-

geneic antibodies (xAb) and monomethoxypolyethyleneglycol (mPEG) induce a prolonged antigen-specific tolerance to the xAb (<500 days) by the generation of suppressor T cells. Only further experiments will prove the usefulness of these strategies and the degree of their effectiveness in relation to immunotherapeutic procedures involving immunotoxins [31]. Therefore, it may be visualized that administration of tolerogenic mPEG conjugates of xenogeneic antitumor IgE antibodies in conjunction with the unmodified antibodies, as described elsewhere [38, 44], may prove an effective strategy for destruction of malignant cells in man.

Acknowledgements. This study was supported by grants from the Medical Research Council of Canada and the George H. Sellers Endowment Trust Fund. The skillful secretarial assistance of Mrs. Jean Sylvester is greatly appreciated.

References

- Allegra J, Lipton A, Harvey H, Luderer J, Brenner D, Mortel R, Demers L, Gillin M, White D, Trautlein J (1976) Decreased prevalence of immediate hypersensitivity (atopy) in a cancer population. *Cancer Res* 36: 3225
- Arbesman CE, Wypych JJ, Reisman RE (1973) Serum IgE in human diseases. In: *Mechanisms in allergy, reagin-mediated hypersensitivity*. Dekker, New York, p 163
- Augustin R, Chandradasa KD (1971) IgE levels and allergic skin reactions in cancer and non-cancer patients. *Int Arch Allergy Appl Immunol* 41: 141
- Bartholomaeus WM, Keast D (1972) Reaginic antibody to tumor and alloantigens in mice. *Nature* 239: 206
- Berci I, Holford-Strevens V, Warsi ZH, McMorris LS, Thorlakson RH, Thorlakson TH, Schon AH (1983) Tumor-reactive IgE antibodies in plasma of patients with gastrointestinal carcinomas. *Cancer Immunol Immunother* 14: 180
- Black MM, Leis HP (1971) Cellular responses to autologous breast cancer tissue. Correlation with stage and lymphoreticuloendothelial reactive. *Cancer* 28: 263
- Black MM, Leis HP Jr (1973) Cellular responses to autologous breast cancer tissue. Sequential observations. *Cancer* 32: 384
- Broom BC, Alexander P (1975) Rat tumor allografts evoke anaphylactic antibody responses. *Immunology* 28: 1033
- Bross IDJ, Natarajan N (1972) Leukemia from low-level radiation. Identification of susceptible children. *N Engl J Med* 287: 107
- Burtin C, Scheinmann P, Salomon JC, Lespinats G, Frayssinet C, Lebel B, Canu P (1981) Increased tissue histamine in tumour-bearing mice and rats. *Br J Cancer* 43: 684
- Burtin C, Scheinmann P, Salomon JC, Lespinats G, Loisillier F, Canu P (1981) The influence of intraperitoneal injections of histamines on tumor growth in fibrosarcoma-bearing mice. *Cancer Lett* 12: 195
- Burtin C, Scheinmann P, Salomon JC, Lespinats G, Canu P (1982) Decrease in tumour growth by injections of histamine or serotonin in fibrosarcoma bearing mice: influence of H1 and H2 histamine receptors. *Br J Cancer* 45: 54
- Burtin C, Noirot C, Paupe J, Scheinmann P (1983) Decreased blood histamine levels in patients with solid malignant tumours. *Br J Cancer* 47: 367
- Burtin C, Ponvert C, Fray A, Scheinmann P, Lespinats G, Loidon B, Canu P, Paupe J (1985) Inverse correlation between tumor incidence and tissue histamine levels in W/W⁻, W/+ and +/+ mice. *JNCI* 74: 671
- Capron M (1988) Eosinophils in diseases: receptors and mediators. In: *Progress in allergy and clinical immunology (Proc. 13th Int Cong Allergy Clin Immunol)*. Hogrefe & Huber, Toronto, p 6

16. Chaudhary VK (1990) A rapid method of cloning functional variable-region antibody genes in *E. coli* as single chain immunotoxins. Second Int Symp Immunotoxins, Lake Buena Vista, Fla.
17. Conrad DH (1989) Low affinity IgE (FcE R II) receptors. Clin Rev Allergy 7: 165
18. Daams JH, Hageman P, Calafat J, Bentvelzen P (1973) Antigenic structure of murine mammary tumour viruses. Eur J Cancer 9: 567
19. Fisherman EW (1960) Does the allergic diathesis influence malignancy? J Allergy 31: 74
20. Galfre G, Milstein C (1981) Preparation of monoclonal antibodies: strategies and procedures. Methods Enzymol 73: 1
21. Gershoni JM (1987) Protein blotting: a manual. Methods Biochem Anal 33: 1
22. Grace JT, Kondo T (1958) Investigations of host resistance in cancer patients. Ann Surg 148: 633
23. Haba S, Ovary Z, Nisonoff A (1985) Clearance of IgE from serum of normal and hybridoma-bearing mice. J Immunol 134: 3291
24. Hirano T, Hom C, Ovary Z (1983) Half life of murine IgE antibodies in the mouse. Int Arch Allergy Appl Immunol 71: 182
25. Hoogenraad N, Helman T, Hoogenraad J (1983) The effect of preinjection of mice with pristane on ascites tumour formation and monoclonal antibody production. J Immunol Methods 61: 317
26. Jacobs D, Landon J, Houry M, Merrett TG (1972) Circulating levels of immunoglobulin E in patients with cancer. Lancet II: 1059
27. Keller R, Ogilvie BM, Simpson E (1971) Tumour growth in nematode-infected animals. Lancet I: 678
28. Kelly KA, Lang GM, Bundesen PG, Holford-Strevens V, Bottcher I, Schon AH (1980) The development of a radioallergosorbent test (RAST) for murine IgE antibodies. J Immunol Methods 39: 317
29. Kenten JH, Molgaard HV, Houghton M, Derbyshire, RB, Viney J, Bell LO, Gould H (1982) Cloning and sequence determination of the gene for the human immunoglobulin ϵ chain expressed in a myeloma cell line. Proc Natl Acad Sci USA 79: 6661
30. Kenten J, Helm B, Ishizaka T, Cattini P, Gould H (1984) Properties of a human immunoglobulin ϵ -chain fragment synthesized in *Escherichia coli*. Proc Natl Acad Sci USA 81: 2955
31. Kernan NA, Byers V, Scannon PJ, Mischak RP, Brochstein MD, Flomenberg N, Dupont B, O'Reilly RJ (1988) Treatment of steroid-resistant acute graft-vs-host disease by in vivo administration of an anti-T-cell ricin A chain immunotoxin. J Am Med Assoc 259: 3154
32. Lee N, Zhang SQ, Testa D (1988) A rapid multicolor Western blot. J Immunol Methods 106: 27
33. Likhite VV (1974) The delayed and lasting rejection of mammary adenocarcinoma cell tumors in DBA/2 mice with use of killed *Bordetella pertussis*. Cancer Res 34: 1027
34. Liu F-T, Bohn JW, Ferry EL, Yamamoto H, Molinaro CA, Sherman LA, Klinman NR, Katz DH (1980) Monoclonal dinitrophenyl-specific murine IgE antibody: preparation, isolation, and characterization. J Immunol 124: 2728
35. Logan J, Saker DN (1953) The incidence of allergic disorders in cancer. NZ Med J 52: 210
36. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265
37. Lynch NR, Salomon JC (1977) Passive local anaphylaxis: demonstration of antitumor activity and complementation of intratumor BCG. JNCI 58: 1093
38. Maiti PK, Lang GM, Schon AH (1988) Tolerogenic conjugates of xenogeneic monoclonal antibodies with monomethoxypolyethylene glycol: I. Induction of long-lasting tolerance to xenogeneic monoclonal antibodies. Int J Cancer [Suppl] 3: 17
39. McKay WD (1966) The incidence of allergic disorders and cancer. Br J Cancer 20: 434
40. Meers PD (1973) Allergy and cancer. Lancet I: 884
41. Metzger H (1988) Molecular aspects of receptors and binding factors for IgE. Adv Immunol 43: 277
42. Reichmann L, Clark M, Waldmann H, Winter G (1988) Reshaping human antibodies for therapy. Nature 332: 323
43. Scheinmann P, Lebel B, Lynch NR, Salomon JC, Paupe JR, Burtin C (1979) Histamine levels in blood and other tissues of male and female C3H mice: II. Mice carrying a 3-methyl-cholanthrene-induced tumor. Agents Action 9: 95
44. Schon AH (1982) Suppression of IgE antibody responses with tolerogenic conjugates of allergens and haptens. Prog Allergy 32: 161
45. Shoenfeld Y, Amnon H, Tal R, Smorodinsky NI, Lavie G, Mor C, Schteren S, Mammon Z, Pinkhas J, Keydar T (1987) Human monoclonal antibodies derived from lymph nodes of a patient with breast carcinoma react with MuMTV polypeptides. Cancer 59: 43
46. Stage DE, Mannik M (1974) Covalent binding of molecules to CNBr-activated agarose: parameters relevant to the activation and coupling reactions. Biochem Biophys Res Commun 343: 382
47. Sweeney WT, Seibel HR (1973) Histamine release from peritoneal mast cells of tumor susceptible rats following periods of tumor growth and sensitization with tumor antigen and *B. pertussis*. Int Arch Allergy 45: 789
48. Tsang VCW, Peralta JM, Simons AR (1983) Enzyme-linked immunoelectro-transfer blot techniques (EITB) for studying the specificities of antigens and antibodies separated by gel electrophoresis. Methods Enzymol 92: 377
49. Waldmann TA, Bull JM, Bruce RM, Broder S, Jost MC, Balestra ST, Suer ME (1974) Serum immunoglobulin E levels in patients with neoplastic disease. J Immunol 113: 379
50. Weatherly NF (1970) Increased survival of Swiss mice given sublethal injections of *Trichinella spiralis*. J Parasitol 56: 748