IgEs Targeted on Tumor Cells: Therapeutic Activity and Potential in the Design of Tumor Vaccines

Eva Reali, John W. Greiner, Angelo Corti, Hannah J. Gould, Federica Bottazzoli, Giovanni Paganelli, Jeffrey Schlom, and Antonino G. Siccardi

ABSTRACT

Surface-bound IgE play a central role in antiparasite immunity; to exploit IgE-driven immune mechanisms in tumor prevention and control, monoclonal IgEs of irrelevant specificity were loaded through biotin-avidin bridging onto tumor cells, either by systemic administration to tumor-bearing mice or pre-loading of tumor cells before inoculation. Here we show that systemic administration of biotinylated IgEs to mice bearing tumors pre-targeted with biotinylated antibodies and avidin significantly decreased tumor growth rate. In addition, as compared with IgG-loaded control cells, inoculation of suboptimal doses of IgE-loaded tumor cells suppressed tumor formation in a fraction of animals and induced protective host immunity by eliciting tumor-specific T-cell responses. Similarly, tumor vaccination experiments showed that irradiated tumor cells (IgE loaded by biotin-avidin bridging) conferred protective immunity at doses 100-fold lower than the corresponding control cells without IgE. Finally, in vivo depletion of eosinophils or T cells abrogated IgE-driven tumor growth inhibition. These results demonstrate that IgEs targeted on tumor cells not only possess a curative potential but also confer long-term antitumor immunity and that IgE-driven antitumor activity is not restricted to the activation of innate immunity effector mechanisms but also results from eosinophil-dependent priming of a T-cell-mediated adaptive immune response. This suggests a potential role for IgEs in the design of new cell-based tumor vaccines.

INTRODUCTION

Increasing evidence indicates that the outcome of an immune response is determined by the context in which antigens are presented to the immune system. In particular, inflammation or cell destruction is required for immune priming, allowing for effective uptake and presentation of tumor antigens by professional APCs1 (1, 2). To this effect, several cancer immunotherapeutic approaches use genetically modified tumor cells expressing proinflammatory cytokines (3–8); in particular, successful outcomes have been obtained with granulocyte macrophage colony-stimulating factor-transduced tumors, which generate systemic immunity by augmenting cross-presentation of tumor antigens, possibly by activating DCs (9–12).

IgE-targeted immediate hypersensitivity and allergic inflammation reactions have been proposed as possible natural mechanisms involved in antitumor immune responses, on the basis of (controversial) epidemiological evidence of an inverse correlation between allergy reactions and that IgE-driven antitumor activity is not restricted to the activation of innate immunity effector mechanisms but also results from eosinophil-dependent priming of a T-cell-mediated adaptive immune response. This suggests a potential role for IgEs in the design of new cell-based tumor vaccines.

MATERIALS AND METHODS

Mice. Animal experiments, both in Milan and in Bethesda, were performed in accordance with institutional and state guidelines. All mice used in this study were 6- to 8-week-old C57BL/6 females bred under pathogen-free conditions.

Antibodies. Anti-2,4,6-trinitrophenol mouse IgEs were obtained from supernatants of TIB142 hybridoma cells (American Tissue Culture Collection, Rockville, MD) cultured in serum-free, protein-free Ultradoma medium (Bio Whittaker, Walkersville, MD) by ammonium sulfate precipitation and dialysis against PBS. IgE levels in the supernatant were evaluated by ELISA. Anti-2,4-dinitrophenol mouse IgEs (mAb SP6) were purchased from Sigma Chemical Co. (St. Louis, MO). Anti-Thy 1.1.19E12 mAb (IgG2a) was purified from mouse ascitic fluid as described (26). COL-1 anti-CEA mAb (IgG2b; Ref. 32) and BL-3 control mAb (IgG2a) were kindly provided by Diane Milenic (Laboratory of Tumor Immunology and Biology, National Cancer Institute/NIH, Bethesda, MD).
Cell Lines. The H-2^d^ T-cell lymphoma RMA is a Rauscher MuLV-induced tumor of C57BL/6 origin. RMA Thy 1.1 was derived from a RMA cell line by transfection with a construct encoding the Thy 1.1 allele, inserted into the mammalian expression vector pRS1-neo. Cells were maintained as described (26). The MC38-CEA-2 CEA^+^ cell line was derived from the parental MC38 (H-2^s^) adenocarcinoma cells by transfection (31). The MC38-CEA-2 cells were routinely >90% positive for surface CEA expression as measured by the binding of an anti-CEA mAb, COL-1-EL-4, a chemically induced T-cell lymphoma, and B16-F1, a murine melanoma of C57BL/6 origin, were provided by Drs. Matteo Bellone and Anna Mondino (San Raffaele Scientific Institute, Milan, Italy), respectively. RBL-13, a rat basophilic leukemia cell line expressing high levels of FCerII, was cultured in DMEM supplemented with 2 mM l-glutamine penicillin-streptomycin and 10% FBS. RBL13 cells were counterstained with FITC-conjugated goat antirat antibody against antimouse IgE mAb (PharMingen, San Diego, CA). After further washing, RBL-13 cells treated with biotinylated IgE were stained with phycoerythrin-conjugated streptavidin; targeting of biotinylated anti-Thy 1.1 mAb 19E12 and anti-CEA COL-1 mAb was performed using 100 ml of biotinylated antibodies at various concentrations ranging from 0.5 to 0.0625 mg/ml to set optimal reaction conditions for achieving the highest level of biotinylation together with unaltered binding to FCerIs.

The best results were obtained with sulfo-NHS-LC-biotin (Pierce Chemical Co., Rockford, IL) solution at concentration 0.125 mg/ml, as determined by FACS analysis on RBL-13 cells. Biotinylated mAbs were diazyl overnight against PBS. The optimal biotin:protein ratio was 10 as determined by Pierce HABA method.

Flow Cytometry. For detection of binding of biotinylated-IgE to FCerIs, 10^5^ RBL-13 rat basophilic leukemia cells were incubated for 1 h at 37°C with 100 ml of 30 mg/ml biotinylated or unconjugated IgE in PBS. As a control, RBL-13 cells were incubated with irrelevant IgG antibody at the same concentration. Cells were washed twice in PBS containing 3% FCS and incubated with rat antiserum IgE mAb (PharMingen, San Diego, CA). After further washing, RBL-13 cells were counterstained with FITC-conjugated goat antirat antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and analyzed by FACSscan (Becton Dickinson, San Jose, CA). Mean fluorescence intensity on RBL-13 cells treated with IgE and biotinylated IgE was compared, and no significant differences were observed, indicating unaltered binding of biotinylated IgE to FCerIs. Alternatively, RBL-13 cells treated with biotinylated IgE were stained with phycoerythrin-conjugated streptavidin (Sigma Chemical Co.).

Biotinylated anti-Thy 1.1 mAb 19E12 and anti-CEA COL-1 mAb were tested for binding to tumor cells (RMA-Thy 1.1 and MC38-CEA-2, respectively) using phycoerythrin-conjugated streptavidin; targeting of biotinylated IgE on tumor cells by biotin-avidin bridging was evaluated by IgE-specific staining as described above.

In Vivo Tumor Targeting with Biotinylated IgEs. Tumor targeting with biotinylated IgE was performed as described (29). Six- to 8-week-old C57BL/6 females were s.c. injected with 3 x 10^5^ MC38-CEA-2 tumor cells. At day 2, the mice were injected i.p. with 40 mg of biotinylated anti-CEA-2 COL-1 mAb to target the tumor. At day 3, 50 mg/mouse of avidin was administered i.p. to chase the unbound mAb, and 4 h later, streptavidin (50 mg/mouse) was injected to create the avidin-avidin bridge. Finally, at day 4, 50 mg/mouse of biotin-conjugated IgEs or IgGs (control group) were administered by i.p. injection, and tumor growth was monitored every 2nd day by measuring two perpendicular diameters with a caliper. Tumor volume was calculated by the formula V = (S x S) x L/2, where S = short diameter and L = long diameter. Mice were euthanized when tumor size was >500 mm^3^.

In Vitro Targeting of Tumor Cells with Biotinylated IgE. Tumor cells were loaded with biotinylated IgE, as described previously (26). Briefly, RMA-Thy 1.1 or MC38-CEA-2 cells were treated with biotin-conjugated 19E12 or COL-1 at 10 mg/ml for 10 min on ice (first step). After washing, cells were further incubated with 10 mg/ml avidin (NeutraAvidin; Pierce Chemical Co.) for 10 min on ice (second step) and then with 30 mg/ml of biotinylated IgEs (TIB-142 or SP6) or IgGs (BL-3) for 30 min on ice (third step). Optimal cell doses for tumor development were 5 x 10^5^ cells for the RMA Thy 1.1 model and 3 x 10^5^ cells for the MC38-CEA-2 model. The tumor cells were suspended in 100-ml 0.9% NaCl solution and injected s.c. in the right flank.

In Vivo Depletion of CD4^+^ and CD8^+^ T Cells and Eosinophils. For in vivo depletion of CD4^+^ and CD8^+^ T-cell subsets in the priming phase of the antitumor immune response, rat antiserum CD4 GK 1.5 and rat antiserum CD8 53.6.72 monoclonal antibodies were used; 300 mg of purified antibodies were administered by i.p. injection at day -6 followed by a second and a third i.p. injection of 200 mg at days -5 and -3 from tumor inoculation. Depletion was maintained during tumor growth by weekly injections of 200 mg of antibody. CD4 or CD8 depletion at the time of tumor inoculation was >90%, as measured by FACS analysis on peripheral blood lymphocytes. Eosinophil depletion was performed by administration of anti-IL-5 antibody TRFK-5 (PharMingen) as described by Garlisi et al. (33). Briefly, 1 week before tumor inoculation, mice were injected i.p. with 20 mg of TRFK-5 mAb followed by a second injection of 20 mg 5 days after tumor inoculation. The treatment is reported to inhibit the release of eosinophils from bone marrow for at least 8 weeks.

CTL Assay. Splenocytes were isolated from IgE-treated, tumor-rejecting mice or from control tumor-developing mice and restimulated in vitro with irradiated (10,000 rad) RMA Thy 1.1 cells at a responder:stimulator ratio of 2:1. After 7 days of culture in RPMI 1640 supplemented with l-glutamine, antibiotics, β-mercaptoethanol, and 10% FCS, the cytotoxic activity of CTLs was tested against RMA-Thy 1.1, parental RMA, and EL-4 or B16 tumor cells. The cytotoxic activity was measured by standard 5-h ^3^HCr release assays as described (34). In some experiments, cytotoxic activity was evaluated with ^111^In-labeled target in an 18-h ^111^In release assay.

T-cell Proliferation Assays. Splenocytes (10^5^) from immunized mice were cocultivated with 5 x 10^5^ irradiated (2000 rad) syngeneic spleen cells in the presence of various concentrations of the T helper peptide (SLTPrCNTAW-NRL), derived from MuLV env antigen and restricted by I-A^b^ molecules (35). After 4 days, 1 μCi [^3^H]thymidine was added to each well for 18 h. The incorporated radioactivity was measured by liquid scintillation counting.

Vaccination Experiments. Vaccination experiments were performed by s.c. administration of IgG- or IgE-loaded irradiated (10,000 rad) tumor cells in the left flank at different doses. For vaccination with MC38-CEA-2 cells, 1–30 x 10^5^ cells were injected twice with a 2-week interval. Two weeks after the last immunization, mice were challenged with 3 x 10^5^ MC38 parental tumor cells. For RMA Thy 1.1 vaccination, 1–50 x 10^5^ irradiated cells were administered once, and after 2 weeks, mice were challenged with 5 x 10^5^ RMA tumor cells.

Anti-CEA Antibody ELISA. Nunc immunoplates (Maxi-Sorb) were coated with 100 ng/ml of purified human CEA or ovalbumin and overcoated with 5% BSA. Sera from mice vaccinated with IgE- or IgG-loaded MC38-CEA-2 vaccines and from normal controls were serially diluted 1:5. Bound immunoglobulins were revealed by a horseradish peroxidase-conjugated rat antiserum immunoglobulin secondary antibody. Absorbance values were subtracted of the background values measured with normal mouse sera. Only the two groups of mice receiving the highest cell doses (one IgE and one IgG group) had antibodies with titers ≥1:125, whereas all other groups were negative at 1:25.

Statistical Analysis. Statistical analysis on tumor growth data was performed using covariance analysis (36). Differences in tumor growth rate were evaluated by analyzing slope differences, which were assessed by including in the model the interaction term between group and day. Statistical analysis on survival data was performed by the Kaplan-Meier method. Differences were assessed using the Log-rank test. ANOVA was evaluated by the Student t test.

RESULTS

In Vivo Three-step Targeting of Tumors with IgEs. The unique high affinity and tetrameric binding of avidin to avidin and biotin offer the opportunity to design a three-step protocol that allows the targeting of different effector molecules to the surface of tumor cells. The first step involves the use of a biotinylated antibody against a tumor-associated antigen. After the localization of the antibody to the tumor, avidin is administered as the second step. The excess avidin and unbound complexes avidin-antibody are cleared by the liver within 4 h; therefore, the only avidin available is the one selectively bound to the tumor. The third and final step is the administration of a biotinylated molecule which becomes selectively localized to the avidin bound to the tumor and initiates the anticipated biological effect (i.e., cytotoxicity, radiimmune-detection). The efficacy and clinical proficiency of the pretargeting three-step method have been conclusively demonstrated (24–29).

To determine whether in vivo targeting of tumor cells with IgEs could affect tumor growth, mice were inoculated with MC38-CEA-2 adenocarcinoma cells (3 x 10^5^ cells/mouse, s.c.) on day 0 and then treated with: (a) biotinylated anti-CEA mAb (40 μg/mouse, i.p., on day 2); (b)
IgE-driven Antitumor Immunity

increase in survival was observed (Fig. 2A). ANOVA gave increasingly significant differences from day 14 ($P = 0.015$) to day 19 ($P = 0.0012$). A significant difference ($P = 0.0098$) in survival was observed (five of five IgE-treated and only one of five IgG-treated mice survived at day 20); however, the mice bore progressive tumors and died within 40 days.

**Effect of in Vitro IgE Loading on in Vivo Tumor Growth.** To study if IgEs could affect the malignant behavior and immunogenicity of tumor cells, $3 \times 10^5$ MC38-CEA-2 tumor cells, loaded *in vitro* by avidin-biotin bridging with IgEs (or with IgGs in the controls), were inoculated s.c. into C57BL/6 mice. The differences in tumor growth rate (Fig. 2A) and in survival (Fig. 2B) between mice bearing tumors derived from IgE- or IgG-loaded tumor cells were highly significant ($P < 0.001$ for tumor growth and $P = 0.0001$ for survival); moreover, 2 of 10 mice injected with IgE-loaded MC38-CEA-2 completely rejected the tumor after 20 days. The 2 tumor-rejecting mice were later rechallenged with $3 \times 10^5$ MC38 cells, and both failed to develop tumors for 60 days, thus showing that protective immunity had been induced. Because the parental (CEA-negative) cell line was used for the rechallenge, it is clear that an anti-CEA response is not involved in the protection mechanism. A control experiment was performed using MC38-CEA-2 cells loaded in the third step with avidin-biotin bridging with IgEs (or with IgGs in the controls). After s.c. inoculation of $5 \times 10^4$ cells into C57BL/6 mice, tumor growth was progressively slower in all mice. However, a significant ($P < 0.001$) and in survival (Fig. 2A) or with control biotinylated IgGs ($n = 5$; ○) and compared for tumor growth rate and survival (i.e., the time to reach the dimensional end point at which animals have to be sacrificed). Statistically significant differences in tumor growth rate ($P < 0.001$; A) evaluated by covariance analysis and in survival ($P = 0.0001$; B) evaluated by Log-rank test were observed. C and D. C57BL/6 mice were inoculated s.c. with $5 \times 10^4$ live RMA-Thy 1.1 cells loaded either with biotinylated IgEs ($n = 5$; ○) or with biotinylated IgGs ($n = 5$; ●). Significance values were: $P = 0.001$ for tumor growth (C) and $P < 0.01$ for survival (D).

Fig. 2. Effect of *in vitro* IgE loading on *in vivo* tumor growth. A and B. C57BL/6 mice were inoculated s.c. with $3 \times 10^7$ MC38-CEA-2 cells loaded by biotin-avidin bridging either with biotinylated IgEs ($n = 10$, ○) or with control biotinylated IgGs ($n = 17$, ●) and compared for tumor growth rate and survival (i.e., the time to reach the dimensional end point at which animals have to be sacrificed). Statistically significant differences in tumor growth rate ($P < 0.001$; A) evaluated by covariance analysis and in survival ($P = 0.0001$; B) evaluated by Log-rank test were observed. C and D. C57BL/6 mice were inoculated s.c. with $5 \times 10^4$ live RMA-Thy 1.1 cells loaded either with biotinylated IgEs ($n = 5$, ○) or with biotinylated IgGs ($n = 5$, ●). Significance values were: $P = 0.001$ for tumor growth (C) and $P < 0.01$ for survival (D).

**Induction of Tumor Protective Immunity by Suboptimal Doses of IgE-loaded Tumor Cells.** To expand the finding that protective immunity could be elicited by targeting IgE on tumor cells, we inoculated mice with a suboptimal dose of MC38-CEA-2 ($3 \times 10^4$ cells s.c./mouse), which caused tumors in two of five mice inoculated with IgE-loaded cells and in one of five mice inoculated with IgG-loaded cells (Fig. 3A). The difference in tumor incidence is statistically not significant; $P = 0.49$). All of the surviving mice were then challenged with $3 \times 10^5$ MC38-CEA-2 cells; four of four mice of the IgG group developed progressively growing tumors, whereas three of three mice of the group primed with IgE-loaded tumor cells did not develop any tumor ($P = 0.0081$; Fig. 3B). The same mice were further rechallenged with the CEA-negative parental cell line MC38, and again, all three developed no tumor (data not shown). Analogous results were obtained in mice administered with suboptimal doses of IgE- or IgG-loaded RMA Thy 1.1 cells (1 x 10^4 s.c. cells/mouse; data not shown).

**Cell Depletion Studies.** To define the mechanism(s) responsible for the acquired antitumor immunity induced by IgEs, we investigated the contribution of eosinophil, CD4, and CD8 cell populations in the inhibition of tumor growth. For eosinophil depletion, mice were treated i.p. with antibodies against IL-5, starting on day 15 before tumor inoculation. For lymphocyte depletions, mice were treated i.p. with antibodies against CD4 or CD8, starting 6 days before tumor inoculation. The lymphocyte depletion remained >90% throughout the experiment, as monitored by FACS analysis of peripheral blood lymphocytes (data not shown). All treated animals were inoculated with $5 \times 10^4$ IgG- or IgE-loaded RMA-Thy 1.1 cells. The significant inhibition of tumor growth by IgE loading observed in untreated animals was completely abrogated by all three kinds of depletions.
significant difference in survival was observed between the two groups. B, parental cells or non-MuLV-induced, H-2 b -positive tumors, such as the IgE-primed mice exhibited cytotoxic activity directed to both versely, B16 and EL-4 tumor cells were not lysed. Similar results cells (data not shown), as compared with control spleen cells. Con-
cytotoxic activity against RMA-Thy 1.1 (Fig. 4 in inoculated with IgE-loaded tumor cells possessed a markedly higher A
and B16 (data not shown). Spleen cells from mice EL-4 (Fig. 4 ) and parental RMA (data not shown). Spleen cells from mice
progressively growing tumors. Spleen cells were stimulated in vitro
with irradiated RMA-Thy 1.1 and after 7 days, tested for cytotoxic
activity against 51 Cr-labeled targets, such as RMA-Thy 1.1 and RMA
parental cells or non-MuLV-induced, H-2 b -positive tumors, such as
EL-4 (Fig. 4 A) and B16 (data not shown). Spleen cells from mice
inoculated with IgE-loaded tumor cells possessed a markedly higher
cytotoxic activity against RMA-Thy 1.1 (Fig. 4 A) and parental RMA
cells (data not shown), as compared with control spleen cells. Con-
versely, B16 and EL-4 tumor cells were not lysed. Similar results
were obtained in the MC38-CEA-2 tumor model; T-cell cultures from
the IgE-primed mice exhibited cytotoxic activity directed to both
CEA + and CEA - adenocarcinoma (data not shown). In the case of
the RMA-Thy 1.1 tumors, a peptide-specific CD4 response could be
studied, because viral-derived antigens presented by MHC class II
molecules have been identified. To detect the presence of tumor-
specific CD4 T cells, we performed a T-cell proliferation assay. Mice
immunized with IgE-loaded RMA-Thy 1.1 tumor cells showed a
dose-dependent proliferative response specific for the MuLV env-
derived Th peptide SLTTPCANTAWNRL, whereas spleen cells from
mice immunized with either IgG-loaded or untreated RMA-Thy 1.1
cells did not (Fig. 4 B).

IgE-loaded Irradiated Tumor Cell Vaccines Elicit Protective Immunity. To understand if IgE-driven antitumor immunity could be exploited for preventive immunization, we prepared cellular vaccines by loading irradiated tumor cells with either IgEs or IgGs by biotin-
avidin bridging. We performed two vaccinations s.c. at days −30 and −15 with three different cell doses (30, 3, and 1 × 10 4 MC38-CEA-2; Fig. 5 A). The vaccinated mice were challenged with 3 × 10 6 MC38 cells on day 0. Within the IgG groups, only mice receiving the highest dose showed protection (significant delay in tumor growth), whereas the other two groups were not protected. All three IgE groups were protected, and 3 of 15 animals remained tumor free (Fig. 5 B). In these groups of mice, we also measured the serum levels of anti-CEA antibodies to evaluate if vaccination with IgE-loaded tumor cells also induced an increased production of tumor-specific antibodies. Anti-
CEA antibodies were only detected in mice vaccinated with the highest dose of cells loaded either with IgG or IgE, thus indicating that IgEs did not increase the tumor-specific antibody response (data not shown).

In the RMA tumor model, protective immunity could be achieved by vaccination with irradiated IgG-loaded cells, at doses of ≥1 × 10 5 cells s.c./mouse but not at lower doses. Conversely, IgE-loaded irradiated cells were highly effective at doses as low as 1 × 10 3 cells s.c./mouse (data not shown).

DISCUSSION

A recent editorial article (39) states that “despite the mixed results, most of the studies have shown a reduced risk for cancer among people who have a history of allergies; one perspective is that allergies are evidence of the competence of the immune system.” It is suggested that the study of allergy-related genetic polymorphisms could clarify the biological basis of the association.

Table 1 Eosinophils, CD4 + , and CD8 T cells requirement for IgE-driven tumor reduction a

<table>
<thead>
<tr>
<th>Tumor Treatment</th>
<th>TI a</th>
<th>Vol</th>
<th>SE</th>
<th>TRF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG-RMA None</td>
<td>5/5</td>
<td>332</td>
<td>55.1</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>IgE-RMA None</td>
<td>5/5</td>
<td>101</td>
<td>16.7</td>
<td>3.29</td>
<td>0.0016</td>
</tr>
<tr>
<td>IgE-RMA Anti-IL5</td>
<td>5/5</td>
<td>359</td>
<td>46.6</td>
<td>0.93</td>
<td>0.7200</td>
</tr>
<tr>
<td>IgE-RMA Anti-CD4</td>
<td>5/5</td>
<td>306</td>
<td>55.7</td>
<td>1.08</td>
<td>0.9526</td>
</tr>
<tr>
<td>IgE-RMA Anti-CD8</td>
<td>5/5</td>
<td>385</td>
<td>57.8</td>
<td>0.86</td>
<td>0.2729</td>
</tr>
</tbody>
</table>

*ANOVA (Student’s t test) was calculated comparing each set of data with the IgG
control.
 a TI, tumor incidence; Vol, average tumor volume (cubic mm) at day 15; TRF, tumor reduction factor.
have been used for a three-step strategy, on the basis of biotin-avidin interactions, which involved in the modulation of acquired immune responses. In humans, thus leading to the hypothesis that IgEs could also be exploited for antigen capture and processing by professional APCs in severe combined immunodeficiency mouse xenograft tumor models at doses 100-fold lower than corresponding control cells and that human DCs and epidermal Langerhans cells can present antigens internalized as IgE-antigen complexes (41, 47, 48). However, mouse DCs have been reported to be devoid of FcεRs (49), thus it is more likely that the mechanism underlying IgE-driven T-cell priming also involves other FcεR-positive cell types, which could facilitate the uptake of tumor antigen by APC in either of two ways, by direct tumor cell killing or by causing allergic and inflammatory reactions at tumor sites.

Vaccination experiments confirm that the T-cell-mediated immunity elicited by IgEs loaded on tumor cells can be effectively exploited for tumor prevention. The presence of IgE immunoglobulin on the surface of irradiated tumor cells conferred protection in both tumor models at doses 100-fold lower than corresponding control cells without IgE.

In conclusion, the results reported here shed new light on a role for antibodies of IgE isotype as an effective mean to elicit immune responses against tumors and suggest that IgEs could be included in the design of cell-based antitumor vaccines.

ACKNOWLEDGMENTS

We thank Dr. Fabrizio Veglia for performing statistical analyses, Dr. Simona Porcellini for mAb purification, and Stefania Baviera for help in some

Fig. 5. IgE-loaded irradiated tumor cell vaccines elicit protective immunity. C57BL/6 mice were vaccinated s.c. at days 30 and 45 with irradiated IgE- (open symbols) or IgG-loaded (solid symbols) MC38-CEA-2 cells at three different cell doses: 30 × 10^3 (circles), 3 × 10^4 (triangles), and 1 × 10^5 (squares). A, on day 0, all vaccinated animals were challenged with 3 × 10^4 MC38 cells. A, all three IgE-loaded cell vaccines and the highest dose of IgG-loaded cell vaccine significantly reduced the growth rate of the tumor challenge. ANOVA at day 15 gave significance values ranging from P = 0.007 to P = 0.022. B, survival curves of the pooled 15 animals vaccinated with IgE-loaded cell vaccines versus the pooled 10 animals vaccinated with the two lower doses of IgE-loaded cell vaccines. The difference in survival was highly significant (P < 0.001).

Two recent reports (18, 40) have shown that exogenous IgE antibodies can activate antitumor effector mechanisms of innate immunity in severe combined immunodeficiency mouse xenograft tumor models. Another study (41) has recently pointed out the capability of IgE to mediate antigen capture and processing by professional APCs in humans, thus leading to the hypothesis that IgEs could also be involved in the modulation of acquired immune responses.

In the tumor therapy model, biotinylated IgE immunoglobulins of irrelevant specificity were administered to tumor-bearing mice using a three-step strategy, on the basis of biotin-avidin interactions, which have been used for in vivo targeting of radioisotopes and cytotoxic molecules on tumor cells in both mice and humans (24–29). A single injection of biotinylated IgEs in mice bearing tumors significantly decreased tumor progression, thus confirming that the IgE potential to trigger inflammatory and allergic reactions at tumor sites can be exploited for therapeutic purposes (18).

Unlike IgGs, which have a serum half-life of 23 days, the half-life of unbound IgE immunoglobulins in serum is ~2 days; therefore, it is very likely that repeated administration of IgE may improve the therapeutic results and even lead to tumor eradication. However, this first study had the aim to characterize the immunological potential of IgE in tumor immunotherapy, and additional studies will be addressed to define the most effective protocols for tumor therapy and vaccination.

Interestingly, we also found that IgEs of irrelevant specificity loaded on tumor cells strongly affected tumorigenicity and drove acquired tumor-specific immunity by priming CD8 and CD4 T-cell responses. We have found that IgE-loaded tumor cells, besides causing a delay in tumor growth, a significant decrease in the rate of tumor progression, and even tumor rejection, did also confer protective immunity against subsequent challenges with untreated tumor cells.

The phenomenon was observed in both our tumor models: (a) the Rauscher-induced rapidly growing RMA lymphoma, known to be immunogenic; and (b) the weakly immunogenic MC38 colon adenocarcinoma. The protective immunity depends upon priming of a T-cell-mediated immune response, because depletion of either CD4 or CD8 T-cell subsets, before the inoculation of IgE-loaded tumor cells, abrogated the inhibition of tumor growth. In vitro experiments on spleen cell cultures derived from IgE-primed tumor-rejecting mice demonstrated an enhanced tumor-specific CTL activity, thus strongly indicating that the induction of CTL responses is one of the mechanisms accounting for IgE-mediated tumor protection. CD4 T-cell proliferation in response to a tumor-derived I-A^b-restricted Th peptide was also observed in spleen cell cultures from mice immunized with IgE-loaded tumor cells.

It is likely that IgE antibodies trigger an inflammatory reaction at the tumor site by recruitment and activation of FcεR-bearing effectors, such as eosinophils, which in turn may favor tumor cell destruction, an essential requirement for an efficient priming of CD4- and CD8-dependent specific immune responses (42–44). Our experiments showed that eosinophil depletion abolished the delay in tumor progression observed after injection of RMA Thy 1.1 cells loaded with IgE, suggesting a role for eosinophils in the induction of tumor-specific immune responses.

It has been reported that IgEs augment antigen presentation by CD23-bearing B cells (45, 46) and that human DCs and epidermal Langerhans cells can present antigens internalized as IgE-antigen complexes (41, 47, 48). However, mouse DCs have been reported to be devoid of FcεRs (49), thus it is more likely that the mechanism underlying IgE-driven T-cell priming also involves other FcεR-positive cell types, which could facilitate the uptake of tumor antigen by APC in either of two ways, by direct tumor cell killing or by causing allergic and inflammatory reactions at tumor sites.

Vaccination experiments confirm that the T-cell-mediated immunity elicited by IgEs loaded on tumor cells can be effectively exploited for tumor prevention. The presence of IgE immunoglobulin on the surface of irradiated tumor cells conferred protection in both tumor models at doses 100-fold lower than corresponding control cells without IgE.

In conclusion, the results reported here shed new light on a role for antibodies of IgE isotype as an effective mean to elicit immune responses against tumors and suggest that IgEs could be included in the design of cell-based antitumor vaccines.

ACKNOWLEDGMENTS

We thank Dr. Fabrizio Veglia for performing statistical analyses, Dr. Simona Porcellini for mAb purification, and Stefania Baviera for help in some
We also thank Donald Hall and Garland Davis for the excellent technical assistance.

REFERENCES

2. Winter, K. D., Schott, M., Hampel, H., and Rieber, P. E. Advances in cancer immuno-
16. Mills, P., Beeson, W., Fraser, G., and Philip, R. Allergy and cancer: organ site-
23. Reali, E., Marzastri, M., Tomatis, R., Masucci, G. M., Tranelli, S., and Gavioli, R. A single specific amino acid residue in peptide antigens is sufficient to activate memory CTLs: potential role of cross-reactive peptides in memory T cell mainte-
IgEs Targeted on Tumor Cells: Therapeutic Activity and Potential in the Design of Tumor Vaccines

Eva Reali, John W. Greiner, Angelo Corti, et al.

Cancer Res 2001;61:5517-5522.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/14/5517

Cited articles
This article cites 47 articles, 28 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/14/5517.full.html#ref-list-1

Citing articles
This article has been cited by 9 HighWire-hosted articles. Access the articles at:
/content/61/14/5517.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.