

# Active Induction of Tumor-Specific IgE Antibodies by Oral Mimotope Vaccination

Angelika B. Riemer,<sup>1,2</sup> Eva Untersmayr,<sup>1</sup> Regina Knittelfelder,<sup>1</sup> Albert Duschl,<sup>4</sup> Hubert Pehamberger,<sup>2</sup> Christoph C. Zielinski,<sup>3</sup> Otto Scheiner,<sup>1</sup> and Erika Jensen-Jarolim<sup>1</sup>

Departments of <sup>1</sup>Pathophysiology, <sup>2</sup>Dermatology, and <sup>3</sup>Internal Medicine I, Medical University of Vienna, Vienna, Austria; and

<sup>4</sup>Department of Molecular Biology, University of Salzburg, Salzburg, Austria

## Abstract

A role of IgE antibodies in cancer surveillance has been implicated for a long time. Studies dealing with IgE antibodies directly targeted to tumor antigens have shown marked anticancer effects mediated by this antibody class. Thus, the basic function of IgE antibodies may be to control tumor growth. Thus far, cancer-specific IgE has only been applied passively. Consequently, the aim of this study was to establish an active vaccination protocol to induce tumor antigen-specific IgE antibodies, and to evaluate functional properties. We previously generated epitope mimics, so-called mimotopes, for the epitope recognized by the anti-HER-2 antibody trastuzumab. Upon i.p. immunizations, IgG antibodies with trastuzumab-like properties could be elicited. In the present study, we immunized BALB/c mice via the oral route with these trastuzumab mimotopes, under simultaneous neutralization and suppression of gastric acid. As shown in preceding experiments, this feeding regimen effectively induces Th2 immune responses. Oral immunizations with trastuzumab mimotopes under hypoacidic conditions indeed resulted in the formation of IgE antibodies towards the HER-2 antigen. Moreover, anti-HER-2 IgE-sensitized effector cells mediated SK-BR-3 target cell lysis in an antibody-dependent cytotoxicity assay. We conclude that directed and epitope-specific induction of IgE against tumor antigens is feasible with an oral mimotope vaccination regimen, and that these antibodies mediate anticancer effects. [Cancer Res 2007;67(7):3406–11]

## Introduction

The concept of immune surveillance was first proposed almost a century ago by Paul Ehrlich (1), and as immunosurveillance hypothesis against malignant disease, was again put forward in 1957 by Francis McFarlane Burnet (2). It suggests that the immune system is continuously searching for and destroying tumor cells as they arise. Evidence for the immune surveillance theory in humans derives from the observation that those who are immunosuppressed exhibit a higher incidence of malignancy (3).

Early on, it was suggested that those with a history of allergy, in particular atopic disorders, may possess an enhanced capacity for immune surveillance. Beginning in 1953, with the study by Logan and Saker (4), and around the 1960s (5–7) and early 1970s (refs. 8–12, reviewed in ref. 13), the first observations were published that allergy could be inversely associated with cancer development.

For several decades, epidemiologic studies have tried to correlate the incidence of allergic disease and diverse tumors, however, the results were mostly controversial and depended on the type of malignancy. In 2005, Turner et al. published the first large prospective cohort study to examine the relationship between atopic disease and cancer mortality, including 1,102,247 U.S. men and women for 18 years of follow-up (14). In this study, they found significantly lowered risks of ~10% for overall cancer mortality and 20% for colorectal cancer mortality among persons with a history of both asthma and hay fever. A history of hay fever alone was associated with a significantly lowered risk of pancreatic cancer mortality, and a history of asthma only was associated with a lowered risk of leukemia mortality.

Also in 2005, Wang and Diepgen published a review of the epidemiologic studies after 1985 (15). Their general conclusion was that allergy is associated with a reduced risk for cancer. This stands especially for colon cancer, pancreatic cancer, childhood leukemia, and brain tumors.

In 2006, the Turner group published another overview of the epidemiologic evidence by using the MEDLINE database since 1966 (16). Again, strong inverse associations were reported for allergy and pancreatic cancer and glioma, whereas lung cancer was positively associated with asthma.

This evidence relates to conditions with overall elevated IgE levels. Only a small number of studies have thus far investigated the effects of tumor-specific IgE (17–21). All of them came to the conclusion that IgE directly targeted to cancer antigens is extremely efficient in eliciting antitumor effects. In 1991, Nagy et al. developed a murine monoclonal IgE against the murine mammary tumor virus. It was applied for passive immunizations of mice, and prevented the development of s.c. tumors in an antigen-specific fashion (17). Kershaw et al. also developed a monoclonal IgE, recognizing a colon carcinoma antigen, and were the first to prove that the antitumor effect of targeted IgE was isotype-dependent (18). In 1999, Gould et al. generated mouse-human chimeric IgG<sub>1</sub> and IgE monoclonal antibodies against the ovarian cancer antigen MOv18 and compared them in a severe combined immunodeficiency mouse xenograft model of ovarian carcinoma. They found that the beneficial effects of tumor-specific IgE were greater and of longer duration than those of IgG<sub>1</sub> (19). In a subsequent study, this group showed that IgE triggered antibody-dependent cellular cytotoxicity (ADCC) via monocytes (20). The last study dealing with IgE directly targeted to tumor antigens postulated that IgE loaded onto tumor cells could affect tumor immunogenicity by activation of the innate immune system directly at the tumor site. They used a biotin-avidin bridging strategy to target IgE to the tumor cells, and found that IgE strongly affects tumor growth *in vivo*, leading, in some cases, to complete tumor rejection and protection against subsequent tumor challenges by a mechanism involving both eosinophils and

**Requests for reprints:** Erika Jensen-Jarolim, Department of Pathophysiology, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria. Phone: 43-1-40400-5120; Fax: 43-1-40400-5130; E-mail: erika.jensen-jarolim@meduniwien.ac.at.  
©2007 American Association for Cancer Research.  
doi:10.1158/0008-5472.CAN-06-3758

T cell-mediated antitumor responses. Moreover, the results showed that IgE targeted to tumor cells not only possess curative potential, but also confer long-term antitumor immunity (21).

From all these data, we believe that IgE, especially IgE targeted to tumor antigens, plays a beneficial role in antitumor immunity. Therefore, we aimed to move the approaches of passive application of tumor-specific IgE a step forward—by developing a vaccine that would induce tumor-specific IgE *in vivo*. To this end, we combined two strategies that were developed in our group during the past few years: first, an epitope-specific vaccination against the HER-2 tumor antigen, rendering antibodies with similar biological properties as the monoclonal antibody trastuzumab (22); second, an oral immunization regimen discovered in food allergy research, resulting in IgE induction (23, 24). Here, we show for the first time the active induction of tumor-specific IgE antibodies, targeting HER-2 as an important model antigen.

## Materials and Methods

**Cell lines and monoclonal antibody.** The HER-2-positive human mammary carcinoma cell line SK-BR-3 (ATCC HTB-30) was grown in McCoy's medium (Life Technologies, Inchinnan, United Kingdom) supplemented with 10% FCS, 1% glutamine, 1% penicillin/streptomycin, and 50 µg/mL of gentamicin sulfate. The human squamous carcinoma cell line A-431 (CRL-1555; American Type Culture Collection, Manassas, VA), which is HER-2-negative, was grown in DMEM (Life Technologies) supplemented as above, and used for control purposes.

Rat basophilic leukemia RBL-2H3 cells were kindly supplied by Dr. Arnulf Hartl, Paracelsus Medical University, Salzburg, Austria. They were grown in RPMI 1640 (Life Technologies), supplemented with 10% FCS, 4 mmol/L of L-glutamine, 2 mmol/L of sodium pyruvate, 10 mmol/L of HEPES, 100 µmol/L of 2-mercaptoethanol, and 1% penicillin/streptomycin. Trastuzumab (Herceptin), a humanized IgG<sub>1</sub> monoclonal antibody targeting HER-2, was purchased from Roche (Hertfordshire, United Kingdom).

**Mimotope characterization and vaccine compound.** Mimotopes mimicking the trastuzumab epitope on HER-2 were previously defined (22, 25). The best peptide mimic of these experiments, the 1,12-cyclic peptide C-QMWAPQWGPD-C, was manufactured synthetically (piChem, Graz, Austria) and coupled via its COOH terminus to a succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate activated immunogenic carrier, keyhole limpet hemocyanin (KLH), through a linker (GPGPG) by S-acetyl-thio-acetate. The conformational accuracy of the coupled mimotope was verified with specific recognition by trastuzumab. As a control, the peptide 1,12-cyclic C-DGGWLSKGSW-C, which did not bind to trastuzumab, was produced and coupled in the same way.

**Immunization of BALB/c mice.** Six-week-old female BALB/c mice ( $n = 7$  per group) were obtained from the Institute for Laboratory Animal Science and Genetics (University of Vienna, Vienna, Austria) and were treated according to European Community rules of animal care with permission no. 66.009/35-BrGT/P2004 from the Austrian Federal Ministry of Education, Science, and Culture.

Mice were immunized according to the oral immunization regimen for IgE induction developed in our group (23), with some modifications. Amounts of antiulcer drugs were correlated with the dosage recommended for use in humans and adjusted for body weight and metabolism of mice. Each animal received 11.6 µg of omeprazole (Losec, AstraZeneca, Vienna, Austria) i.v. on days 1, 2, and 3 of each immunization cycle. On day 3, omeprazole was applied 2 h and 15 min before intragastric immunization. One hundred micrograms of the respective antigen were mixed with 2 mg of sucralfate (Ulcogant; Merck, Vienna, Austria) and administered intragastrically in a final volume of 100 µL. The verum group was immunized with the C-QMWAPQWGPD-C mimotope-KLH conjugate, and the control group received the carrier KLH alone. Immunizations were done on days 3, 17, 45, 59, and 87. Blood was taken from the tail vein

on day 0 (preimmune serum), and on days 27, 69, and 97 (first, second, and third mouse immune serum).

**Titer determination.** IgE and IgG titers of sera from the immunized BALB/c mice against KLH and against the mimotope peptide were determined by incubation of serial dilutions of pooled group sera with DotBlots of KLH, the C-QMWAPQWGPD-C mimotope peptide, and the control peptide C-DGGWLSKGSW-C. In short, antigens were solubilized in PBS/20% dimethylformamide, and dotted onto nitrocellulose membrane at 1 mg/mL. Blot strips were incubated with mouse serum dilutions, and bound antibodies detected with rat anti-mouse IgG or rat anti-mouse IgE antibodies (PharMingen, San Diego, CA), that were again detected by a peroxidase-conjugated sheep anti-rat immunoglobulins (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom) using the ECL chemoluminescent detection protocol (Amersham). Trastuzumab was used as a positive control and detected with a peroxidase-conjugated goat anti-human IgG antibody (Jackson ImmunoResearch, West Grove, PA). Titers were given as the highest serum dilution at which antigen reactivity was still detectable in these DotBlot experiments.

**β-Hexosaminidase release assay from RBL-2H3 cells.** RBL-2H3 cells, which express rodent FcεRI as their only antibody receptor, were used to determine functional HER-2-specific IgE. Cells were incubated with individual third immune sera of the mimotope-immunized or the control mice to allow for binding of serum IgE to FcεRI. Loaded RBL-2H3 cells were then incubated with SK-BR-3 cells as a source of natural HER-2, or with A-431 cells as controls. IgE recognition of its target antigen leads to cross-linking of FcεRI, rat basophilic leukemia (RBL) cell degranulation, and thus, β-hexosaminidase release. Released enzyme was detected by 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide (4-MUG), and the resulting fluorescence measured at 465 nm (excitation wavelength 360 nm). For 100% release, RBL cells were lysed with Triton X-100, and relative experimental releases determined as follows:

$$\% \beta\text{-hexosaminidase release} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100$$

**Antibody-dependent cytotoxicity assay.** The ADCC potential of the IgE antibodies induced by mimotope vaccination was assessed with a modified CytoTox 96 nonradioactive cytotoxicity assay (Promega, Madison, WI). SK-BR-3 and A-431 cells were used as target cells; with A-431 cells as controls. The number of target cells was optimized to  $2 \times 10^5$  cells/mL according to the instructions of the manufacturer. For the ADCC experiments, an effector to target (E/T) ratio of 100:1 was found to yield optimal specific lysis in a series of preliminary tests. RBL-2H3 cells were used as effector cells, after ascertaining that even at full ionomycin-induced degranulation, these cells do not release lactate dehydrogenase, the indicator of target cell lysis measured by a color reaction in this ADCC assay. Ten microliters of the third immune sera of the mimotope-immunized or the control mice were used for ADCC experiments. All assay procedures and readouts were done according to the instructions of Promega. The percentage of cytotoxicity was calculated after correcting for background absorbance values according to the following formula:

$$\% \text{ cytotoxicity} = \frac{\text{experimental} - \text{effector spontaneous} - \text{target spontaneous}}{\text{target maximum} - \text{target spontaneous}} \times 100$$

Maximal cytotoxicity caused by fully degranulated RBL cells against SK-BR-3 cells was set to 100% RBL-mediated lysis, and resulting cytotoxicities were adjusted accordingly. All titer determinations, β-hexosaminidase release, and ADCC assays were done in triplicate, and repeated for reproducibility.

**Statistical analyses.** Statistical analyses were done using two-tailed Student's *t* test, as immune responses in syngeneic mice were considered to be distributed normally.  $P < 0.05$  was considered statistically significant, and  $P < 0.01$  was considered highly statistically significant.

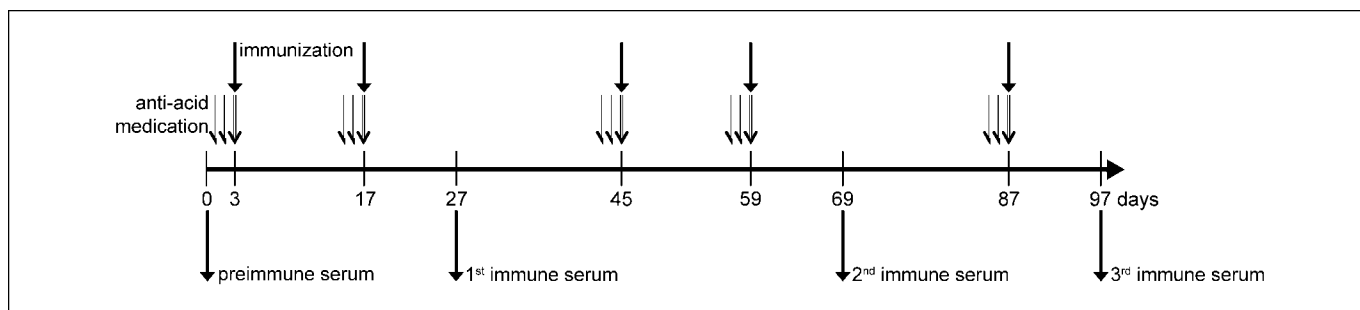


**Figure 1.** Oral immunization regimen. Timeline of antacid medication, antigen gavage, and blood sampling.

## Results

**Immune responses against the vaccine construct induced by oral mimotope vaccination.** Mice were immunized with the mimotope C-QMWAPQWGPDC or the carrier molecule KLH as shown in Fig. 1. As mimotopes on their own are too small to elicit an immune response, the immunogenic carrier KLH was used in designing the vaccine construct (26, 27). Groups were further designated as “QMW-KLH” and “KLH.” To ensure hypoacidity during the gastric passage, mice received the proton pump inhibitor omeprazole once daily 2 days preceding each immunization, and twice on the day of immunization. Additionally, the antigens were dispersed in the protective film-building ulcer medication sucralfate. This combined regimen has been shown to effectively elevate gastric pH,<sup>5</sup> and lead to the formation of IgG and, importantly, IgE antibodies against food proteins fed under these conditions (23, 24). In contrast, proper digestion of dietary proteins favors immune tolerance or ignorance (28).

IgG and IgE titers against the carrier and trastuzumab mimotope were determined by DotBlot analysis. IgG titers reached a plateau after two courses of two immunizations, as no booster effect was seen between the second and third immune serum (see Fig. 1; Table 1). IgE was only first observed after these four immunizations, and could be boosted by an additional immunization (Table 1). A representative example of a DotBlot titer determination can be seen for the third immune sera in Fig. 2. Interestingly, both IgG and IgE responses were stronger against the mimotope than the carrier (in the case of IgE, there was no response against the carrier detectable at all, see Table 1).

**IgE formation against the original antigen, HER-2.** As shown in our previous work, a crucial point in mimotope immunizations is the induction of antibodies not only recognizing the applied mimotope, but also the original antigen that is mimicked. For anti-HER-2 IgG antibodies, we could show this cross-reactivity in Western blots (22). For IgE antibodies induced by the oral vaccination regimen, we employed another experimental setup. We applied an assay commonly used in allergology, i.e., a release assay from RBL cells. These cells only carry the high-affinity receptor for IgE, FcεRI, and can thus be used to determine IgE levels in sera in which a high content of IgG antibodies against the same antigen would otherwise interfere in assay sensitivity. When specific IgE is bound to the high-affinity receptors, and brought in contact with cognate antigen, FcεRI cross-linking and RBL degranulation occurs. The released enzyme β-hexosaminidase

can be detected by a fluorescence reaction, and corresponds with the specific IgE content of the applied sera.

To show that the induced antibodies recognize the mimicked original antigen, HER-2, we used the HER-2-overexpressing breast cancer cell line SK-BR-3 as the source of antigen in the RBL assay. The HER-2-negative squamous cell carcinoma cell line A-431 was used as a negative control. IgE from mice immunized with QMW-KLH consistently led to highly significant degranulation of RBL cells after incubation with SK-BR-3 cells, but not with A-431 cells. KLH-immunized mice showed no reactivity with either cell line (Fig. 3). This indicates that the induced antimimotope IgE antibodies indeed specifically recognize the tumor antigen HER-2. Additionally, a successful RBL release assay was the first hint of the functionality of the induced IgE antibodies, as they have been shown to be capable of cross-linking FcεRI molecules.

**IgE-mediated cytotoxicity.** To further investigate the biological effects of the induced anti-HER-2 IgE antibodies, we adapted a classic nonradioactive cytotoxicity assay. RBL cells were used as effector cells, and sensitized with IgE from individual third immune sera from the QMW-KLH- and KLH-immunized mice. In this RBL ADCC assay, the mimotope-induced IgE antibodies exhibited RBL-mediated lysis in the range of 20% to 100% of SK-BR-3 cells (Fig. 4). RBL-ADCC ranging from 0% to 55% was seen with antibodies from the KLH-immunized control group. Although these ranges are broad and overlapping, the difference was found to be statistically significant ( $P \leq 0.01$ ). When antimimotope antibodies were tested on A431 control cells, no specific cytotoxicity was observed (data not shown).

## Discussion

The physiologic role of IgE antibodies is still not fully understood. Nevertheless, evidence is accumulating that these antibodies could be involved in the prevention of malignant disease

**Table 1.** Antibody titers of mimotope-immunized mice against the mimotope and the carrier protein

|                     | IgG      |         | IgE   |     |
|---------------------|----------|---------|-------|-----|
|                     | QMW      | KLH     | QMW   | KLH |
| Preimmune serum     | —        | —       | —     | —   |
| First immune serum  | 1:1,000  | 1:1,000 | —     | —   |
| Second immune serum | 1:10,000 | 1:5,000 | 1:100 | —   |
| Third immune serum  | 1:10,000 | 1:5,000 | 1:500 | —   |

<sup>5</sup> E. Untermayer, unpublished data.

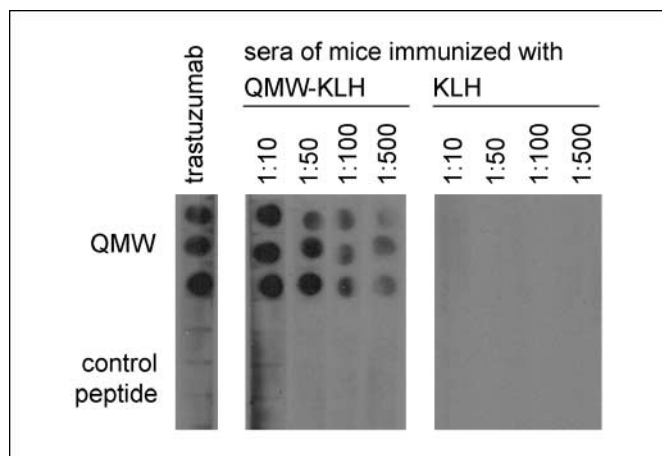


**Figure 2.** Oral immunization under acid suppression with the mimotope conjugate elicits IgE antibodies recognizing the immunogen. Mice were immunized with C-QMWAPQWGPDC-KLH (QMW-KLH) or KLH alone. IgE titer determination by DotBlot against the peptide C-QMWAPQWGPDC, and against a control peptide. Serum dilutions are shown.

(14–16). IgE is present in only low concentrations in the serum of normal individuals ( $\sim 30$  ng/mL; ref. 18), but is highly increased in two situations: the case of parasite infestation and in allergic disease. For both conditions, there is evidence of a negative association with cancer: Experimental data from mice show that animals infested with nematodes were resistant to syngeneic mammary adenocarcinoma (29), or showed decreased incidence of spontaneous mammary tumors (30). Huge epidemiologic studies and metaanalyses have been conducted on the topic of atopic disease and cancer risk, and a significant, if small, negative association has been found (14–16). The latest bit of evidence for the inverse association between allergy and malignant disease stems from the first clinical trials with the anti-IgE monoclonal antibody, omalizumab. Omalizumab is a human/murine chimeric monoclonal IgG antibody that prevents IgE from attaching to effector cells, thereby blunting IgE-mediated inflammatory responses. It is known to result in a marked reduction in serum levels of free IgE and down-regulation of IgE receptors on circulating basophils. When IgE was inhibited with this anti-IgE antibody, 1 in 200 asthmatic patients developed breast, skin, prostate or parotid gland malignancies during the median observation period of 1 year, whereas in the control group, the incidence was 1:500.<sup>6</sup>

The picture becomes clearer when one focuses on IgE specific for cancer cell antigens. There are early observations of “natural” IgE-mediated mechanisms in cancer patients. In 1977, Rosenbaum and Dwyer pointed out that evidence was published prior to the recognition of IgE that suggested that IgE may recognize tumor-specific antigens (13). A study in cancer patients in 1958 reported that eight tumor patients (five breast, two Hodgkin’s, one lymphoma) showed wheal and flare skin reactions upon intradermal injections of lysates of their own tumor. This apparently was an immediate hypersensitivity response typically mediated by IgE. Moreover, this reaction could be transferred to a healthy volunteer in a classic Prausnitz-Küstner reaction (31). In 1972, Bartholo-

maeus and Keast observed that mice could produce IgE specific for a tumor, in this case, B16 melanoma (32).

Four groups have thus far targeted IgE to tumor-specific antigens, i.e., the mouse mammary tumor virus (17), a surface antigen of human colorectal carcinoma (18), the ovarian cancer antigen MOv18 (19, 21), and—via biotin-avidin bridging—to a lymphoma and an adenocarcinoma antigen (20). All found that IgE antibodies directly targeted to tumor antigens—in contrast with overall elevated IgE levels—cause a marked effect on tumor development and growth. For example, monoclonal IgE raised against murine mammary tumor virus were able to protect syngeneic mice from a lethal dose of mammary carcinoma cells (17), and as little as 1  $\mu$ g of colon carcinoma-specific IgE per mouse was sufficient to inhibit COLO 205 tumor growth (18). However, all these strategies used passive applications of IgE. A vaccination regimen aimed at active induction of tumor-specific IgE seemed to be a promising approach.

Recently, we have developed an active immunization regimen for inducing tumor-inhibitory antibodies against HER-2, which is a member of the epidermal growth factor receptor (also known as the ErbB) family. This receptor is overexpressed in  $\sim 30\%$  of breast cancer patients and confers a detrimental prognosis in the course of early as well as advanced breast cancer (33). The monoclonal antibody trastuzumab (Herceptin) targets this receptor, and

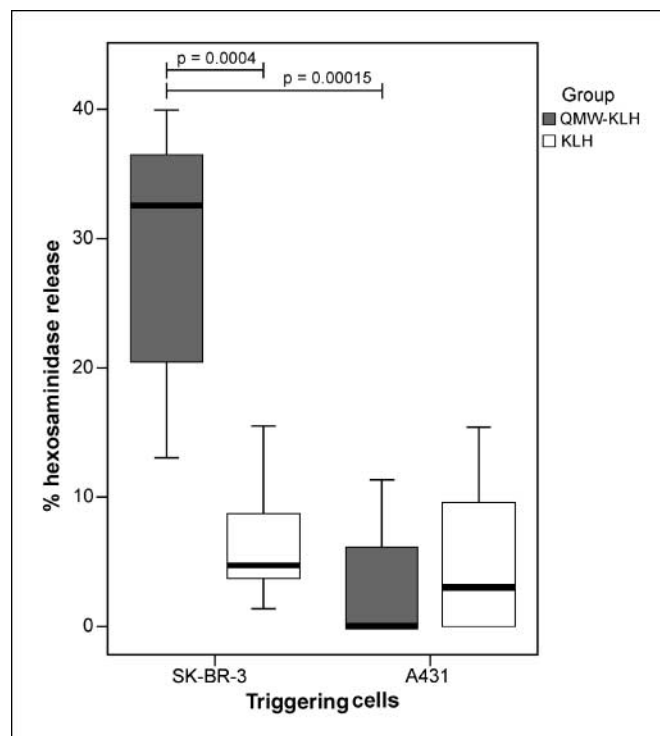


**Figure 3.** Mimotope-induced IgE antibodies recognize the original antigen, HER-2, expressed on SK-BR-3 breast cancer cells. In a  $\beta$ -hexosaminidase release assay with RBL-2H3 cells, IgE from mice immunized with C-QMWAPQWGPDC-KLH (QMW-KLH) led to significant degranulation of RBL cells after incubation with HER-2-overexpressing SK-BR-3 cells, but not with the HER-2-negative control cell line, A-431. KLH-immunized mice show no reactivity with either cell line. Box plots, median, 25th, and 75th percentiles of  $\beta$ -hexosaminidase release mediated by sera of individual mice in each group ( $n = 7$ ). This release correlates with the anti-HER-2 IgE levels of the respective groups. Both the difference of the mimotope-immunized group in SK-BR-3/A-431 recognition, as well as the difference between mimotope and control group in SK-BR-3 recognition, were found to be highly statistically significant ( $P < 0.01$ ) by two-tailed Student’s *t* test.

<sup>6</sup> Food and Drug Administration Briefing Document on Safety BLA STN 103976/0. [http://www.fda.gov/ohrms/dockets/ac/03/briefing/3952B1\\_02\\_FDA-Xolair-Safety.pdf](http://www.fda.gov/ohrms/dockets/ac/03/briefing/3952B1_02_FDA-Xolair-Safety.pdf).

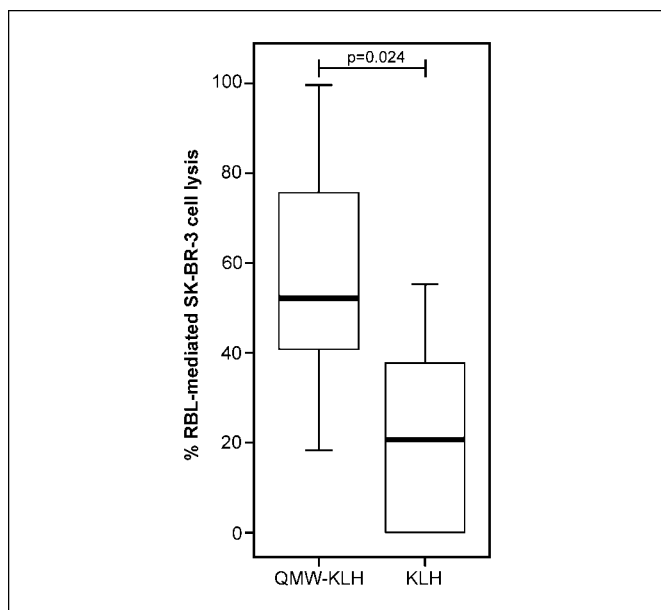


**Figure 4.** HER-2-specific IgE antibodies lead to SK-BR-3 breast cancer cell lysis via ADCC mechanisms. In a nonradioactive cytotoxicity assay, IgE-loaded RBL cells were used as effector cells. Mimotope-induced IgE antibodies targeting HER-2 mediated significant ADCC lysis of SK-BR-3 cells, when compared with control sera from KLH-immunized mice. Box plots, median, 25th, and 75th percentiles of relative target cell lysis ( $n = 7$  per group). The difference between verum and control group was found to be statistically significant ( $P < 0.05$ ) by two-tailed Student's  $t$  test.

beneficially influences the progression of early and advanced HER-2 overexpressing tumors. Consequently, we used trastuzumab to generate peptide mimics (so-called mimotopes) of its binding site on HER-2. These mimotopes induced trastuzumab-like IgG antibodies in mice upon parenteral (i.p.) immunization. Importantly, these actively induced antibodies showed similar biological antitumor features as the original antibody itself (22).

A second experience in our group stems from our research on food allergy mechanisms. We found that food proteins can effectively lead to IgE formation and sensitization when they persist the gastric passage undegraded (28). Gastric digestion is impaired in conditions of hypochlorhydria as, e.g., under antilulcer treatment. Consequently, an oral immunization regimen was developed that leads to the induction of a Th2 bias, i.e., high levels of food protein-specific IgG and IgE antibodies, eosinophilia, and hypersensitivity of skin and mucosa, when antigens are fed under concomitant gastric acid suppression (23, 24, 34).

In the present study, we combined our two fields of experience, and could show that feedings of trastuzumab mimotopes under antiacidic conditions indeed induced HER-2-specific IgE in BALB/c mice. These antibodies proved to be functional in an allergologic mediator release assay, where mimotope-induced IgE was found to react highly-specifically with HER-2-overexpressing breast cancer cells. To the best of our knowledge, this is the first active induction of tumor-specific IgE *in vivo*.

IgE is known to be capable of mediating ADCC of parasitic larvae in combination with effector cells bearing Fc $\epsilon$ RI. Indeed, these mechanisms play a major role in immune defense against parasites (35). Thus, IgE specifically targeted to tumor antigens could function in a similar way: by cross-linking Fc $\epsilon$ RI it would activate potent effector cells. Already in the first study on tumor-specific IgE, it was hypothesized that IgE-mediated cytotoxic

mechanisms may play an immunologically specific antitumor surveillance role (17). Gould et al. also thought that IgE could mediate anticancer ADCC, and therefore created chimeric IgE and IgG<sub>1</sub> antibodies recognizing an ovarian cancer antigen to directly compare between the two subclasses. Indeed, in their *in vivo* model, only IgE, and not IgG<sub>1</sub> caused sustained inhibition of tumor growth until the end of the experiments (day 48). The inhibitory effect of tumor-specific IgE was substantial (83%) and highly significant (19). In a more recent publication, they were able to show that human monocytes were active in IgE-dependent ADCC *in vivo*. Tumor cell killing was proportional to the expression of Fc $\epsilon$ RI on monocytes, and they observed phagocytosis of tumor cells by the monocytes *in vitro*. Testing basophils, they observed targeted exocytosis of the granules at the site of contact with cancer cells, and overall, an accumulation of basophils at the site of tumors (21).

We therefore did an assay evaluating the ADCC-mediating potential of the induced anti-HER-2 IgE antibodies towards breast cancer cells. As effectors, we used the rat basophilic leukemia cell line RBL-2H3, which expresses the rodent Fc $\epsilon$ RI as its only antibody receptor. Thus, we were sure that no other antibodies contained in the immune sera could interfere in this assay. These cells were incubated with individual serum samples of mimotope and KLH immunized mice. Indeed, specific IgE-mediated lysis of target cells could be observed. The degree of IgE-ADCC mediated by the sera correlated with the level of HER-2-specific IgE detected by the  $\beta$ -hexosaminidase assay.

Generally, IgE may have two major advantages over other isotypes in the therapy of cancer. One aspect may be the prolonged duration of antitumor effects. Whereas the serum half-life of unbound IgE in humans is only  $\sim 2$  days, the half-life of IgE in skin is in the order of several months (36). Thus, IgE-mediated effector functions at tissue tumor sites could be expected to last for a long time. The extended lifetime of receptor-bound IgE may allow cells to locate and kill tumor cells in solid tissues more effectively than IgG.

The second aspect is the high affinity of the interaction between the Fc portion of IgE and Fc $\epsilon$ RI, which may allow effective arming of potential effector cells that would then be able to extravasate and penetrate tissue without losing surface-bound antibody (18). Indeed, it is well known that there is an infiltration of developing tumors by various Fc $\epsilon$ RI-bearing immune cells. More importantly, on-site production of IgE by plasma cells could contribute to beneficial effects. Indeed, a high number of IgE-positive cells was found in most squamous cell carcinomas of the head and neck when compared with normal mucosa (37).

In general, the special role of IgE is to act as a very potent inducer of mediator release and cytokine production from eosinophils, basophils, macrophages, and mast cells. Among the released substances are histamine, heparin, the enzymes tryptase and chymase, as well as the proinflammatory cytokines granulocyte macrophage colony-stimulating factor, interleukin 4, interleukin 5, and tumor necrosis factor  $\alpha$  (38, 39). Moreover, several lines of evidence indicate a crucial role for eosinophils and macrophages in tumor eradication through a mechanism involving the production of reactive oxygen metabolites and nitric oxide (20). But in humans, it has to be considered that the high-affinity receptor Fc $\epsilon$ RI is also expressed on antigen-presenting cells as Langerhans cells and monocytes (40). IgE is capable of mediating antigen capture, processing, and presentation by professional antigen-presenting cells, facts known from allergic patients. Thus, IgE-driven antitumor activity is not restricted to the activation of innate

immunity effector mechanisms, but could also initiate efficient antigen presentation and thus priming of a T cell-mediated adaptive immune response (41). It is likely that IgE antibodies trigger an inflammatory reaction at the tumor site by recruitment and activation of FcεR-bearing effector cells, which in turn, may favor tumor cell destruction, an essential requirement for efficient priming of CD4- and CD8-dependent immune responses (42, 43).

Taken together, there is growing evidence that IgE might have a role in cancer surveillance and suppression. The negative association between cancer incidence and conditions with elevated IgE levels could indicate that atopic individuals possibly have some antitumor protection due to a tendency to form IgE antibodies, and among them, the beneficial tumor-specific ones as well. The slightly increased cancer risk seen in patients exposed to the anti-IgE antibody omalizumab<sup>6</sup> might thus be explained by both a

reduction of possible tumor-specific IgE antibodies, but even more so, by its reduction of FcεR-bearing effector cells.

We conclude that IgE specifically targeted to tumor antigens elicits effective antitumor immune responses and that it is feasible to induce tumor antigen-specific IgE antibodies by active immunization.

## Acknowledgments

Received 10/12/2006; revised 12/15/2006; accepted 1/24/2007.

**Grant support:** P18238-B13 of the Austrian Science Fund (FWF) and BioLife Science GmbH, Vienna, Austria. A.B. Riemer and E. Untersmayr are recipients of Hans and Blanca Moser scholarships.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The authors thank H. Kurz and S. Gruber for excellent technical assistance.

## References

- Ehrlich P. Über den jetzigen Stand der Karzinomforschung. *Ned Tijdschr Geneesk* 1909;5:273–90.
- Burnet M. Cancer: a biological approach. I. The processes of control. *Br Med J* 1957;1:779–86.
- Markiewicz MA, Gajewski TF. The immune system as anti-tumor sentinel: molecular requirements for an anti-tumor immune response. *Crit Rev Oncol* 1999;10:247–60.
- Logan J, Saker D. The incidence of allergic disorders in cancer. *N Z Med J* 1953;52:210–2.
- Fisherman EW. Does the allergic diathesis influence malignancy? *J Allergy* 1960;31:74–8.
- Mackay WD. The incidence of allergic disorders and cancer. *Br J Cancer* 1966;20:434–7.
- McKee WD, Arnold CA, Perlman MD. A double-blind study of the comparative incidence of malignancy and allergy. *J Allergy* 1967;39:294–301.
- Ure DM. Negative association between allergy and cancer. *Scott Med J* 1969;14:51–4.
- Augustin R, Chandradasa KD. IgE levels and allergic skin reactions in cancer and non-cancer patients. *Int Arch Allergy Appl Immunol* 1971;41:141–3.
- Shapiro S, Heinonen OP, Siskind V. Cancer and allergy. *Cancer* 1971;28:396–400.
- Meers PD. Allergy and cancer. *Lancet* 1973;1:884–5.
- Allegra J, Lipton A, Harvey H, et al. Decreased prevalence of immediate hypersensitivity (atopy) in a cancer population. *Cancer Res* 1976;36:3225–6.
- Rosenbaum JT, Dwyer JM. The role of IgE in the immune response to neoplasia: a review. *Cancer* 1977; 39:11–20.
- Turner MC, Chen Y, Krewski D, Ghadirian P, Thun MJ, Calle EE. Cancer mortality among US men and women with asthma and hay fever. *Am J Epidemiol* 2005;162:212–21.
- Wang H, Diepgen TL. Is atopy a protective or a risk factor for cancer? A review of epidemiological studies. *Allergy* 2005;60:1098–111.
- Turner MC, Chen Y, Krewski D, Ghadirian P. An overview of the association between allergy and cancer. *Int J Cancer* 2006;118:3124–32.
- Nagy E, Berczi I, Sehon AH. Growth inhibition of murine mammary carcinoma by monoclonal IgE antibodies specific for the mammary tumor virus. *Cancer Immunol Immunother* 1991;34:63–9.
- Kershaw MH, Darcy PK, Trapani JA, MacGregor D, Smyth MJ. Tumor-specific IgE-mediated inhibition of human colorectal carcinoma xenograft growth. *Oncol Res* 1998;10:133–42.
- Gould HJ, Mackay GA, Karagiannis SN, et al. Comparison of IgE and IgG antibody-dependent cytotoxicity *in vitro* and in a SCID mouse xenograft model of ovarian carcinoma. *Eur J Immunol* 1999;29:3527–37.
- Karagiannis SN, Wang Q, East N, et al. Activity of human monocytes in IgE antibody-dependent surveillance and killing of ovarian tumor cells. *Eur J Immunol* 2003;33:1030–40.
- Real E, Greiner JW, Corti A, et al. IgEs targeted on tumor cells: therapeutic activity and potential in the design of tumor vaccines. *Cancer Res* 2001;61:5517–22.
- Riemer AB, Klinger M, Wagner S, et al. Generation of peptide mimics of the epitope recognized by trastuzumab on the oncogenic protein Her-2/neu. *J Immunol* 2004;173:394–401.
- Untersmayr E, Schöll I, Swoboda I, et al. Antacid medication inhibits digestion of dietary proteins and causes food allergy: a fish allergy model in BALB/c mice. *J Allergy Clin Immunol* 2003;112:616–23.
- Schöll I, Untersmayr E, Bakos N, et al. Antiulcer drugs promote oral sensitization and hypersensitivity to hazelnut allergens in BALB/c mice and humans. *Am J Clin Nutr* 2005;81:154–60.
- Riemer AB, Kraml G, Scheiner O, Zielinski CC, Jensen-Jarolim E. Matching of trastuzumab (Herceptin) epitope mimics onto the surface of Her-2/neu—a new method of epitope definition. *Mol Immunol* 2005;42: 1121–4.
- Riemer AB, Kurz H, Klinger M, Scheiner O, Zielinski CC, Jensen-Jarolim E. Vaccination with cetuximab mimotopes and biological properties of induced anti-epidermal growth factor receptor antibodies. *J Natl Cancer Inst* 2005;97:1663–70.
- Riemer AB, Forster-Wald E, Brämswig KH, et al. Induction of IgG antibodies against the GD2 carbohydrate tumor antigen by vaccination with peptide mimotopes. *Eur J Immunol* 2006;36:1267–74.
- Untersmayr E, Jensen-Jarolim E. The effect of gastric digestion on food allergy. *Curr Opin Allergy Clin Immunol* 2006;6:214–9.
- Ogilvie BM, Simpson E, Keller R. Tumour growth in nematode-infected animals. *Lancet* 1971;1:678–80.
- Weatherly NF. Increased survival of Swiss mice given sublethal infections of *Trichinella spiralis*. *J Parasitol* 1970;56:748–52.
- Grace JT, Jr., Kondo T. Investigations of host resistance in cancer patients. *Ann Surg* 1958;148:633–41.
- Bartholomaeus WN, Keast D. Reaginic antibody to tumour and alloantigens in mice. *Nat New Biol* 1972;239: 206–7.
- Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* 2001;2:127–37.
- Untersmayr E, Ellinger A, Beil WJ, Jensen-Jarolim E. Eosinophils accumulate in the gastric mucosa of food-allergic mice. *Int Arch Allergy Immunol* 2004;135:1–2.
- Gounni AS, Lamkhouiou B, Ochiai K, et al. High-affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature* 1994;367:183–6.
- Asai K, Kitaura J, Kawakami Y, et al. Regulation of mast cell survival by IgE. *Immunity* 2001;14:791–800.
- Neuchrist C, Kornfehl J, Grasl M, et al. Distribution of immunoglobulins in squamous cell carcinoma of the head and neck. *Int Arch Allergy Immunol* 1994; 104:97–100.
- Plaut M, Pierce JH, Watson CJ, Hanley-Hyde J, Nordan RP, Paul WE. Mast cell lines produce lymphokines in response to cross-linkage of Fc epsilon RI or to calcium ionophores. *Nature* 1989;339:64–7.
- Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. *Nat Immunol* 2005;6:135–42.
- Bieber T. Fc epsilon RI-expressing antigen-presenting cells: new players in the atopic game. *Immunol Today* 1997;18:311–3.
- Adams GP, Weiner LM. Monoclonal antibody therapy of cancer. *Nat Biotechnol* 2005;23:1147–57.
- Rovere P, Vallinoto C, Bondanza A, et al. Bystander apoptosis triggers dendritic cell maturation and antigen-presenting function. *J Immunol* 1998;161:4467–71.
- Albert ML, Sauter B, Bhardwaj N. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature* 1998;392:86–9.