AllergoOncology: High innate IgE levels are decisive for the survival of cancer-bearing mice

Josef Singer, Gertrude Achatz-Straussberger, Anna Bentley-Lukschal, Judit Fazekas-Singer, Gernot Achatz, Sophia N. Karagiannis, Erika Jensen-Jarolim

Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University Vienna, Austria
Department of Internal Medicine II, University Hospital Krems, Karl, Landsteiner University of Health Sciences, Krems an der Donau, Austria
The Interuniversity Messerli Research Institute of the University of Veterinary Medicine Vienna, Medical University Vienna and University Vienna, Austria
Department of Molecular Biology, University of Salzburg, Austria
St. John’s Institute of Dermatology, School of Basic and Medical Biosciences, King’s College London, Guy’s Hospital; London, UK
Division of Cancer Studies, Faculty of Life Sciences & Medicine; King’s College London, Guy’s Hospital, London, UK

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ABSTRACT

Background: Atopics have a lower risk for malignancies, and IgE targeted to tumors is superior to IgG in fighting cancer. Whether IgE-mediated innate or adaptive immune surveillance can confer protection against tumors remains unclear.

Objective: We aimed to investigate the effects of active and passive immunotherapy to the tumor-associated antigen HER-2 in three murine models differing in Epsilon-B-cell-receptor expression affecting the levels of expressed IgE.

Methods: We compared the levels of several serum specific anti-HER-2 antibodies (IgE, IgG1, IgG2a, IgG2b, IgA) and the survival rates in low-IgE \( \Delta M1M2 \) mice lacking the transmembrane/cytoplasmic domain of Epsilon-B-cell-receptors expressing reduced IgE levels, high-IgE KN1 mice expressing chimeric Epsilon-Gamma1-B-cell receptors with 4-6-fold elevated serum IgE levels, and wild type (WT) BALB/c. Prior engrafting mice with D2F2/E2 mammary tumors overexpressing HER-2, mice were vaccinated with HER-2 or vehicle control PBS using the Th2-adjuvant Al(OH)\(_3\) (active immunotherapy), or treated with the murine anti-HER-2 IgG1 antibody 4D5 (passive immunotherapy).

Results: Overall, among the three strains of mice, HER-2 vaccination induced significantly higher levels of HER-2 specific IgE and IgG1 in high-IgE KN1 mice, while low-IgE \( \Delta M1M2 \) mice had higher IgG2a levels. HER-2 vaccination and passive immunotherapy prolonged the survival in tumor-grafted WT and low-IgE \( \Delta M1M2 \) strains compared with treatment controls; active vaccination provided the highest benefit. Notably, untreated high-IgE KN1 mice displayed the longest survival of all strains, which could not be further extended by active or passive immunotherapy.

Conclusion: Active and passive immunotherapies prolong survival in wild type and low-IgE \( \Delta M1M2 \) mice engrafted with mammary tumors. High-IgE KN1 mice have an innate survival benefit following tumor challenge.

Background

Amongst the clinically-applied monoclonal antibodies targeting tumor-associated antigens (TAA), especially trastuzumab (Herceptin\textsuperscript{®}, Roche) is indispensable in the treatment of metastatic breast cancer and other cancer entities overexpressing the human epidermal growth factor-
This humanized IgG1 antibody leads to improved overall response rates and better progression-free as well as overall survival. Trastuzumab is a derivative of the mouse monoclonal antibody 4D5, an IgG1 antibody clone targeting human HER-2. Because of its favorable binding affinity and tumor growth inhibition, the complementarity-determining regions of 4D5 were inserted into the backbone of a human IgG1 antibody with increased affinity to HER-2 as a positive side-effect. All antibodies currently FDA-approved are of the IgG isotype, although nature has equipped the human immune system with five different immunoglobulin classes.

IgE antibodies have gained attention in the past for their potential protective role in cancer. IgE antibodies purified from pancreatic cancer patients were capable of mediating antibody-dependent cell-mediated cytoxicity (ADCC) against pancreatic cancer cells in vitro. In vitro, higher levels of ADCC could be triggered by IgE antibodies, compared to their IgG counterparts. This points towards a superior function of IgE in ADCC, compared to IgG, which is known to likely induce antibody-dependent cellular phagocytosis (ADCP). 

In order to achieve specific immunity to HER-2, mice were vaccinated subcutaneously four times at 2-week intervals with 50 μg of rHER-2 admixed with aluminum hydroxide solution (Al(OH)3, Alum, Alu-Gel®; Cat-No.: 12261.01,Serva, Heidelberg, Germany). Control groups received phosphate buffered saline (PBS). Before the immunization trial and after each round of immunization serum samples were taken to monitor the immune response (Fig. 1, PIS ... Enzyme linked immunosorbent assay (ELISA)

Immune responses in mice were evaluated by ELISA. The tumor-associated antigen rHER-2 was coated on 96-well microtiter plates (ImmuNo Maxi-Sorp™, Nunc, Cat-No: M9410-1CS, Roskild, Denmark) at a concentration of 1 μg/ml. Unspecific binding was blocked with 1% dried milk powder (DMP) in TRIS-buffered saline, 0.05% Tween20 (TBST). Sera of treated mice, diluted in TBST/0.1% DMP, were allowed to adhere overnight on 4 °C. Bound murine immunoglobulins were detected with rat anti-mouse IgM (Cat-No: 553405), IgG1 (Cat-No: 553440), IgG2a (Cat-No: 553387), IgG2b (Cat-No: 553392), IgA (Cat-No: 556966) and IgE (Cat-No: 553416) antibodies (Pharmingen™, BD Biosciences, Franklin Lakes, New Jersey, USA), followed by detection of bound antibodies with a horseradish-peroxidase labeled goat anti-rat IgG antibody (Amersham ECL, GE Healthcare Europe GmbH; Cat-No: NA935; diluted 1:3000 in TBST + 0.1% DMP, Buckinghamshire, United Kingdom). For detection, 3,3',5,5'-tetramethylbenzidine (TMB, BD OptEIA TMB Solution, BD Biosciences; Cat-No: 5535440), IgG2a (Cat-No: 553387), IgG2b (Cat-No: 553392), IgA (Cat-No: 556966) and IgE (Cat-No: 553416) antibodies (Pharmingen™, BD Biosciences, Franklin Lakes, New Jersey, USA), followed by detection of bound antibodies with a horseradish-peroxidase labeled goat anti-rat IgG antibody (Amersham ECL, GE Healthcare Europe GmbH; Cat-No: NA935; diluted 1:3000 in TBST + 0.1% DMP, Buckinghamshire, United Kingdom). For detection, 3,3',5,5'-tetramethylbenzidine (TMB, BD OptEIA TMB Solution, BD Biosciences; Cat-No: 555214, 100μl/well) was added and the optical density (OD) was measured at 450 nm with 620 nm as reference. An integrated absorbance value was calculated for each sample. The optical density was directly proportional to the concentration of murine immunoglobulins, since the same quantity of enzyme conjugate was used for each sample. The resulting values were analyzed in a statistical program and compared to a standard curve. The sensitivity of the assay was 10 μg/ml for IgM, 50 μg/ml for IgG1, 75 μg/ml for IgG2a, 150 μg/ml for IgG2b, 150 μg/ml for IgA and 75 μg/ml for IgE.

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Fig. 1. Schematic overview of immunization plan and tumor graft trial. Mice were immunized 4 times with rHER-2 or PBS + Al(OH)3 in bi-weekly intervals (top panel, bottom panel). Mouse sera were taken to monitor the immune response (PIS = Pre-ImmuneSerum, MIS 1-4 = Mouse Immune Serum). Subsequently mice were grafted 2x10^6 HER-2 over-expressing D2F2/E2 cells s.c.; tumor growth was measured daily by caliper measurement. Passive immunotherapy groups received the murine anti-HER-2 antibody 4D5 i.p. 1 day prior to tumor grafting and every 7 days after tumor transplantation (middle panel). MIS5 was taken at day 14 of the tumor trial. Mice were sacrificed when tumor volume reached 300mm^3. MIS6 was taken prior to sacrifice (at individual time points).
Table 1
Overview on treatment groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Active Immunotherapy</th>
<th>Passive Immunotherapy</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT BALB/c</td>
<td>rHER-2</td>
<td>4D5</td>
<td>PBS</td>
</tr>
<tr>
<td>ΔM1M2 (low IgE)</td>
<td>rHER-2</td>
<td>4D5</td>
<td>PBS</td>
</tr>
<tr>
<td>KN1 (high IgE)</td>
<td>rHER-2</td>
<td>4D5</td>
<td>PBS</td>
</tr>
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(Cat-No.: 0103-01), mouse IgG2b clone A-1 (Cat-No.: 0104-01), mouse IgA clone S107 (Cat-No.: 0106-01) and mouse IgE clone 15.3 (Cat-No.: 0114-01).

Results

High IgE-expressing mice respond to HER-2 immunization by enhancing anti-HER-2 IgE and IgG1, while low-IgE ΔM1M2 mice develop higher IgG2a levels.

In mice of all three strains immunoglobulins could be specifically induced by the HER-2 vaccine (Fig. 2). IgG1 was by far the most prominent immunoglobulin isotype response in all three strains (Fig. 2A). After 4 immunizations (MS4, Fig. 3), WT BALB/c mice displayed significantly less HER-2 specific IgG1 than high-IgE expressing KN1 (p < 0.0001, Fig. 3A), and more than low-IgE expressing ΔM1M2 mice (n.s., p = 0.346) mice. ΔM1M2 mice developed significantly higher levels of anti-HER-2 IgG2a (Fig. 2B) than either WT BALB/c (p < 0.0001, Fig. 3B) or KN1 (p < 0.001, Fig. 3B). Both WT BALB/c and KN1 mice (Fig. 2C) displayed comparable (p = 0.5633), but significantly higher IgG2b levels than ΔM1M2 mice (p < 0.0001 for WT BALB/c; p = 0.0023 for KN1; Figs. 3C). For HER-2 specific IgA, the serum levels did not significantly differ between the three strains (WT BALB/c: ΔM1M2: p = 0.388; WT vs KN1: p = 0.9819; KN1: ΔM1M2: p = 0.2725; Figs. 2D and 3D). Notably, after 4 rounds of immunization, HER-2 specific IgE levels were highest in the KN1 animals (p < 0.0001) but did not differ between normal-IgE WT BALB/c vs low-IgE ΔM1M2 mice (p = 0.8993; Figs. 2E and 3E). The induction of HER-2 specific IgE occurred more slowly in ΔM1M2 mice (Fig. 2E).

Together, these findings suggest that high-IgE expressing mice develop a prominent IgE-enhanced response following HER-2 vaccination.

Active and passive immunotherapy do not further prolong the survival rates in high-IgE mice

Following tumor challenge, WT HER-2-vaccinated mice had a significant survival benefit over PBS-treated control mice (Fig. 4A, left, p < 0.0001), which exceeded the effect of passive immunotherapy with the HER-2 specific monoclonal IgG1 antibody 4D5 (Fig. 4A, middle, p = 0.0428). 4D5 treatment, however, provided a significant survival benefit compared to PBS sham treatments. (Fig. 4A, right, p = 0.0013).

In low-IgE ΔM1M2 mice, the HER-2 vaccine (p = 0.0001) and 4D5 (p = 0.0081) prolonged survival compared to PBS (Fig. 4B left and middle). Again, survival of the HER-2 vaccinated mice was more pronounced than in mice treated with 4D5 (Fig. 4B right: HER-2 vs. 4D5 treated, p = 0.0251).

In the high-IgE KN1 group, neither HER-2 immunization (Fig. 4C left, p = 0.1557) nor 4D5 mediated any additional survival benefits compared to naïve animals (Fig. 4C middle, p = 0.8475).

Notably, untreated high-IgE KN1 mice displayed significantly longer survival after tumor grafting than both WT and ΔM1M2 mice (Fig. 5A, middle and right).

Discussion

Promising approaches employ alternate immunoglobulin classes, such as IgA or IgE, to the commonly used IgG for cancer immunotherapy.19,34-46 A trastuzumab-like IgE with the same variable region and differing only in the constant region, mediated higher levels of ADCC in a side-by-side comparison with the corresponding IgG1 in vitro.19 These functional studies in parallel with data from epidemiological meta-analyses describing a negative correlation of specific and total IgE levels in allergies and atopes with the occurrence of specific cancers suggest that elevated levels of IgE antibody responses such as in allergy and atopy, may offer protection from cancer initiation.23-26 In accordance with these observations, recent retrospective studies demonstrate that vice versa low levels of total IgE in 4488 patients of the 2005–2006 US National Health and Nutrition Examination Survey (NHANES) termed “IgE-deficient”, correlated with a higher frequency of occurrence of cancer, compared with normal, high, or very high IgE levels.34 In another study, the authors showed that in fact 2.7% of 2339 allergies visiting an outpatient allergy clinic correlated with significantly higher rates of diagnosis of any type of cancer during the observation period 2010–2015 (33% vs 8.7%; odds ratio 5.51, 95% confidence interval 3.07e9.88).32

In the present study, we mimic the human setting of high, atopic IgE levels, versus low IgE levels employing three different mouse strains differing in their ε-B-cell receptor expression. Our findings confirm that IgE antibodies have a protective effect, in a model of HER-2 positive mammary cancer, even in a non-antigen specific manner.

In high-IgE KN1 mice, higher expression of the ε-BCR and of IgE seem to play a decisive role in significantly enhancing tumor-free survival time independent of active or passive immunotherapy. In WT and low-IgE ΔM1M2 mice, both passive and active immunotherapy significantly prolonged mouse survival, compared to sham treatments. These findings suggest the merit of anticancer immunotherapies targeting the tumor-associated antigen HER-2. Notably, high-IgE mice survived significantly longer than normal- and low-IgE mice following mammary tumor challenge when no immunotherapy was made. In the high-IgE KN1 mice, mimicking the atopic state in humans (Fig. 5A), the “innate” effects of high IgE could not be further increased by specific anti-HER-2 vaccination, or by passive anti-HER-2 IgG1 antibody treatment using 4D5. This is in favor of previous work demonstrating an innate “adjuvant function” of IgE in cooperation with FcεRI expressed by cytotoxic effector cells.28 In concordance, a gene expression signature incorporating the high affinity IgE receptor FcεRI was shown to correlate with improved prognosis in lung adenocarcinoma.47 These findings mirrored numerous epidemiologic studies highlighting an inverse correlation of high total or allergen-specific IgE in atopic and allergic patients, with the reduced risk of developing different cancers including those of the breast.

When mice were treated with passive or active immunotherapy, the advantage of innate IgE in KN1 was lost and all strains had a similar survival benefit (Fig. 5B and C). Based on these data we propose that low-IgE patients might have the greatest advantage of immunotherapies, while atopic patients already naturally exploit innate IgE-mediated mechanisms against malignancies.

Our study has several limitations: first of all, the sample size for each group is small, however, this is an exploratory pilot study addressing a novel aspect in cancer immunology, which may represent the basis for...
Fig. 2. Antibody responses of different mouse strains. A: HER-2 specific IgG1 antibody levels are displayed during the course of the immunization trial. Rectangle symbols depict the groups immunized with rHER-2, triangle symbols represent the groups receiving PBS. Serum was taken prior to immunization (PIS) and after each immunization round (MIS 1–4). Left panel depicts WT mice, middle panel ΔM1M2 mice and right panel KN1. Displayed are mean immunoglobulin levels in ng/ml ± SEM. B: HER-2 specific IgG2a antibody levels. C: HER-2 specific IgG2b. D: HER-2 specific IgA. E: HER-2 specific IgE.
Fig. 3. Comparison of antibody responses in different mouse strains after 4 rounds of immunizations. A: HER-2 specific IgG1 levels in WT, ΔM1M2 and KN1 mice. B: HER-2 specific IgG2a antibody levels. C: HER-2 specific IgG2b. D: HER-2 specific IgA. E: HER-2 specific IgE. Displayed are mean values in ng/ml ± SEM. ns ... not significant, * ... p < 0.05, ** ... p < 0.01, *** ... p < 0.001, **** ... p < 0.0001.

Fig. 4. Survival curves of different treatment groups after tumor challenge. A: Survival curves of wild type animals distributed by different treatment groups. Left panel: HER-2 immunized animals compared to naive (PBS treated) mice, middle: mice treated with the anti-HER-2 antibody 4D5 compared to naive mice, right: comparison of the active (HER-2 immunized) and passive (4D5 treated) immunotherapy groups. B: Survival curves of different treatment groups in the ΔM1M2 mouse strain. Left panel: HER-2 treated mice compared to naive, middle: 4D5 treated animals compared to naive, right: HER-2 immunized mice compared to 4D5 treated. C: Survival curves of differently treated mice in the KN1 strain. Left: HER-2 treatment vs. naive, middle: 4D5 treatment vs. naive, right: HER-2 immunization vs. 4D5. Full lines: naive groups, dashed lines: HER-2 immunized groups, dotted lines: 4D5 treated groups; ns ... not significant, * ... p < 0.05, ** ... p < 0.01, *** ... p < 0.001, **** ... p < 0.0001.
further research studies into this field. Second, in the absence of a recombinant mouse anti HER-2 IgE antibody of the same specificity as 4D5, a side-by-side comparison of IgE versus IgG isotypes could not be done to compare the potential of IgG versus IgE-based immunotherapies. Furthermore, adoptive transfer experiments of sera from high-IgE to low-IgE mice could have further elucidated the role of IgE in immuno-surveillance and defense of cancer. Finally, the mouse immune system does not fully mirror the human setting and generally such experiments would only constitute an impression of the human immune system setting. For instance, murine dendritic cells do not express FcRI, which hinders conclusions on the IgE-mediated antigen presentation capacity of the used transgenic mouse models.8

Conflict of interest

Sophia N. Karagiannis is scientific founder of IGEM Therapeutics, a UK-based Immuno-Oncology company. The other authors declare no conflict of interest.

References


