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

Josef Singer \textsuperscript{a,b,c}, Gertrude Achatz-Straussberger \textsuperscript{d}, Anna Bentley-Luksch \textsuperscript{a}, Judit Fazekas-Singer \textsuperscript{b,c}, Gernot Achatz \textsuperscript{d}, Sophia N. Karagiannis \textsuperscript{e,f}, Erika Jensen-Jarolim \textsuperscript{a,c,*}

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

ARTICLE INFO

Keywords:
AllergoOncology
Cancer vaccine
IgE
HER-2
Onco-immunology

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 \textsuperscript{Δ}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)\textsubscript{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, while low-IgE \textsuperscript{Δ}M1M2 mice had higher IgG2a levels. HER-2 vaccination and passive immunotherapy prolonged the survival in tumor-grafted WT and low-IgE \textsuperscript{Δ}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 \textsuperscript{Δ}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-

Abbreviations: ADCC, Antibody-dependent Cell-mediated Cytotoxicity; ADCP, Antibody-dependent Cellular Phagocytosis; BCR, B-Cell Receptor; HER-2, Human Epidermal Growth Factor Receptor-2, ErbB-2; IgA, Immunoglobulin A; IgE, Immunoglobulin E; IgG, Immunoglobulin G; TAA, Tumor-Associated Antigen; WT, wild type.

* Corresponding author. Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University Vienna, Waehringer Gürtel 18-20, 1090 Vienna, Austria.

E-mail address: erika.jensen-jarolim@meduniwien.ac.at (E. Jensen-Jarolim).

Deceased.

https://doi.org/10.1016/j.waojou.2019.100044
Received 16 January 2019; Received in revised form 22 May 2019; Accepted 12 June 2019
1939-4551/© 2019 Published by Elsevier Inc. on behalf of World Allergy Organization. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
2, HER-2.1 This humanized IgG1 antibody leads to improved overall response rates and better progression-free as well as overall survival.2

Trastuzumab is a derivative of the mouse monoclonal antibody 4D5, an IgG1 antibody clone targeting human HER-2.3 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.4 All antibodies currently FDA-approved are of the IgG isotype,5 although nature has equipped the human immune system with five different immunoglobulin classes.6

IgE antibodies have gained attention in the past for their potential protective role in cancer.7,8 IgE antibodies purified from pancreatic cancer patients were capable of mediating antibody-dependent cell-mediated cytoxicity (ADCC) against pancreatic cancer cells in vitro.9 In vitro, higher levels of ADCC could be triggered by IgE antibodies, compared to their IgG counterparts.10,11 This points towards a superior function of IgE in ADCC, compared to IgG, which is known to likely induce antibody-dependent cellular phagocytosis (ADCP).12-19 Large epidemiological studies demonstrated inverse correlations between elevated IgE levels in atopic and malignant diseases, including colo-

rectal, pancreatic, and gynecological cancers as well as gliomas, and childhood leukemia.16,20

Elevated IgE levels in atopics and malignant diseases, including colorectal, pancreatic, and gynecological cancers as well as gliomas, and childhood leukemia.16,20 However, it remains unclear whether serum IgE levels and IgE-mediated innate or adaptive immune surveillance can confer protection against tumors,21 or vice versa, low IgE levels are in favor of tumor development.22,23 Therefore, we investigated the impact of IgE against cancer at low-, normal- or high-IgE conditions in a mouse model. In order to address these aspects, we compared the levels of specific IgE and other serum specific anti-HER-2 antibodies (IgG1, IgG2a, IgG2b, IgG3) along with the survival rates between three different mouse strains engrafted with HER-2 overexpressing tumors, differing in their Epsilon-B-cell receptor expression and IgE expression levels: i) low-IgE ΔM1M2 mice lacking the transmembrane/cytoplasmic domain of the ε-B-cell receptor (BCR),39 ii) high-IgE KN1 mice expressing chimeric ε-γ1-BCRs with a 4- to 6-fold elevated mean serum IgE level,40 compared to iii) wild type (WT) BALB/c mice with “normal” IgE levels.

**Materials and methods**

**Cell lines, recombinant proteins and monoclonal antibodies**

D2F2/E2 is a mouse mammary carcinoma cell line derived from D2F2 cells transplanted with human HER-2. This cell line was established41 and kindly provided by Prof. Wei-Zen Wei (Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan, USA). D2F2/E2 cells were grown in a humidified atmosphere of 5% CO2 at 37°C in IMDM medium supplemented with 10% fetal calf serum (FCS), penicillin (100 U/mL), streptomycin (100 μg/mL) and 1 mg/mL gentamicin (G418). Recombinant human HER-2 (rHER-2) was produced as previ-

ously described42,43 in Lec-1 cells (a kind gift of Prof. Daniel J. Leahy, The University of California, California, USA). Recombinant human HER-2 (rHER-2) was produced as previously described42,43 in Lec-1 cells (a kind gift of Prof. Daniel J. Leahy, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA). Lec-1 cells were kept in DMEM/F12 supplemented with 5% FCS, 100 μM methotrexate and 10 μg/mL gentamicin sulfate. Trastuzumab (Herceptin®), a humanized IgG1 monoclonal anti-HER2-2 (HER-2) anti-

body, was obtained from Roche (Basel, Switzerland). Rituximab (Mab-
Theon®), a chimeric IgG1 anti-CD20 monoclonal antibody, was employed as isotype control in ELISAs. Clone 4D5, the murine IgG1 precursor of trastuzumab was kindly provided by Genentech (South San Francisco, California, USA).

**Mouse strains**

Mice with different degrees of IgE-responses to foreign antigens were employed in this study. BALB/c mice served as wild type animals (WT). ΔM1M2 mice, which lack the transmembrane and cytoplasmic domain of IgE39 served as the IgE-reduced (IgE low) strain. KN1 mice, which express chimeric ε-γ1 B-cell receptors40 served as the IgE-overexpressing (high-IgE) model. Both transgenic mouse strains are of BALB/c background.

All mice were kept on the basis of authorization of the Animal Ethics Committee of the Medical University according to the European Union and Federation for Laboratory Animal Science Associations (FELASA) guidelines for animal care and protection (GZ: BMWF-J/0009/ 0086-C/CT/2007).

**Immunization scheme and tumor graft trial**

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-adjuvanted with aluminum hydroxide solution (Al(OH)3, Alum, Alu-

Gel-S®, 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... Pre-Immune Serum, MIS 1–4... Mouse Immune Serum 1–4). For overview on mouse groups, strains and treatments see Table 1.

On day 1 of tumor challenge experiments, immunized mice were grafted subcutaneously into their left flanks with 2 × 106 HER-2-over-

expressing D2F2/E2 mouse mammary carcinoma cells, resuspended in 100 μl of IMDM (without supplements). Tumor size was measured daily by caliper measurement and tumor volume was calculated according to formula V(mm³) = d² x D (mm)/2, where d stands for the smallest and D for the largest diameter of the tumor. When tumors reached a volume of 300 mm³, mice had to be sacrificed and tumors were taken for histologic evaluation of HER-2 expression.

In order to treat one group with passive anti-HER-2 immunotherapy, respective mice received 100 μg 4D5 antibody intraperitoneally (i.p.) 1 day before the tumor graft and every 7 days after tumor transplantation. MIS5 (Mouse Immune Serum) was taken at day 14 of the tumor trial. Mice were sacrificed when tumor volume reached 300 mm³ MIS6 was taken prior to sacrifice (at individual time points; see Fig. 1).

**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: 556960) and IgE (Cat-No: 553416) antibodies (PharMingen™, BD Bio-

sciences, 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, 3,5,5′,5′-tetramethylbenzidine (TMB, BD OptiELA 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 wavelength with a multiwell plate reader (infinite M200 PRO, Tecan Group AG, Maennedorf, Switzerland). For standard dilution curves, the following purified murine immunoglobulins from Southern Biotechnologies were used: mouse IgM clone 11E10 (Cat-No: 0101-01), mouse IgG1 clone 15H6 (Cat-No: 0102-01), mouse IgG2a clone HOPC.
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-Immune Serum, 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).
IgA, the serum levels did not signifi
0.0001 for WT BALB/c; p
(WT BALB/c: Both WT BALB/c and KN1 mice (Fig. 2C) displayed comparable (p
than either WT BALB/c (p
0.5633), but signifi
mice developed signifi
and more than low-IgE expressing
Δ
HER-2 speci
Δ
mice had a sig-
advantage of innate IgE in KN1 was lost and all strains had a similar
in high-IgE mice
Together, these
When mice were treated with passive or active immunotherapy, the
advantage of innate IgE in KN1 was lost and all strains had a similar
in high-IgE mice
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 immunosurveillance 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 FcεRI, which hinders conclusions on the IgE-mediated antigen presentation capacity of the used transgenic mouse models.8

Conclusion

This study highlights: i) that anticancer vaccines targeting HER-2 provide a significant survival benefit and delay disease progression in an immunocompetent syngeneic mouse model of cancer, ii) that vaccination was superior to passive antibody therapy with the anti-HER-2 IgG1 antibody 4D5 in WT and low-IgE expressing mice, and iii) the protective effects of elevated natural IgE levels after tumor challenge compared with normal- or low-IgE expressing mice who did not undergo any intervention. IgE therefore has a natural surveillance function against malignant cells, together with its cytotoxic cellular effector arm. Epideimiologic studies indicate that atopics with elevated IgE may benefit from its innate immune surveillance and functions. We propose that in non-atopic patients, the benefits of IgE can be exploited and improved by targeting recombinant IgE antibodies to cancer antigens. While low-IgE conditions pose a higher risk for progressive cancer, these individuals may benefit from anticancer immunotherapies. Based on our study and in line with the AllergoOncology concept, we propose that IgE should be reinvented to target cancer antigens.7,13,36,48

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


24. Ferastraoaru D, Rosenstreich D. IgE de...