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Genetic regulation of the response to Her-2 DNA vaccination in human Her-2 transgenic mice ¹

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Abstract

Genetic regulation of immune reactivity to Her-2 vaccination and the consequent anti-tumor effect was tested in human Her-2 transgenic (Tg) mice in C57BL/6 (B6), BALB/c (BALB) and (B6x BALB) F1 (F1) background. Mice were electrovaccinated with Her-2 DNA with or without pre-treatment with CD25 mAb to remove CD25^{hi} regulatory T cells. When CD25⁺ T cells were intact, BALB Her-2 Tg mice were more responsive than the other two strains in both humoral and cellular immunities and their tumor growth was significantly delayed. B6 Her-2 Tg mice responded poorly and F1 mice showed modest immune reactivity, but tumor growth did not change in either strain. Depletion of CD25^{hi} T cells before vaccination significantly improved protection from tumor challenge in F1 Her-2 Tg mice. This was associated with elevated levels of Her-2 IgG1, IgG2a and IgG2c antibodies (Ab) and some mice also showed IFN- γ producing T cell response. The same treatment induced modest improvement in B6 Her-2 Tg mice. In BALB Her-2 Tg mice, however, depletion of CD25^{hi} T cells did not further improve anti-tumor efficacy. Although their Her-2 specific IgG1 and IL-5 secreting T cells increased, the levels of IgG2a and IFN- γ secreting T cells did not change. These results are the first to demonstrate genetic regulation of the response to a cancer vaccine and an unequal effect of removing CD25^{hi} T cells on anti-tumor immunity. These results warrant individualized treatment plans for patients with heterogeneous genetic backgrounds and possibly differential intrinsic immune reactivity to tumor antigens.

Keywords

Her-2; DNA vaccine; cancer vaccine; transgenic mice; regulatory T cell

Introduction

Active vaccination is both attractive and promising for cancer control, but results from clinical trials and pre-clinical animal testing have been highly variable and the determining factors of the response are poorly defined. Since MHC class II haplotype is the primary determining factor in autoimmunity (1), it is possible that immune reactivity to a tumor associated self-antigen is also regulated via MHC. We have reported previously that HLA-DR3, which dictates

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the severity of experimental autoimmune thyroiditis, does not significantly affect Her-2 immunity (2). But, it remains unclear if cancer vaccines would function equally in individuals of heterogeneous MHC haplotypes. In this study, the efficacy of Her-2 DNA vaccine is tested in Her-2 transgenic mice of BALB/c (BALB), C57BL/6 (B6) and BALBxB6 F1 (F1) background to determine genetic regulation of Her-2 vaccine response.

ErbB-2, a member of the erbB receptor tyrosine kinase family, is weakly to moderately expressed in normal adult tissues (3). Human and rat homologues are designated as Her-2 and neu, respectively (4). Transphosphorylation of tyrosine residues in the cytoplasmic domain is triggered when Her-2 is dimerized with any erbB family member. The consequent signal transduction leads to cell proliferation, migration, adhesion and transformation (5-8). Dysregulated signal transduction from over-expressed or mutated Her-2 leads to tumorigenesis (9). In 20–30% of human breast cancers, over-expression of Her-2 is correlated with more aggressive disease, resistance to chemo and hormone therapy and poor survival (4,10-12).

Vaccination of wild type mice with Her-2 DNA induces robust immune response and protects mice from tumor growth (13), with vaccines advancing to clinical trials (14). With the expected immune tolerance to self-Her-2 in humans, several transgenic (Tg) mouse strains expressing rat neu had been used to evaluate Her-2 vaccines (15,16). But their immune reactivity to Her-2 did not mimic the response in humans because neu transgenic mice responded to Her-2 as a foreign, rather than self-antigen (17). We previously showed that antibody (Ab) induced by Her-2 and neu DNA vaccines recognized only cognate antigen, although T cell responses to Her-2 and neu were cross-reactive (17). This disparate cross-reactivity may contribute to some of the confusions in the literature.

To evaluate immunoreactivity to Her-2 as a self-antigen, we generated Her-2 Tg mice by expressing the human Her-2 gene under the whey acidic protein (WAP) promoter (18). Her-2 is expressed in mammary epithelia during pregnancy and lactation and constitutively in the Bergman glia cells within the molecular layer of the cerebellum, but overt neoplastic transformation has not been detected in any tissue. In Her-2 Tg mice, Her-2⁺ tumors grow progressively without inducing Her-2 Ab, showing immune tolerance to Her-2 in these mice (18).

We tested DNA vaccination in Her-2 Tg mice of BALB/c (BALB), C57BL/6 (B6) and BALBxB6 F1 (F1) backgrounds and they showed different response profiles. Treatment with CD25 mAb to deplete regulatory T cells (Treg) is beneficial only in mice of B6 or F1 background that have more profound tolerance. These new findings warrant a personalized vaccination regimen for the heterogeneous patient population to achieve clinical benefit.

Materials and Methods

DNA vaccine constructs

pCMV/E2TM (pE2TM) encoding the extracellular and transmembrane domains of human ErbB-2 (Her-2) was previously described (2,17). pEFBos/GM-CSF (pGMCSF) encoding murine GM-CSF was provided by Dr. N. Nishisaki at Osaka University, Osaka, Japan. The blank vector, pCMV, was used as a control.

Mice

All animal procedures were conducted in accordance with accredited institution guidelines and the US Public Health Service Policy on Humane Care and Use of Laboratory Animals. C57BL/6 (B6), BALB/c (BALB) and (BALBxB6) F1 (F1) female mice were purchased from Charles River Laboratory (Frederick, MD). Heterozygous C57BL/6 Her-2 Tg mice (B6 Her-2 Tg), which expressed the full-length, wild type human Her-2 under the whey acidic protein (WAP)

promoter were generated in our laboratory and have been maintained by breeding with normal B6 mice (18). B6 Her-2 Tg mice were back-crossed onto the BALB/c background >13 generations to establish syngeneic, heterozygous BALB/c Her-2 Tg (BALB Her-2 Tg) mice. (BALBxB6) F1 Her-2 Tg mice (F1 Her-2 Tg) were generated by crossing BALB Her-2 Tg mice with wild type B6 mice or vice versa. Transgene positive mice were identified by PCR as we previously described (19).

Cell lines

BALB/c mouse mammary tumor line D2F2 was established from a spontaneous tumor that arose in a prolactin-induced hyperplastic alveolar nodule line, D2 (20). The D2F2/E2 cell line was generated by stable transfection of D2F2 cells with the wild type Her-2 (13,17). EO771/E2 was generously provided by Dr. Daniel Allendorf (James Graham Brown Cancer Center, Louisville, Kentucky) and was generated by transfecting the C57BL/6 mammary tumor EO771 with wild type Her-2. BALB/c APCs 3T3/KB expressed H2K^d and B7.1 (CD80), and 3T3/EKB expressed H2K^d, CD80 and Her-2 (17). C57BL/6 APC TC-1 cells (generously provided by Dr. T.C. Wu, The Johns Hopkins University, Baltimore, MD) (21) expressed endogenous H2K^b and B7.1 (17). TC-1/E2 expressed wild type Her-2. SKOV3, a human ovarian cancer cell line with amplified Her-2 (22), was purchased from ATCC (Manassas, VA). The hybridoma line PC61 (ATCC), which produces rat mAb to mouse CD25 was propagated in SCID mice. All cell lines were maintained as previously described (2,17).

Analysis of Her-2 expression in cerebellum

Her-2 expression in the cerebellum of Her-2 Tg mice and transgene-negative littermates were analyzed by Western blot as we previously described (19). Briefly, cerebellum tissue from individual animals was finely cut and lysed in tissue lysis buffer containing protease inhibitors. Three mg of total protein were used for each immunoprecipitation reaction, and Her-2 protein was immunoprecipitated from the tissue lysates by incubation with trastuzumab (Genentech, South San Francisco, CA) and protein A/G-agarose (Santa Cruz Biotechnology, Santa Cruz, CA). Proteins were eluted in 2x sample buffer and boiled for 5 min before fractionation in 4–20% gradient SDS- PAGE. Proteins were electrotransferred to Immobilon-P (Millipore, Bedford, MA) polyvinylidene difluoride (PVDF) membranes. Membranes were hybridized with mAb 42 (BD Biosciences, San Jose, CA), which specifically recognizes human Her-2, followed by HRP-conjugated secondary antibody and detected by chemiluminescence using Kodak Imaging Station.

Immunization

Mice received DNA electrovaccination as we previously described (17). Briefly, 50 µg each of individual or mixtures of plasmid DNA in a total volume of 50 µl was injected into the quadriceps muscle. DNA injection was followed immediately by square wave electroporation over the injection site using a BTX830 (BTX Harvard Apparatus, Holliston, MA). A tweezer electrode was used to deliver 8 pulses at 100V for 25 msec per pulse. Wild type mice were immunized twice and Her-2 Tg mice 4 times at two-week intervals. To deplete CD25^{hi} regulatory T cells (Treg), indicated groups of Her-2 Tg mice received i.p. 0.25 mg of CD25 mAb, PC61, 10 d. prior to the 1st electrovaccination. Depletion of CD25^{hi} T cells was verified by flow cytometry (data not shown).

Measurement of Her-2 Ab by flow cytometry

Her-2 Ab levels were determined as previously described (23). Briefly, SKOV3 cells were incubated with serially diluted mouse sera, and PE-conjugated goat anti-mouse IgG Fcγ was the secondary Ab (Jackson ImmunoResearch, West Grove, PA). Her-2 mouse mAb TA-1 (EMD Chemicals, Inc, San Diego, CA) was used to generate standard binding curves. The

concentrations of Her-2 IgG in the test sera were calculated by regression analysis (24). Differences in Ab levels were analyzed by 2-tail Student's *t* test. To determine the IgG isotype, SKOV3 cells were incubated with immune sera at 1:150 dilution, followed by FITC-conjugated goat anti-mouse IgG1, IgG2c, or IgG2c (Jackson ImmunoResearch). Flow cytometric analysis was performed with a FACScalibur (BD Biosciences), and the results were expressed as mean channel fluorescence (MCF). Differences in Her-2 Ab isotype levels were analyzed by Student's *t* test.

Measurement of T cell response by IFN- γ and IL-5 ELISpot

All ELISpot reagents were purchased from BD Biosciences, unless otherwise specified. Her-2 reactive T cell levels were enumerated by IFN- γ and IL-5 ELISpot assay as we previously described (17). Briefly, 96-well HTS IP plates (Millipore, Bedford, MA), were pre-coated with 2.5 μ g/ml rat anti-mouse IFN- γ (clone R4-6A2) or rat anti-mouse IL-5 (clone TRFK5). Immune spleen cells (SC) were added to the wells in the presence of TC-1, TC-1/E2, 3T3/KB, or 3T3/EKB at APC:lymphocyte ratio of 1:10. Following 48 h incubation, cells were removed, and captured IFN- γ or IL-5 were detected with biotinylated rat anti-mouse IFN- γ (clone XMG1.2) or rat anti-mouse IL-5 (clone TRFK4), avidin-HRP and the substrate 3-amino-9-ethylcarbazole. The visualized cytokine spots were enumerated with the ImmunoSpot analyzer (CTL, Cleveland, OH), and the results were expressed as number of cytokine-producing cells per 10^6 spleen cells (SC). Data were analyzed using Student's *t* test.

Tumor growth inhibition assay

Her-2 Tg mice were inoculated s.c. in the flank with 2×10^5 EO771/E2 or D2F2/E2 cells at 2 wks after the 4th electrovaccination. Tumor growth was monitored by weekly palpation, and tumor size was measured weekly in two perpendicular dimensions. Mice were sacrificed when any one dimension of the tumor reached 20 mm. Difference in tumor incidence was analyzed by the log-rank test.

Results

Establishment of Her-2 transgenic (Tg) mice in B6, BALB and F1 backgrounds

To test Her-2 vaccination in mice of different genetic backgrounds, Her-2 Tg mice were established in the syngeneic B6 background (18), then back-crossed with BALB/c mice to generate syngeneic BALB Her-2 Tg mice. F1 Her-2 Tg mice were produced by mating BALB Her-2 Tg mice with wild type B6 mice or vice versa. Regardless of their genetic background, all mice should have the same Her-2 transgene insertion. We previously showed by Western blotting and immunohistochemistry the constitutive expression of recombinant Her-2 in the Bergman glia cells within the molecular layer of the cerebellum (18). To verify that recombinant Her-2 is also expressed in BALB and F1 Her-2 Tg mice, cerebella were removed and tissue extract prepared. Recombinant Her-2 in the tissue extract was immunoprecipitated and analyzed by Western blot analysis. Fig. 1A shows the 185 kDa human Her-2 in the cerebella of Her-2 Tg mice in B6, BALB and F1 backgrounds. Tissue extracts from non-transgenic littermates were negative (Fig. 1B). Overt neoplastic transformation was not detected in any of the Her-2 Tg mice (not shown). Therefore, Her-2 Tg mice in B6, BALB and F1 background all expressed recombinant Her-2 as a self-antigen, without inducing neoplastic transformation.

Antibody (Ab) response induced by Her-2 DNA vaccine

Reactivity to Her-2 was tested by electrovaccinating Her-2 Tg mice with pE2TM and pGM-CSF, i.m., 3 times, at two-week intervals. DNA electrovaccination induces humoral and cellular immune responses to the entire repertoire of antigenic epitopes without using confounding exogenous elements and immunized mice reject tumor challenge (2,14,17). Sera

were collected 2 wks after the third vaccination and Ab levels were analyzed by flow cytometry as we previously reported (24). IgG concentrations were calculated by regression analysis. B6, BALB and F1 Her-2 Tg mice produced 4 ± 3 , 35 ± 30 , and 9 ± 13 $\mu\text{g/ml}$ anti-Her-2 IgG, respectively (Fig. 2, open circles), thus BALB Her-2 Tg mice were most responsive. Still, the response level was reduced when compared with wild type BALB/c mice which produce >50 $\mu\text{g/ml}$ Her-2 Ab after 2 vaccinations (17). Removal of $\text{CD4}^+\text{CD25}^{\text{hi}}$ T cells before vaccination amplified Her-2 Ab response to 19 ± 15 , 82 ± 41 , and 55 ± 41 $\mu\text{g/ml}$ in B6, BALB and F1 Her-2 Tg mice, respectively (Fig. 2, filled circles), showing control of Her-2 immunity by Treg as we previously reported (2). Overall, BALB Her-2 Tg mice produced more Her-2 Ab than B6 or F1 Her-2 Tg mice whether CD25^{hi} cells were removed or not. This difference is not due to an intrinsic inability of B6 mice to produce Her-2 Ab as wild type B6 mice produce the same or more Her-2 Ab than wild type BALB/c mice (Figure 6A).

Analysis of Her-2 Ab isotypes showed IgG1 production in all test strains, indicating Th2 activation (Fig. 3). IgG2c was produced by B6 and IgG2a by BALB Her-2 Tg mice, showing concurrent Th1 activation (25) (Fig. 3A and 3B). F1 mice produced both IgG2a and IgG2c, consistent with the inheritance of genetic traits from both parents (Fig. 3C). As with total IgG, BALB Her-2 Tg mice produced the highest level of IgG1 and IgG2a Ab.

Treatment with CD25 mAb before vaccination elevated IgG1 and IgG2c response significantly in B6 Her-2 Tg mice. Similar amplification of IgG1, IgG2a and IgG2c production was also induced in F1 Her-2 Tg mice. In BALB Her-2 Tg mice, however, increased levels were observed in IgG1, but not IgG2a. Overall, Her-2 tolerance was overcome in part by DNA electrovaccination, with BALB Her-2 Tg mice being most responsive to vaccination. Humoral response was amplified by depleting CD25^{hi} T cells before vaccination, with the exception that the level of IgG2a in BALB Her-2 Tg mice remained the same whether CD25^{hi} cells were depleted or not. The disparate Ab response in the most responsive BALB Her-2 Tg mice may indicate the elimination of some Th1 effector T cells or selective amplification of Th2 cells after CD25 mAb treatment.

T cell response induced by Her-2 DNA vaccine

To analyze T cell response, immune spleen cells (SC) from individual mice were prepared 2 wks after the final vaccination. $\text{IFN-}\gamma$ and IL-5 producing T cells were enumerated by ELISPOT to evaluate Th1/Tc1 vs Th2 responses. B6 immune SC were incubated with TC-1/E2 cells which were MHC I^+ , CD80^+ and stably transfected with Her-2 to generate TC-1/E2 (17). BALB/c immune SC were incubated with 3T3/EKB cells which were 3T3 cells stably transfected with Her-2, H2K^{d} and CD80. T cells from F1 mice would respond to either TC-1/E2 or 3T3/EKB.

Vaccinated B6 Her-2 Tg mice showed little to no T cell response (Fig. 4A/B, left panels) as we reported (17), whether or not mice received CD25 mAb, demonstrating their profound T cell tolerance to Her-2. In BALB Her-2 Tg mice, $\text{IFN-}\gamma$ producing T cells were induced at $50\pm49/10^6$ SC, but IL-5 response was not detected (Fig. 4A/B, middle panel). Depletion of CD25^{hi} T cells before vaccination did not change $\text{IFN-}\gamma$ producing T cell response, but IL-5 producing T cells were significantly amplified (Fig. 4B, middle panel). This preferential amplification of Th2 over Th1/Tc1 response is consistent with the increase in IgG1, but not IgG2a. These results may suggest the depletion of activated Th1 or selective enhancement of Th2 cells in BALB Her-2 Tg mice by treatment with 0.25 mg CD25 mAb.

In F1 Her-2 Tg mice, $\text{IFN-}\gamma$ producing T cells were induced sporadically and only after Treg depletion; 1/5 mice responded to TC-1/E2 and 2/5 mice responded to 3T3/EKB (Fig. 4A, right panel). IL-5 producing cells were not induced in any of the test mice, regardless of CD25 mAb treatment or the APC used for stimulation (Fig. 4B, right panel).

Interestingly, BALB Her-2 Tg, but not B6 Her-2 Tg or F1 Her-2 Tg mice, showed low level anti-Her-2 IFN- γ producing T cells ($\sim 20/10^6$ SC; data not shown) after CD25^{hi} T cell depletion, without vaccination, suggesting pre-existence of Her-2 reactive T cells. But combined treatment with CD25 mAb and DNA vaccination may not result in a net gain of IFN- γ producing T cells because these pre-existing reactive T cells may be eliminated by CD25 mAb.

In summary, BALB Her-2 Tg mice were the most responsive to vaccination in both the humoral and cellular compartments. Their Th2, but not Th1/Tc1 response was amplified when CD25^{hi} T cells were removed. B6 Her-2 Tg mice were very tolerant to Her-2 and their T cell response was not detectable whether or not CD25^{hi} T cells were depleted, although both IgG1 and IgG2c were amplified in CD25 mAb treated mice, indicating T cell involvement. To determine if treatment with CD25 mAb resulted in comparable Treg depletion in different mouse strains, supplemental Figure 1 showed the kinetics of depletion and recovery of Foxp3⁺ cells in BALB/c and C57BL/6 mice which were treated with varying doses of CD25 mAb. At 0.25 mg of CD25 mAb, which was the dose used throughout this study, depletion of CD25^{hi}Foxp3⁺ cells was achieved in both mouse strains. Partial recovery of these cells was observed at wk 3 in C57BL/6, but not BALB/c mice. The more sustained depletion of CD25^{hi}Foxp3⁺ cells in mice of BALB/c background may result in some loss of pre-existing effector T cells in BALB Her-2 Tg mice with little gain in the levels of Th1/Tc1 and IgG2a.

Compared to B6 Her-2 Tg mice, F1 Her-2 Tg mice were more responsive. After CD25 mAb treatment, B6 and F1 Her-2 Tg mice produced comparable levels of IgG1 and IgG2c, but F1 mice also produced IgG2a. About 40% of F1 Her-2 Tg mice had detectable IFN- γ T cell response. These results demonstrated the influence of genetic background on immune reactivity to Her-2 vaccine. Treatment with CD25 mAb amplified immune reactivity and favors Th1/Tc1 response in F1 Her-2 Tg mice. In BALB Her-2 Tg mice, treatment with CD25 mAb increased Th2 response without a net gain in Th1 response, the latter may be due to partial loss of activated T cells.

Anti-tumor immunity in Her-2 Tg mice

The efficacy of DNA vaccination on tumor growth inhibition was tested by electrovaccinating mice 4 times with pE2TM and pGM-CSF before s.c. tumor challenge. B6 Her-2 Tg mice were inoculated with 2×10^5 EO771/E2 which was a B6 mammary tumor line stably transfected with Her-2. BALB and F1 Her-2 Tg mice received 2×10^5 D2F2/E2, which was a BALB/c mammary tumor line stably transfected with Her-2.

All control mice which received blank vector, regardless of their strain or challenging tumor cells, developed palpable tumors in 2 wks which grew progressively (Fig. 5A). DNA vaccination was not protective against EO771/E2 tumor in B6 Her-2 Tg mice (Fig. 5A, left panel). Vaccination also failed to protect F1 Her-2 Tg mice from D2F2/E2 tumor (Fig. 5A, right panel). A significant delay of D2F2/E2 tumor growth was observed only in vaccinated BALB Her-2 Tg mice, although 10 of 12 mice eventually succumbed to tumors (Fig. 5A, middle panel). Therefore, anti-tumor immunity was poor in B6 and F1 Her-2 Tg mice, but was significant in BALB Her-2 Tg mice.

In F1 Her-2 Tg mice, vaccination after CD25 mAb treatment induced the most significant increase in protection and 8 of 12 mice (67%, $P < 0.005$) rejected D2F2/E2 tumor challenge (Fig. 5B, right panel). The same treatment showed a modest improvement in B6 Her-2 Tg mice, and 2 of 5 (40%) mice rejected tumor challenge, compared to 0 of 4 mice in vector control group (Fig. 5B, left panel, $P < 0.05$). BALB Her-2 Tg mice, however, showed little gain after CD25 mAb treatment, with 4 of 15 mice rejecting tumor challenge. These results suggested that CD25 mAb depleted Treg in B6 and F1 Her-2 Tg mice to amplify their anti-Her-2 and

anti-tumor immunity. In BALB Her-2 Tg mice, CD25 mAb may deplete both regulatory and effector T cells, resulting in little improvement of vaccine activity.

Immune reactivity to Her-2 vaccine in wild type mice

The difference in Her-2 reactivity may be due to intrinsic differences in BALB and B6 mice. To test this possibility, age-matched wild type B6, BALB and F1 female mice were electrovaccinated twice with pE2TM and pGM-CSF without Treg depletion. B6 mice produced significantly more Her-2 Ab than BALB mice and Ab levels in F1 mice were not significantly different than those in B6 or BALB mice (Fig. 6A). Therefore, when Her-2 was presented as a foreign antigen, B6 mice were equally or more responsive than other mouse strains.

To measure T cell response, SC from individual immunized animals were incubated with the corresponding APCs for 48 hr. IFN- γ producing T cells were detected at $590 \pm 149/10^6$ and $658 \pm 75/10^6$ SC in wild type B6 and BALB/c mice, respectively. IL-5-secreting T cells were at $179 \pm 126/10^6$ and $129 \pm 66/10^6$ SC for B6 and BALB mice, respectively (Fig. 6B and 6C). F1 mice showed 53 ± 63 and $1147 \pm 337/10^6$ SC IFN- γ T cells when stimulated with TC-1/E2 and 3T3/EKB, respectively (Fig. 6B), suggested a preferential activation of H2^d restricted Th1/Tc1 cells in F1 mice. This preferential response to BALB APC was not observed in IL-5 response which may be mediated by APC in the immune spleen cells after they take up Her-2 during the incubation (Fig. 6C).

Overall, wild type BALB and B6 mice showed equivalent T cell response to foreign Her-2 with B6 mice producing higher levels of Ab. The disparate response in B6 and BALB Her-2 Tg mice was not due to intrinsic difference of the mice, but may result from differential thymic selection of Her-2-reactive cells during T cell development.

Discussion

All Her-2 Tg mice, regardless of their genetic background, lived a normal life span and expressed recombinant Her-2 without developing spontaneous tumors. When vaccinated with Her-2 DNA, BALB Her-2 Tg mice were most responsive and B6 Her-2 Tg mice least responsive. When CD25^{hi} T cells were intact before vaccination, significant protection against tumor growth was achieved only in BALB Her-2 Tg mice. To the best of our knowledge, this is the first demonstration of genetic regulation of Her-2 immune reactivity. MHC class II has been strongly associated with susceptibility to autoimmune diseases, i.e. immune reactivity to self antigens. We previously reported that mice expressing human HLA-DR3, which dictates susceptibility to autoimmune thyroiditis, did not show increased reactivity to Her-2 DNA vaccines (2), demonstrating that genetic regulation of Her-2 immunity does not parallel that of thyroid antigens.

We showed previously that Her-2/neu immunity was under the control of CD25^{hi} Treg and was amplified by Treg depletion (2,17,26). In BALB Her-2 Tg, but not B6 or F1 Her-2 Tg mice, low level T cell reactivity to self-Her-2 could be detected by simply removing CD25^{hi} T cells without vaccination, indicating pre-existing anti-Her-2 T cells in these mice (not shown). When 0.25mg CD25 mAb was administered before DNA vaccination, their Th2 response was amplified, but their Th1/Tc1 activity indicated by IgG2a and IFN- γ production did not change. Since CD25 mAb removed Treg as well as activated effector T cells, these results may indicate a loss of pre-activated Th1/Tc1 cells in BALB Her-2 Tg mice when mice were treated with CD25 mAb. Since there was no net gain in tumor rejection, the increase in Th2 response was of little benefit. Therefore, in hosts with endogenous Her-2 reactivity like BALB Her-2 Tg mice, specific measures for Treg depletion is warranted.

In B6 Her-2 Tg mice, immune reactivity to Her-2 was poor and moderate anti-tumor activity was achieved in Treg depleted, vaccinated mice. Although cytokine producing T cells were not detected, Th1 and Th2 mediated production of IgG2c and IgG1, respectively, indicated the activation of T cell which may be further amplified by additional immune modulating regimen.

When vaccinated after CD25 mAb treatment, F1 Her-2 Tg mice showed the most profound increase in tumor growth inhibition, compared to either parental strain. They generated IgG1, IgG2a and IgG2c as well as some IFN- γ producing T cells, but no detectable IL-5 producing cells. Although their T cell response was not as uniform as that in BALB Her-2 Tg mice, the ability to recognize antigenic epitopes presented by both H2^b and H2^d and the broader spectrum of Ab isotypes, combined with Th1/Tc1 activation may contribute to greater tumor rejection. These results may suggest more effective anti-tumor immunity in individuals with broader HLA phenotype and greater Th1/Tc1 activation.

Vaccinated mice live a normal life span without overt toxicity or autoimmunity. However, concurrent exposure to vaccine-independent, self-antigens during Treg depletion or tumor regression can increase their risk of autoimmune diseases to other self-antigens as we reported, and this should be closely monitored (2,14,26).

We previously demonstrated that D2F2/E2 or E0771/E2 which were transfected with Her-2, but not dependent on Her-2 signaling for survival, were controlled primarily by T cells with lesser contribution from Ab (17). Consistent with this finding, activation of T cells in BALB and F1 Her-2 Tg mice was associated with greater tumor rejection, although contribution from innate immunity could not be ruled out.

Since wild type B6, BALB and F1 mice all show excellent reactivity to foreign Her-2 and B6 mice actually produce more Ab than BALB mice, there is no intrinsic deficiency in B6 mice to recognize or respond to Her-2. Different Her-2 immune responses in B6 Her-2 Tg and BALB Her-2 Tg may be the culmination of several factors. B6 and BALB/c Her-2 Tg express MHC H2^b and H2^d, respectively, resulting in the presentation of different Her-2 epitopes and different repertoire of T cells through positive and negative thymic selection. It is well established that MHC class II haplotype is the primary determining factor in autoimmune diseases (1). B6 mice express only one MHC class II allele, IA, whereas BALB/c mice express both IA and IE. The greater diversity of MHC class II alleles in BALB/c versus B6 mice could result in effector or regulatory CD4 T cells that are of different functional strength. Also, B6 and BALB/c mice produce a different spectra of IgG. B6 mice secrete IgG2c, but not IgG2a (Figure 3). BALB/c mice secrete IgG2a, but not IgG2c and F1 mice produce both. Such discrepancy in IgG isotypes may result in different functionality. With F1 mice inheriting the genetic traits from both parents, their response falls in between the two parental strains.

In addition, activation of IL-5 producing Th2 cells was observed only in CD25 mAb treated BALB Her-2 Tg mice, but their IFN- γ producing T cells did not change. BALB/c mice may have pre-existing Th1/Tc1 Her-2 reactivity and the observed results may represent combined effect of removing a portion of Treg as well as pre-existing effectors. Further analysis of genetic regulation is warranted to bring cancer vaccine to fruition.

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Figure 1.

Expression of Her-2 transgene in three Her-2 Tg mouse strains. Cerebella were removed from mice. Tissue extracts were prepared, immunoprecipitated and analyzed by Western blot as described in the Materials and Methods. (A) extracts from Her-2 Tg mice. Lanes 1–4: B6 Her-2 Tg, lanes 5–6: BALB Her-2 Tg, and lanes 7–8: F1 Her-2 Tg. (B) extract from non-transgenic littermates. Lanes 1–3: B6, lanes 4–5: BALB, and lanes 6–8: F1 littermates. Lysate of SKOV3 cells overexpressing Her-2 was the positive control (+). The arrow points to the 185-kDa human Her-2.

Figure 2.

Her-2 antibody response in three Her-2 Tg mouse strains. Her-2 Tg mice were electrovaccinated every 2 wks with pE2TM and pGM-CSF, starting 10 days after they received 0.25 mg CD25 mAb (filled circles) or without mAb treatment (open circles). Sera were collected at 2 wks after the 3rd vaccination, and Her-2 Ab levels were measured. * $P < 0.05$, ** $P < 0.001$ between indicated groups by Student's t test. *** indicates $P < 0.001$ when compared to similarly treated B6 or F1 Her-2 Tg mice.

Figure 3.

Her-2 antibody isotypes in Her-2 Tg mice. Sera were collected from immunized B6 (A), BALB (B), and F1 (C) Her-2 Tg mice as described in Fig. 2 legend. Ab isotypes were determined by flow cytometry using isotype-specific FITC-conjugated secondary Ab and expressed as mean channel fluorescence (MCF). The results were analyzed by Student's *t* test. * $P < 0.001$ compared to mice receiving the same vaccine without CD25 mAb treatment.

Figure 4.

Her-2 T cell response in Her-2 Tg mice. B6, BALB, and F1 Her-2 Tg mice were electrovaccinated 4 times with pE2TM and pGM-CSF with or without 0.25 mg CD25 mAb given 10 days before 1st vaccination. SC of 5–7 mice per group were collected individually, 2 wks after the final vaccination and incubated with Her-2 positive APC TC-1/E2 or 3T3/EKB for 48h. The number of IFN- γ -secreting (A) or IL-5-secreting (B) T cells was measured by ELISpot assay. The number of spots in wells receiving control APC TC-1 or 3T3/EKB were subtracted from the corresponding test groups and the values of these control wells were under 10 spots/10⁶ SC.

Figure 5.

Anti-tumor immunity in Her-2 Tg mice. Her-2 Tg mice were electrovaccinated with (B) or without (A) CD25 mAb treatment as described in Fig. 4 legend. At 2 wks after the final vaccination, B6 Her-2 Tg mice were implanted s.c. with 2×10^5 E0771/E2 cells (left panels). BALB (middle panels) and F1 (right panels) Her-2 Tg mice were implanted s.c. with 2×10^5 D2F2/E2 cells. Tumor growth was monitored by weekly palpations, and difference between the curves was analyzed by log-rank test.

Figure 6.

Immune reactivity to Her-2 in wild type mice. Wild type B6, BALB, and F1 mice ($n=5/\text{group}$) were electrovaccinated twice with pE2TM and pGM-CSF. (A) sera were collected 2 wks after the 2nd vaccination, and Her-2 Ab levels were determined. Splenocytes were collected 2 wks after the 2nd vaccination, and incubated with corresponding APC for 48 hours. The number of IFN- γ -secreting (B) or IL-5-secreting (C) T cells was measured by the ELISPOT assay. There were less than 6 spots/ 10^6 splenocytes after incubation with control APC TC-1 or 3T3/KB and the value was subtracted from the corresponding test groups. Experiment was repeated twice with similar results. * indicates $P < 0.001$ by Student's t test.