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Activity of DNA vaccines encoding self or heterologous Her-2/neu in Her-2 or neu transgenic mice ☆

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Abstract

To assess the efficacy of self versus heterologous ErbB-2 vaccines, the reactivity to human and rat ErbB-2 (Her-2 and neu, respectively) DNA vaccines were tested in normal, Her-2 or neu transgenic mice. When immunized with either Her-2 or neu DNA, normal BALB/c and C57BL/6 mice produced cross-reactive T cells, but only antigen specific antibodies. In Her-2 Tg mice, weak to no anti-Her-2 response was induced by either self Her-2 or heterologous neu DNA, demonstrating profound tolerance to Her-2 and the inability to induce anti-Her-2 immunity with either vaccine. In NeuT mice, vaccination with self neu but not heterologous Her-2 DNA induced anti-neu antibodies and delayed spontaneous tumorigenesis. Both neu and Her-2 DNA induced anti-neu T cell response, but depletion of CD8 T cells did not change the delay in tumorigenesis. Therefore, in NeuT mice, both self and heterologous DNA activated anti-neu T cells, although T cell response did not reach sufficient level to suppress spontaneous tumorigenesis. Rather, induction of anti-neu antibodies by self neu DNA is associated with the delay in spontaneous tumor growth. Overall, NeuT mice were more responsive to DNA vaccination than Her-2 Tg mice and this may be associated with the continuous production of neu by the 10 mammary glands undergoing tumor progression.

Keywords: Tumor vaccine; DNA vaccine; Her-2; Neu; Transgenic mice

1. Introduction

Her-2/neu[1] over-expression, which occurs in about 25% of breast cancers [1], is associated with poor prognosis [2], making Her-2/neu an important target of cancer therapy and active vaccination. Several forms of Her-2/neu based vaccines are under active investigation, such as Her-2 DNA developed in our lab [3,4], T cell reactive peptides [5,6], tumor cells expressing GM-CSF [7] or MHC II transactivator [8], dendritic cells loaded with apoptotic tumor cells [9], etc. Although encouraging results are observed in both experimental and clinical

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[1] *Abbreviations used:* APC, antigen presenting cell; 3T3/EKB, 3T3 cells transfected to express Her-2, K^d and CD80; 3T3/NKB, 3T3 cells transfected to express neu, K^d and CD80; DNA, deoxyribonucleic acid; DMEM, Dulbecco's modified Eagle's medium; E2, human ErbB-2 (Her-2); ELISPOT, enzyme linked immuno spot; FITC, fluorescein isothiocyanate; PE, phycoerythrin; GM-CSF, granulocyte–monocyte colony stimulating factor; Her-2, human ErbB-2; Her-2 Tg, Her-2 transgenic mice; MHC, major histocompatibility complex; neu, rat ErbB-2; NeuT, BALB NeuT mice; PBL, peripheral blood leukocytes; TM, transmembrane domain; TRP, tyrosinase-related protein; WAP, whey acidic protein promoter.

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studies, further improvement of the vaccine regimen is still warranted.

Transgenic mice expressing either human Her-2 [10] or rat neu [11–13] have been established. BALB NeuT mice (NeuT) express a transforming rat neu in their mammary glands and females develop spontaneous mammary tumors around 17 weeks of age [11]. Her-2 transgenic mice (Her-2 Tg) express human Her-2 in the mammary gland and cerebellum under the control of whey acidic protein (WAP) promoter [10]. Unlike NeuT mice, Her-2 Tg mice do not develop spontaneous mammary tumors. Tolerance in Her-2 Tg mice was previously reported by our group [10]. Only 30% of Her-2 Tg mice rejected tumors after five time immunizations with DNA encoding a secreted form of Her-2 together with pGM-CSF, whereas all non-transgenic littermates rejected tumors. The tolerant status and immune reactivity to Her-2/neu vaccine in different model systems have not been directly compared and seemingly conflicting results in the literature can be problematic in designing further studies.

Immunization with cross-reactive microbial antigen has been shown to induce autoreactive antibodies or T cells, resulting in autoimmune diseases [14]. Heterologous antigens are commonly used to induce autoimmune diseases in the animal models. In the murine model of multiple sclerosis, immunization of mice or rats with bovine or guinea pig myelin basic protein leads to demyelination, resembling disease in humans [15,16]. Collagen-induced arthritis, the murine model of rheumatoid arthritis, can be induced by bovine, porcine, or human collagen, generating complement-fixing autoantibodies [17]. Autoantibodies are generated in experimental autoimmune myasthenia gravis following immunization with the *Torpedo californica* acetylcholine receptor antigen [18].

Vaccination with heterologous tumor-associated antigens has been tested by immunizing mice with human melanoma associated antigen gp100. Mice developed T cell response to self gp100 with significant anti-tumor activity [19]. Similarly, immunization with DNA encoding human tyrosinase-related protein (TRP), but not the self protein, induced both antibody and T cell responses to self TRP, leading to protection from B16F10 tumor growth [20].

In this study, we measured Her-2 and neu reactivity to DNA vaccination in normal, Her-2 Tg and NeuT mice. NeuT mice were much more responsive to neu DNA vaccine than Her-2 Tg mice to Her-2 DNA vaccine. When NeuT mice were immunized with self neu, but not heterologous Her-2 DNA, there was a delay in spontaneous tumorigenesis which was independent of CD8 T cells.

2. Materials and methods

2.1. Mice and cell lines

BALB/c and C57BL/6 (6–8 week old) female mice were purchased from Charles River Laboratory (Frederick, MD). Heterozygous NeuT mice, which express a transforming neu under the control of the mouse mammary tumor virus promoter were maintained by breeding with BALB/c mice [11,12]. Heterozygous Her-2 Tg, which express the full-length Her-2 under the whey acidic protein (WAP) promoter were maintained by breeding with C57BL/6 mice [10]. Transgene positive mice were identified by PCR. All animal procedures were performed in accordance with the regulation of Wayne State University, Division of Laboratory Animal Resources, following the protocols approved by the Animal Investigation Committee.

All cell lines were maintained *in vitro* in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% heat-inactivated cosmic calf serum (Hyclone, Logan, UT), 5% heat-inactivated fetal calf serum (Sigma, St. Louis, MO), 10% NCTC 109 medium (Invitrogen, Carlsbad, CA), 2 mM L-glutamine, 0.1 mM MEM non-essential amino acids, 100 units/ml penicillin, and 100 μg/ml streptomycin. All tissue culture reagents were purchased from Invitrogen (Gaithersburg, MD) unless otherwise specified.

D2F2 is a mouse mammary tumor cell line derived from a spontaneous mammary tumor that arose in a BALB/c hyperplastic alveolar nodule line D2 [21]. D2F2 cells were co-transfected with pRSV2/neo and either pCMV/neu, which encodes wild-type rat *neu*, or pCMV/E2, which encodes wild-type Her-2 [3]. Stable clones of D2F2/neu or D2F2/E2 were established and the expression of neu or Her-2 protein on the cell surface was verified by flow cytometry. Transfected cell lines were maintained in medium containing 0.8 mg/ml G418 (Geneticin, Invitrogen). The TUBO line was cloned from a spontaneous mammary tumor in a NeuT female and the cells express neu protein [22]. TUBO cells grow progressively in normal BALB/c mice and give rise to tumors which are histologically similar to those seen in NeuT mice [23].

Antigen presenting cells (APC) 3T3/NKB and 3T3/EKB were generated as previously described [24]. Briefly, BALB/c NIH 3T3 cells were transfected with K^d, B7.1, and Her-2 (EKB) or neu (NKB). Stable clones were selected, and maintained in supplemented DMEM (as above) with the addition of 0.8 mg/ml neomycin and 0.8 mg/ml zeocin. TC-1/E2 and TC-1/neu were generated by transfecting C57BL/6 TC-1 cells (generously provided by Dr. T.C. Wu, The Johns Hopkins University, Baltimore, MD) with pMSCV/puro and pCMV5/neu or pCMV5/E2. Stable clones were selected, and maintained in supplemented DMEM with 7.5 μg/ml puromycin. TC-1 is a tumor cell line derived by transforming lung epithelial cells with human papilloma virus-16 E6, E7 and ras oncogene [25]. Expression of neu or Her-2, K^d or K^b and B7.1 was measured by flow cytometry (Fig. 1E).

2.2. Peptides

Peptide corresponding to K^d restricted, dominant neu epitope, N63 (TYVPANASL) [26] was synthesized by New England Peptide, Inc. (Gardner, MA) and dominant Her-2 epitope, E63 (TYLPTNASL) by Quality Controlled