Award Number: DAMD17-00-1-0380

TITLE: The Use of Venezuelan Equine Encephalitis Replicons Encoding the HER-2/neu Tumor Associated Antigen for the Prevention and Treatment of Breast Cancer

PRINCIPAL INVESTIGATOR: Brian R. Long
Roland M. Tisch

CONTRACTING ORGANIZATION: University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27599-1350

REPORT DATE: May 2004

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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The Use of Venezuelan Equine Encephalitis Replicons Encoding the HER-2/neu Tumor Associated Antigen for the Prevention and Treatment of Breast Cancer

Brian R. Long
Roland M. Tisch

University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27599-1350

E-Mail: blong@med.unc.edu

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

Overexpression and amplification of the Her-2/neu proto-oncogene has been implicated in the development of aggressive human breast cancer. Consequently, Her-2/neu provides a potential target for immunotherapy. Indeed, Her-2/neu specific cytotoxic T lymphocytes (CTL) can be detected in patients with breast and ovarian cancer. Nevertheless, the observed response to Her-2/neu is inadequate to prevent tumor progression. Our overall goal is to determine whether genetic vaccination is a feasible strategy to enhance Her-2/neu specific T cell activity, and in turn prevent and/or treat breast cancer. Using Venezuelan Equine Encephalitis virus replicons encoding Her-2/neu we have been able to generate neu specific cytotoxicity in vaccinated mice. Further, we have demonstrated in vivo efficacy in mice challenged with neu expressing tumor cells, and have noted a delay in the onset of spontaneous adenocarcinoma in vaccinated neu transgenic mice.
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Overview:

Our project has focused developing a vaccination protocol using Venezuelan Equine Encephalitis (VEE) replicons (VRP) encoding the Her-2/neu tumor associated antigen to mediate anti tumor immunity in Her-2/neu transgenic (Tg) mice. Initial work demonstrated that vaccination with the VRP encoding full-length Her2/neu elicited robust Her2/neu-specific cytotoxic T lymphocyte (CTL) reactivity in the non-Tg parental strain (FVB/n) of mice. However, no significant Her2/neu-specific CTL reactivity was detected in cultures prepared from the FVB/neu Tg mice. The results demonstrated that an element of self tolerance to Her2/neu exists in the transgenic mice. We postulated that due to the size of the cDNA encoding full length Her2/neu (greater than 4 kb), levels of in vivo expression were not sufficient to elicit a CD8+ T cell response in FVB/neu Tg mice. Accordingly, a VRP encoding the extracellular and transmembrane domains (VRP EC-TMD) spanning approximately 2 kb was established. More recently, a VRP encoding the intracellular domain (VRP ICD) has also been generated. To compare the in vivo efficacy of these various VRPs to elicit neu specific immunity, FVB/neu Tg mice were immunized with 5.0 x 10^5 IU VRP and challenged with NT2.5 tumor cells, derived from a spontaneously arising tumor in an FVB/neu Tg mouse. These cells express high levels of both MHC class I (H2-K^d) and neu protein on their cell surface (Figure 2A). In this example, treatments with VRPs encoding Her-2/neu were unable to completely prevent engraftment and growth of neu expressing tumor cells (Figure 1). However, an inhibited rate of growth was noted for recipient mice vaccinated with either the full length or EC-TMD VRPs.

As we were encouraged with delayed NT2.5 tumor growth in figure 2, we attempted to enhance in vivo immunity by using a ‘prime-boost’ strategy in which mice are first immunized with plasmid DNA (pDNA) encoding Her2/neu (the prime) and subsequently boosted two weeks later with VRP encoding full length Her2/neu. Mice were then challenged ten days following the boost with 2.0 x 10^6 NT2.5 tumor cells injected subcutaneously into the left flank (Figure 2B). Again, tumor engraftment was not prevented, however there was a delay associated with priming with pDNA encoding neu versus priming with irrelevant pDNA (encoding Influenza HA protein), mice primed and boosted with VRP, or untreated mice. These results demonstrate that administration of plasmid DNA in conjunction with VRP can elicit T cell reactivity sufficient to delay tumor engraftment in FVB/neu Tg mice.

Previously, we had evaluated the efficacy of VRP vaccination to prevent spontaneous tumor development in FVB/neu Tg mice. These mice were immunized at 6 and 7 weeks of age with VRP encoding full length Her-2/neu and monitored for tumor development on a weekly basis. Normally, tumors first begin to appear around 7 months of age (200 days). Here we noticed a delay in the onset of tumor formation in the immunized FVB/neu Tg mice (p = 0.04, log rank) and this data was submitted in last years report. For a larger long-term experiment, we settled on an immunization protocol in which mice were immunized three times at two-month intervals beginning at 8 weeks of age. The therapeutic efficacy of VRPs encoding full length Her-2/neu and the truncated version of the protein (EC-TMD and ICD) were compared to untreated mice and mice treated with an irrelevant control VRP encoding HA (Figure 3). Although several mice treated with the full length neu and EC-TMD encoding VRP did remain tumor free for the duration of the experiment (3 of 14 and 6 of 23 respectively), none of the treatment protocols were able to reliably prevent spontaneous tumor formation in FVB/neu Tg mice. Encouragingly though, treatment with both the full-length neu and EC-TMD encoding VRPs displayed a statistically significant delay in tumor onset (p = 0.0135 and p = 0.0475 by log
rank test respectively). Median time to tumor onset was 279 days for the EC-TMD VRP and 309 days for the full-length neu VRP versus 259 days for untreated mice.

Our plans are to further establish the efficacy of VRP vaccination using a series of in vitro assays to determine the cellular phenotype mediating anti tumor protection in the FVB/neu Tg mice. The relative contribution of CD4\(^+\) Th and CD8\(^+\) CTL will be evaluated. We are currently assessing the in vivo therapeutic efficacy higher titer replicon immunization (5.0 x 10\(^5\) versus 5.0 x 10\(^6\) IU) as well as coimmunization with cytokine encoding VRP. Finally, we plan to assess whether VRP immunization can prevent or inhibit the metastatic progression of transferred tumor to the lung. Spontaneous adenocarcinomas arising in FVB/neu Tg mice have been shown to metastasize to the lung and assay protocols have been developed in which cultured tumor cells are transferred to recipient mice I.V. and tumor engraftment in the lung is evaluated microscopically following india ink inflation of the lung.

**Key Accomplishments:**

- We have completed a tumor challenge experiment demonstrating the in vivo efficacy of using a ‘prime – boost’ strategy. Mice are first vaccinated with plasmid DNA encoding Her-2/neu and subsequently boosted with Her-2/neu encoding VRP, to inhibit the engraftment of transferred neu expressing tumor cells.

- Tumor challenge experiments comparing VRP encoding full length Her-2/neu with VRP encoding truncated versions of the protein have been completed. Two versions of truncated VRP were used. One encoding the extracellular and transmembrane portions (EC-TMD) or just the intracellular domain (ICD) of Her-2/neu.

- A large long term experiment to evaluate the efficacy of VRP encoding Her-2/neu to prevent the spontaneous development of breast adenocarcinoma in FVB/neu Tg mice has been completed. These results demonstrate VRP encoding Her-2/neu, either the full length protein, or a truncated version encoding only the extracellular and transmembrane domains, can delay the onset of spontaneous mammary adenocarcinoma.

**Reportable outcomes:**

**Research Awards and Fellowships:**

**Source of support:** The Graduate School of the University of North Carolina

*Dissertation Completion Fellowship*

**Title:** Genetic Vaccination Using Venezuelan Equine Encephalitis Replicons Encoding Her-2/neu for the Prevention and Treatment of Breast Cancer

**Period of Support:** August, 2003 to April, 2004
Oral Communication and Poster Presentation

Brian Long, Randall Friedline, Thi Bui and Roland Tisch.

A manuscript describing out results is currently in preparation.

Figures and Tables

Figure 1. Comparison of tumor growth in VRP immunized mice. FVB/neu Tg mice were immunized I.P. twice at 14-day intervals with 5.0 x 10^5 I.U. VRP. 10 Days later, mice received 2.0 x 10^6 NT2.5 tumor cells subcutaneously in the left flank. Challenged mice were monitored for tumor growth every 2-3 days and the major and minor diameters of palpable tumors measured using a Vernier type caliper. Tumor volume was calculated as [(minor diameter)^2 X (major diameter/2)].
Figure 2. Tumor challenge assay following prime-boost immunization. A. Cultured NT2.5 tumor cells expressed high levels of both MHC Class I (H2-K^d) and Her-2/neu prior to transfer. B. FVB/neu Tg mice initially immunized with plasmid DNA encoding Her-2/neu and subsequently boosted with full length Neu VRP (Neu FL VRP) displayed delayed tumor growth compared with untreated control mice, mice primed with an HA encoding plasmid and mice primed and boosted with Neu FL VRP. Mice were immunized with 50ng plasmid DNA (JW4303) encoding full length Her-2/neu or Influenza HA protein in each hind leg. Mice were boosted with 5.0 x 10^7 IU VRP injected i.p. 2 weeks later. 10 Days following the last immunization, 2.0 x 10^6 NT2.5 tumor cells were transferred subcutaneously into the left flank of recipient mice. Tumor growth was monitored every 2-3 days and the major and minor diameters of palpable tumors were measured using a Vernier type caliper. Tumor volume was calculated as [(minor diameter)^2 X (major diameter/2)].
Figure 3. Spontaneous tumor development in VRP immunized FVB/neu Tg mice. Treated mice were immunized 3X with 5.0 x 105 IU of indicated VRP at 2, 4 and 6 months of age. Mice were examined weekly for the presence of palpable breast adenocarcinoma. While none of the treatments was sufficient to reliably prevent tumor development in recipient mice, a significant delay in onset was noted in mice immunized with VRP encoding either full length Her-2/neu (FL Neu, p = 0.01) or encoding only the extracellular and transmembrane portions of the protein (Neu EC-TMD, p < 0.05).

Summary

We have completed much of the work described for Specific Aim 1 in which we set out to establish and characterize VRP vaccination to induce Her-2/neu specific CD4+ Th1 and CD8+ CTL responses. We have completed all in vitro testing and packaging of replicons including two truncated Her-2/neu replicons encoding the extracellular and transmembrane domains, and encoding the intracellular domain of the protein.

For the second specific aim, we wished to determine the efficacy VRP vaccination for the prevention and/or treatment of mammary adenocarcinoma in FVB/neu Tg mice. We have made progress in evaluating the therapeutic efficacy of VRP toward preventing the progression of adenocarcinoma in mice challenged with NT2.5 tumor cells. Specifically, we have demonstrated delayed onset and inhibited growth in challenged mice immunized with the truncated VRP-EC/TMD. We have evaluated the efficacy of Her-2/neu encoding VRP in preventing the spontaneous development of mammary adenocarcinoma in FVB/neu Tg mice. These results demonstrate mice vaccinated with either VRP encoding full length neu or neu EC-TMD display a delay in the development of spontaneous mammary adenocarcinoma as compared to untreated control mice.

Comparative analyses are ongoing to determine the magnitude of CD4+ and CD8+ T cell reactivity induced in FVB/neu Tg mice following vaccination with VRP-EC/TMD plus/minus cytokine encoding VRPs. Depending on their adjuvant effect, VRP encoding the appropriate cytokine will be co-administered with VRP-EC/TMD and the efficacy of this regime to suppress the progression of established tumors in the challenge model will be investigated. Studies will continue to evaluate the immunotherapeutic efficacy of VRP-EC/TMD in the prevention of spontaneous tumor formation in FVB/neu Tg mice.

References
