Award Number: DAMD17-98-1-8225

TITLE: The Use of Her2/neu-specific Genetic Vaccines for the Prevention and Treatment of Breast Cancer

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REPORT DATE: July 2000

TYPE OF REPORT: Annual

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 Her2/neu-specific Genetic Vaccines for the Prevention and Treatment of Breast Cancer

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13. ABSTRACT (Maximum 200 Words)
We are investigating the feasibility of using HER2/neu encoding genetic vaccines to induce potent CD4+ and CD8+ T cell reactivity for the prevention and treatment of breast cancer. Two strategies of genetic vaccination, each with distinct advantages, will be assessed. The first strategy entails the use of alphavirus Venezuelan equine encephalitis virus (VEE)-replicons which selectively infect dendritic cells in vivo. Dendritic cells are characterized by a highly potent capacity to activate naïve T cells. Our second strategy is to employ a plasmid DNA (pDNA) vaccine encoding HER2/neu. pDNA vaccination has been shown to be effective in eliciting persistent T cell reactivity in various experimental model systems. VEE-replicon and pDNA vaccines encoding cytokines such as IL-12 and IL-18, will also be used to enhance anti-HER2/neu-specific CD4+ Th1 cell and CD8+ CTL activity.

To directly assess the efficacy of our approach to prevent tumor cell growth and/or eradicate established tumors, mice transgenic for a rat HER2/neu gene will be employed. These mice develop mammary tumors and pulmonary metastatic lesions. Furthermore, we will use mice transgenic for HLA-A2.1 to investigate CD8+ CTL responses to HER2/neu-specific peptide epitopes which have been proposed as targets for immunotherapy in a clinical setting.

14. SUBJECT TERMS
Breast Cancer

17. SECURITY CLASSIFICATION OF REPORT
Unclassified

18. SECURITY CLASSIFICATION OF THIS PAGE
Unclassified

19. SECURITY CLASSIFICATION OF ABSTRACT
Unclassified

20. LIMITATION OF ABSTRACT
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INTRODUCTION.

The long-term objective of this proposal is to establish a safe, effective, and persistent form of tumor antigen-specific immunotherapy for the prevention and treatment of breast cancer. Specifically, we are investigating the efficacy of tumor antigen encoding genetic vaccines to induce CD4+ Th cell and CD8+ CTL reactivity. We believe that recruitment of both CD4+ and CD8+ T cells is necessary if not essential, to mediate potent and long-term immunity to tumor antigens, which typically are weak immunogens. Our model antigen is HER2/neu, an oncoprotein which is overexpressed in 30% of all breast and ovarian cancers. Two strategies of HER2/neu-specific genetic vaccination are being investigated, namely VEE-replicon- and pDNA-based vaccines. Each strategy will be assessed separately, however, we hypothesize that a combination of VEE-replicon and pDNA vaccination will prove to be the most effective form of immunotherapy necessary for prevention and eradication of established tumors. The rationale is to complement the strengths and weaknesses associated with each strategy. For example, administration of a VEE-replicon provides a unique approach to selectively infect dendritic cells in vivo, and in turn initiate potent T cell immunity. However, expression mediated by a VEE-replicon is only transient. On the other hand, the stability and long-term expression of an immunogen encoded by pDNA typically results in persistent T cell and antibody immunity. Yet, it is unlikely that the magnitude of the T cell response elicited via pDNA vaccination will be sufficient to eradicate established tumors. We predict that these two strategies together will elicit a robust and persistent T cell response. In a further attempt to enhance immunotherapeutic efficacy, VEE-replicon and pDNA vaccines encoding cytokines such as IL-12 and IL-18 known to promote CD4+ Th1 and CD8+ CTL reactivity, will be employed. To directly determine the immunotherapeutic efficacy of the treatment regimes, we have established a unique model system incorporating mice transgenic for HER2/neu and HLA-A2.1. In this way, we can: i) critically assess the effectiveness of targeting immunity to a self antigen, ii) directly determine the efficacy of our treatment regimes to prevent tumor progression and eradicate established tumors, and iii) analyze CTL reactivity to HER2/neu-specific epitopes which may have direct application for immunotherapy. In summary, this proposed work should provide the necessary insight to establish a rational, effective, and safe form of HER2/neu-specific genetic vaccination for the long-term prevention and treatment of breast cancer. In addition, this work will generate therapeutic reagents which if effective, can be directly applied to a clinical setting.

BODY.

Specific Aim 1. Determine the optimal conditions for pDNA and VEE-based replicon vaccination to induce HER2/neu-specific CD4+ Th cell and CD8+ CTL reactivity.


As reported previously, we have established pDNA and VEE-replicons encoding rat HER2/neu and shown that the respective recombinants are functional in terms of protein expression.

We have established a panel of eight constructs encoding recombinant rat HER2/neu which span the entire length of the protein. The recombinant protein is required to detect HER2/neu-specific CD4+ T cell responses following genetic vaccination. An E.coli based expression system employing the pTRCHIS vector was utilized. Of the eight constructs, six were shown via SDS-PAGE analysis to express protein consisting of approximately 220 amino acids of the rat HER2/neu molecule. Importantly, when FVB or FVB/neu mice were immunized with the recombinant proteins, CD4+ T cell proliferation was detected in vitro in response to the corresponding fragments. These results demonstrate that the fragments are indeed immunogenic.
Task 2. Complete construction and testing of pDNA and VEE-replicon recombinants expressing IL-12 and IL-18.

As detailed in the previous progress report, we have established and detected protein expression for pDNAs encoding IL-12, IL-18 or both, and VEE-replicons encoding IL-12 or IL-18. We have since established and detected protein expression for a VEE-replicon encoding both IL-12 and IL-18.

We are currently establishing additional pDNA and VEE-replicon recombinants encoding murine IL-2, IFNγ, GM-CSF, and soluble FLT-3 ligand in a further attempt to increase the immunogenicity of the HER2/neu recombinants. IL-2 and IFNγ are expected to enhance Th1 cell development and expansion, whereas GM-CSF and FLT-3 ligand should promote proliferation of dendritic cell precursors and subsequent differentiation (1).

Task 3. Characterize and optimize CD4+ Th cell and CD8+ CTL reactivity in FVB/A2.1/Kb mice following treatment with HER2/neu- and cytokine-specific pDNA and VEE-replicon encoding recombinants.

As described in the previous progress report, we have demonstrated that a HER2/neu-specific response can be induced in FVB/A2.1/Kb and FVB/neu transgenic mice following pDNA-neu immunization. Experiments have been carried out comparing HER2/neu-specific CD8+ CTL reactivity in FVB/A2.1/Kb mice immunized with pDNA-neu plus/minus pDNA-IL-12/18. To date, no significant enhancement in the CD8+ CTL response has been observed upon co-immunization with equal amounts (i.e. 100 μg) of pDNA-IL12/18. Accordingly, the amounts of pDNA-IL-12/18 used for immunization are being varied to determine whether “optimal” conditions can be achieved to increase CD8+ CTL reactivity. Nevertheless, it is possible that HER/neu-specific CD4+ Th1 reactivity is selectively enhanced using current conditions of pDNA-neu and pDNA-IL-12/18 co-immunization. With the establishment of HER/neu recombinant fragments (Task 1), the nature and magnitude of the CD4+ Th cell response following immunization with pDNA-neu plus/minus pDNA-IL-12/18 is currently being assessed.

HER2/neu-specific CD8+ CTL reactivity can also be elicited in mice immunized with the VEE-replicon encoding HER2/neu (pVR2-neu). FVB/A2.1/Kb or FVB/neu mice received two immunizations of 2x10^6 infectious units (IU) of pVR2-neu, and 14 days after the final footpad injection CTL assays were established. As demonstrated in Figures 1 and 2, significant HER2/neu-specific CD8+ CTL reactivity restricted to endogenous H-2b was detected. Importantly, the finding that CD8+ CTL reactivity is detected in FVB/neu mice provides further evidence that self tolerance to HER2/neu in the FVB/neu is incomplete. Experiments are currently underway to investigate the type and magnitude of HER2/neu-specific CD4+ and CD8+ T cell reactivity in FVB/A2.1/Kb and FVB/neu transgenic mice following co-immunization with the VEE-replicons encoding HER2/neu plus/minus IL-12, IL-18 or both. Once available, VEE-replicons (and pDNAs) encoding IL-2, IFNγ, GM-CSF and soluble FLT3-ligand will also be included to assess their effect on pVR2-neu (and pDNA-neu) elicited T cell reactivity.

Specific Aim 2. Determine the efficacy of HER2/neu-specific pDNA and VEE-replicon vaccination to prevent and eradicate breast tumor cell growth in HER2/neu transgenic mice.

Task 1. Determine efficacy of VEE-replicon and pDNA administration to prevent tumor progression FVB/neu x A2.1/Kb mice.

In parallel with work being carried out in Specific Aim 1, experiments are underway in which groups of five FVB/neu x A2.1/Kb mice have been immunized at four weeks of age with pDNA-neu or pVR2-neu plus/minus the IL-12/IL-18 encoding recombinants. The animals are being monitored for mammary gland tumor progression. The age of the treatment groups is too young (12-18 weeks old) at this time to determine the immunotherapeutic efficacy of the vaccines.

Task 2. Determine efficacy of VEE-replicon and pDNA administration to eradicate established tumor foci in the mammary glands and pulmonary metastatic lesions in FVB/neu x A2.1/Kb mice.
Depending on the results we obtain in Task 1 of this Specific Aim in addition those obtained in Specific Aim 1, FVB/neu x A2.1/K^b mice exhibiting mammary gland and pulmonary tumor lesions will be treated under the appropriate conditions with the pDNA and VEE-replicon vaccines and assessed for inhibition or regression of tumor mass.

**KEY RESEARCH ACCOMPLISHMENTS.**
- Establishment and purification of overlapping HER2/neu recombinant protein fragments which elicit CD4^+ T cell reactivity upon immunization.
- Demonstration that significant CD8^+ CTL reactivity can be elicited in FVB/A2.1/K^b and FVB/neu transgenic mice immunized with the VEE-replicon encoding HER/neu.
- Demonstration that self tolerance to HER2/neu is incomplete in FVB/neu mice.

**REPORTABLE OUTCOMES.**
Currently not applicable.

**CONCLUSIONS.**
We have established that administration of either pDNA-neu and pVR2-neu to FVB/A2.1/K^b or FVB/neu transgenic mice can elicit significant CD8^+ CTL reactivity. Under current conditions being employed no significant effect on CD8^+ CTL reactivity was observed between animals immunized with pDNA-neu alone versus co-immunization with pDNAs encoding HER2/neu plus IL-12 and IL-18. This finding does not, however, rule out the possibility that CD4^+ Th1 reactivity may in fact be increased thereby enhancing the overall immunotherapeutic efficacy of the strategy.

**REFERENCES.**
APPENDICES.

Figure 1. Immunization with pVR2-neu elicits a CD8+ CTL response in FVB/A2.1/Kb mice. Three FVB/A2.1/Kb mice were immunized twice with pVR2-neu (2x10^6 IU). Cultures were established from individual mice, and H-2^k restricted CD8^+ CTL effector function determined by varying the effector to target (E:T) ratio and measuring percent specific killing via ^51^Cr release of target cells consisting of either control NIH3T3 or NIH3T3 transfectants expressing HER2/neu (NIH3T3-neu).

Figure 2. Immunization with pVR2-neu elicits a CD8^+ CTL response in FVB-neu mice. Three FVB-neu mice were immunized twice with pVR2-neu (2x10^6 IU). Cultures were established from individual mice, and H-2^k restricted CD8^+ CTL effector function determined by varying the E:T ratio and measuring percent specific killing via ^51^Cr release of control NIH3T3 or NIH3T3-neu target cells.