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

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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 naive 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 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.
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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.
Task 2. Complete construction and testing of pDNA and VEE-replicon recombinants expressing IL-12 and IL-18.

As reported previously, we have established pDNA and VEE-replicons encoding IL-12 and IL-18 and shown that the respective recombinants are functional.

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. To date, under various conditions co-immunization of FVB/A2.1/Kb mice with pDNA-neu and pDNA-IL-12/18 has failed to enhance HER2/neu-specific CD8+ or CD4+ T cell reactivity relative to treatment with pDNA-neu alone. We are currently assessing the potential adjuvant effect of pDNAs encoding IL-2, IFNγ, GM-CSF and soluble FLT3-ligand. Various reports in different model systems have demonstrated that these cytokines can enhance CD8+ CTL and CD4+ Th1 cell reactivity (1-4).

As described in the previous report, HER2/neu-specific CD8+ CTL reactivity can also be elicited in FVB/neu or FVB/A2.1/Kb mice immunized with the VEE-replicon encoding HER2/neu (VRP-neu). We have found that the magnitude of the CD8+ CTL response induced in either FVB/A2.1/Kb or FVB/neu mice is greater than that elicited following pDNA-neu treatment. This may reflect the preferential infection of dendritic cells in vivo by VEE-replicons. Similar to our pDNA experiments, co-immunization with VRP-neu and VRP-IL12/IL-18 had no significant enhancing effect on HER2/neu-specific T cell reactivity versus immunization with VRP-neu alone in FVB/A2.1/Kb or FVB/neu mice. As above, we are currently determining whether co-immunization with VEE replicons encoding IL-2, IFNγ, GM-CSF and soluble FLT3-ligand can enhance HER2/neu-specific T cell reactivity.

As a further attempt to enhance the immunogenicity of the HER2/neu encoding recombinants, we have established pDNA and VEE-replicon constructs encoding the extracellular domain of HER2/neu (pDNA-exdneu, VRP-exdneu). Our rational is that the level of expression of full length HER2/neu may be limiting since it’s size is large in terms of mRNA transcript (~4.0 kilobases) and protein (~185 kilodaltons). The HER2/neu extracellular domain which contains relevant CD8+ CTL epitopes (5) is approximately half the size of full length HER2/neu with regard to mRNA and protein. Consequently, in vivo expression and the relative immunogenicity of these recombinants may be increased. Experiments are underway in which FVB/neu transgenic mice have been immunized with either pDNA-exdneu or VRP-exdneu versus pDNA-neu or VRP-neu in combination with the respective recombinants encoding IL-2, IFNγ, GM-CSF and soluble FLT3-ligand.

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.
We immunized groups of 5 FVB/neu x A2.1/K^b female mice at four weeks of age with pDNA-neu or VRP-neu plus/minus the IL-12/IL-18 encoding recombinants. No significant difference in mammary tumor onset was detected between control and the respective treatment groups. Noteworthy, these animals received a single round of immunizations with the respective recombinants. Specifically, mice received either weekly injections of pDNA over 4 weeks or 2 injections of VEE replicons over 2 weeks. To induce long-term protection it may in fact be necessary to immunize animals on a continuous basis i.e. single monthly injections. Alternatively, the use of pDNA and VEE replicons encoding extracellular HER2/neu coupled with the extended panel of cytokines may prove to be more effective. These possibilities are currently being further investigated.

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/K^b mice.**

We have carried out short-term experiments employing a HER2/neu expressing tumor cell line (F-H2N) established in the laboratory from a mammary tumor excised from FVB/neu mice. Upon adoptive transfer, F-H2N cells are tumorigenic in FVB/neu mice. Interestingly, reduced F-H2N tumor progression and limited vascularization of the tumors was observed in FVB/neu mice immunized with VRP-neu (independent of VRP-IL12/IL18 co-immunization) relative to animals treated with VRP encoding an irrelevant antigen. These results suggest that under the conditions employed, an immunotherapeutic effect can be elicited. However, as discussed above, we feel that the immunogenicity of the recombinants can be enhanced which should translate into increased immunotherapeutic efficacy. We are currently assessing the immunotherapeutic efficacy of recombinants encoding extracellular HER2/neu and the extended panel of cytokines in the adoptive transfer model.

**KEY RESEARCH ACCOMPLISHMENTS.**

- Demonstration that VEE replicon immunization elicits enhanced HER2/neu-specific T cell reactivity relative to pDNA vaccination.

- Demonstration that pDNA and VEE-replicon encoded IL-12 and IL-18 fails to enhance HER2/neu-specific T cell reactivity.

- Establishment of second generation pDNA and VEE-replicon recombinants encoding: 1) the extracellular domain of HER2/neu, and 2) cytokines (IL-2, IFNγ, GM-CSF and soluble FLT3-ligand).

**REPORTABLE OUTCOMES.**
Currently not applicable.
CONCLUSIONS.
Our results suggest that the current conditions of pDNA and VEE-replicon administration are less than optimal to elicit sufficient T cell reactivity to prevent mammary tumor progression in FVB/neu x A2.1/Kb female mice. Nevertheless, in a tumor cell adoptive transfer model, VRP-neu vaccination was found to elicit modest protection. Our current efforts are focused on enhancing the overall immunogenicity of the genetic vaccines. This entails the use of pDNA and VEE-replicon recombinants encoding the extracellular domain of HER2/neu, in addition to an extended panel of cytokines. Our hope is that with this panel of “second generation” recombinants coupled with a modified immunization schedule, HER2/neu-specific T cell reactivity and immunotherapeutic efficacy can be enhanced.

REFERENCES.