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# Abstract

HER-2/*neu* (neu) transgenic mouse model (neu-N mice) overexpress neu in a mammary-specific fashion and develop spontaneous focal neu-expressing tumors. The neu-N mouse model is among the most informative pre-clinical models available for the development of immunotherapeutic strategies for breast cancer treatment and prevention, and insights gained through the use of neu-N mice can be readily applied to clinical trial development. The goal of the present work is to test the hypothesis that reproductive hormones can modulate immunity to breast cancer antigens in neu-N mice. We will explore this hypothesis by assessing vaccine-induced immunity a specific stages of the mouse estrous cycle, as well as during mammary gland involution. Results from these studies will lay the foundation for more detailed investigation of the mechanisms by which hormonal changes during the reproductive cycle modulate our ability to induce immunity to breast cancer antigens, and to identify the optimal stage of the reproductive cycle for vaccination. Due to a serious problem that we detected in our neu-N mouse breeding colony, we were forced to invalidate all of our early data in these studies and seek a no-cost extension of this project, which was approved. We have now re-established the neu-N mouse colony and will begin these studies anew when the first shipment of mice arrives.

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**Introduction**

While a role for reproductive hormones in breast cancer risk has been an area of intense study, interest in their effects on breast cancer treatment outcomes is only just emerging. Indeed, hormonal influences may underlie the observation that the phase of the menstrual cycle at the time of breast cancer surgery may influence survival (1, 2). In addition to their effects on tumor cell growth and metastasis, reproductive hormones may also influence our ability to induce breast cancer-specific immunity. Reproductive hormones are known to regulate the function of lymphocytes, and can exacerbate autoimmunity in women (3). Although there has been growing interest in the effects of reproductive hormones on autoimmunity, there are currently no data evaluating their influence on breast cancer vaccine efficacy. Our lab is interested in vaccine-mediated immunity against HER-2/neu (neu) in neu transgenic (neu-N) mice, a pre-clinical model for breast cancer vaccine development. We have observed that neu-specific immunity is poorly induced after vaccination in neu-N mice, similar to what is seen in patients with neu-expressing breast cancer. Intriguingly, a small proportion of vaccinated neu-N mice respond vigorously and can reject their tumors. Among a large cohort of neu-N mice given neu-targeted vaccination, we found that animals that responded vigorously were housed together. After controlling for a number of variables, and considering that mice housed within a cage tend to synchronize their estrous cycles, we suspect that the estrous stage at vaccination may influence the animal's ability to mount a potent antitumor immune response.

**Body**

In order to test the hypothesis that reproductive hormones can modulate immunity to breast cancer antigens, we proposed the following series of experiments:

*1) Do hormonal changes during estrous in the mouse affect the ability to induce immunity against a foreign antigen?* First, we proposed to characterize the generation of immunity to the influenza nuclear protein (NP) in neu-N mice as a function of the stage of estrous. We proposed to use ELISA to monitor cytokine production by $CD4^+$ T cells in response to NP (to evaluate skewing of the T-helper response), and quantify the development of NP-specific $CD8^+$ T cells (4, 5). These studies will allow us to determine whether there is global immunosuppression and/or enhancement at different points during estrous.

*2) Is the response to a tumor rejection antigen affected by hormone-associated structural changes within the mammary epithelium?* In neu-N mice, neu is expressed in both normal mammary cells and mammary tumors. Apoptosis within the mammary epithelium, induced during estrous and mammary gland involution after weaning, may release large amounts of neu protein. Protein released in this way (i.e. in the absence of pro-inflammatory cytokines) would be expected to diminish the response to vaccine. We will evaluate neu-specific T cell responses in the mammary-draining lymph nodes and the spleen (4, 5) during estrous in virgin neu-N females, and after weaning of pups from neu-N breeder females. Estrous stage will be determined by vaginal cytology. Apoptosis and neu expression within mammary gland whole-mounts will be assessed by immunohistochemistry.

HER-2/*neu* (neu) transgenic mice (neu-N mice) overexpress neu in a mammary-specific fashion and develop spontaneous focal neu-expressing tumors. Tumors that arise in neu-N mice bear a striking resemblance to human breast cancer both developmentally and histologically. Importantly, these mice demonstrate immune tolerance to the neu-expressing mammary tumors that is similar to the immune tolerance observed in patients with neu-expressing cancers. Collectively, these attributes make the neu-N mouse model among the most informative pre-clinical models available for the development of immunotherapeutic strategies for breast cancer treatment and prevention. Unlike many other model systems, insights gained through the use of neu-N mice can be readily applied to clinical trial development.

In a series of experiments unrelated to this proposal, we recently developed evidence demonstrating that the modulation of T cell immunity to neu in neu-N mice, where immune tolerance mechanisms limit vaccine-induced immunity to neu, has a much greater effect than that measured for the same immune-modulatory mechanism on the induction of immunity against NP, which is perceived as a foreign antigen in neu-N mice. These data suggest that measuring the effects of estrous stage on the induction of immunity to neu may be more informative than determining the effects of estrous stage on NP-specific immune induction, as we had originally proposed. Therefore, in our initial experiments we first used vaginal cytology to successfully determine the estrous stage of individual neu-N mice. Next, the mice were vaccinated against either the exogenous NP antigen or the mammary-expressed neu antigen. Two weeks following vaccination the mice were euthanized and $CD8^+$ T cell responses to NP and neu were quantified using intracellular cytokine staining (ICS). As anticipated, immune responses to the exogenous NP antigen were strongly induced in all animals. However, we also measured strong T cell responses to neu in the majority of animals, regardless of estrous stage. The measurement of a significant immune response to neu-targeted vaccination in a majority of neu-N mice is in stark contrast to a great deal of previous data from our lab. This caused us to question the genotype of the neu-N transgenic mice used in these studies, as non-transgenic FVB mice (the parental strain on which the neu-N mice were founded) typically mount potent T cell responses to neu-targeted vaccination.

Over the course of several months, we determined that a non-transgenic mouse had been introduced into our neu-N transgenic mouse colony. During that time our colony had been housed and maintained for us by a commercial organization, and it is not clear how a non-neu-N mouse was introduced in the breeding colony. The problem was not detected during our quarterly genotyping of the colony, however we subsequently determined that a significant proportion of the colony had reverted to a neu-negative genotype. Because the use of the neu-N model is critical to this research project, and because we could not be certain of the genotype of the neu-N mice we had utilized for our experiments, we were forced to invalidate all the data obtained with the questionable mice. We then set about re-establishing a homozygous breeding colony for the production of neu-N mice for our experimental requirements. We were forced to request a No-cost extension of this project to allow us to complete the experiments now that the neu-N mouse colony has been re-established. The no-cost extension was granted. We will receive our first shipment of experimental neu-N mice during the first week of August, and will start the project anew at that time.

## Key Research Accomplishments / Reportable Outcomes

Due to the problems with our transgenic mouse colony, we have no reportable outcomes at present.

## Conclusions

We can draw no conclusions regarding the immunomodulatory role of reproductive hormones at present.

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## Appendices

None