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FOREWORD

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Dr. Jennifer Czarneski 06/29/00

PI - Signature Date

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Progress Report: Year Two of Three

Mammary Specific Expression of Cre Recombinase Under the Control of an Endogenous MMTV LTR: A Conditional Knock-Out System

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Introduction

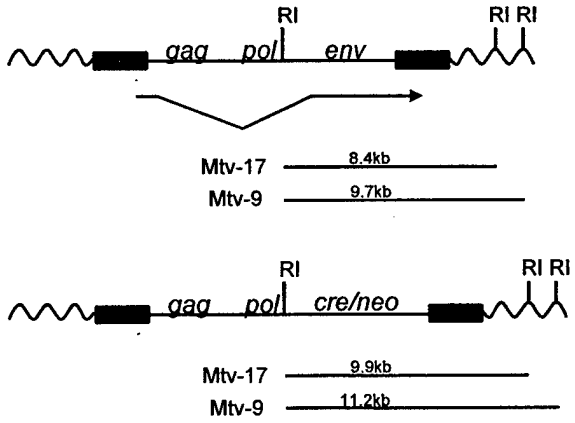
The proposed project was to test the expression of Cre recombinase directed by the Mtv-17 locus is in its second of three years of funding. The hypothesis of the project was to develop a novel breast cancer model using the tissue-specific expression of the Mtv-17 locus, which was previously shown by this lab to only be expressed in the mammary gland. We proposed knocking-in the Cre recombinase gene at the Mtv-17 env region, so that this enzyme would only be expressed in the mammary gland. Mice that carried the Mtv-17 Cre fusion vector would then be mated to mice that had the p53 locus flanked by loxP sites, resulting in the tissue-specific loss of p53 in the mammary gland only. The prediction is that these animals would only develop mammary and not other tumors. We believed that the cre-transgenic mice would also prove useful for other investigators in the mammary gland development and tumorigenesis field.

During the past year, we concentrated on getting an ES cell line that had the Cre-recombinase gene targeted to the Mtv-17 locus. Our efforts in this aspect of the project are described in the first part of the report. In the second part of the report, we describe recent progress in a new area that was not described in the original proposal, which involves understanding the trafficking of lymphocytes to mammary gland and mammary tumors. This second part of the proposal takes advantage of some vectors that were constructed by the original P.I. on this grant, Dr. Reb Russell. These studies will also utilize the mice with targeted expression of cre recombinase in their mammary gland (see below).

Targeting the Cre recombinase gene to the Mtv-17 locus

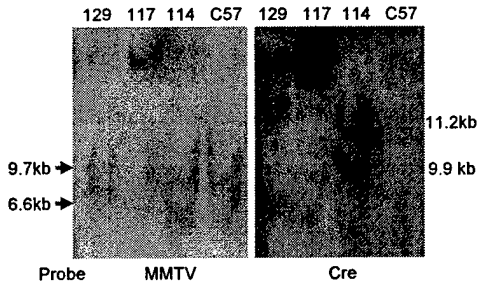
In the previous year, a vector was constructed which placed the cre recombinase gene in the envelope gene of *Mtv*-17. This construct was electroporated into C57Bl/6 embryonic stem cells and these were selected for neomycin resistance. The clones were first screened by polymerase chain reaction using one primer in virus flanking sequences not present in the targeting clone and the other primer in the neomycin resistance gene (Fig. 1). Four clones were positive by this assay. These clones were

Fig. 1



then expanded and DNA was isolated from each of them. This DNA was digested with EcoRI and probed with either probe specific for MMTV or for the Cre recombinase gene. One of the four clones, #114, has the correct band of about 10kb, that hybridizes both with the MMTV and cre probes (Fig. 2). This clone also has an additional band of about 11kb. We are currently using clone #114 to create chimeras. Once mice with germline integrations of the knockout are made, we will separate the 2 integration sites by genetic crossing. The mice will then be crossed with lox P-β-gal reporter mice (a gift from David Anderson) to test whether the cre recombinase is expressed and causes

Fig. 2



recombination in mammary gland (*Mtv*-17 insertion).

Lymphocyte Trafficking to the Mammary Gland

Although it is now well established that mammary gland is part of mucosal-associated lymphoid tissue (MALT), little is known about how lymphoid cells home to this tissue or to mammary tumors. Similar to gut associated lymphoid tissue (GALT), there is a much larger percentage of γδ+ T cells than are found in peripheral lymphoid sites (10% in mammary gland MALT and GALT vs. 1% at other sites). In addition, during lactation mammary gland is a major site for the production of immunoglobulin A, also produced by B cells in GALT. Lymphocytes emigrate from the vasculature to sites within tissues by use of cell surface adhesion molecules. These adhesion molecules interact with specific cell surface molecules expressed on the target tissue. Two members of the integrin family of adhesion molecules have been implicated in the migration of naive and mature B and T lymphocytes to the GALT. One member, α4β7, mediates the adherence of lymphocytes to the high endothelial venules of PP, while another member, αEβ7, is responsible for adherence of intra-epithelial lymphocytes to the intestinal epithelium. The major vascular addressin for α4β7 is MadCAM-1, which is highly expressed in GALT, while E-cadherin is thought to be responsible for binding to αEβ7-bearing lymphocytes. The mucosal vascular addressin, MadCAM-1, is expressed in mammary gland endothelial tissue while E-cadherin is expressed on a wide variety of epithelial cells, including mammary tissue.

Many viruses are acquired through gut mucosal tissue and it is now well established that a number of retroviruses, including mouse mammary tumor virus (MMTV), feline

and human immunodeficiency virus (FIV, HIV) and HTLVI are transmitted through milk. MMTV transmission from lactating mothers to their nursing females was discovered in the 1920's and has served as a prototype for this form of retroviral infection. MMTV first infects B cells in the Peyer's patches of the gut. A viral protein encoded in its long terminal repeat (LTR), termed the superantigen (Sag), enables it to first amplify in gut lymphocytes and then spread systemically. This allows MMTV to traffic from the gut to the mammary gland via infected lymphocytes.

We are using MMTV infection to track lymphocyte migration to the mammary gland and to mammary tumors. Dr. Reb Russell, the original P.I. on this grant, constructed MMTV-based vectors that encode a marker gene, green fluorescent protein (GFP). Cells expressing this vector can be distinguished by fluorescence activated cell sorter (FACS) analysis or by fluorescence microscopy. We have used these vectors to create transgenic mice that express GFP in lymphoid cells and mammary tissue and to create pseudoviruses that can be used to infect mice. We have found that by fluorescence microscopy that GFP-positive lymphocytes can be found in all lymphoid organs of the transgenic mice (spleen, Peyer's patches, lymph nodes) and scattered in the mammary gland. We are currently using the viruses produced by these mice to study the pathway of MMTV infection, i.e. how lymphocytes traffic from the gut to the mammary gland.

Recently, mice with targeted disruption of the E-cadherin gene have been generated by Dr. Rolf Kemler's group. These mice have the E-cadherin gene flanked by lox sites. In the presence of the cre-recombinase enzyme, this gene will be deleted. The E-cadherin-targeted mice are currently being bred at the Jackson Laboratory by Dr. Barbara Knowles. In collaboration with Dr. Knowles, we will breed our mice with mammary gland specific expression of cre-recombinase specific with the E-cadherin-floxed mice. We will then test whether there is appropriate homing of lymphocytes to mammary gland of the double transgenic mice. We will study homing using cell surface markers and by infecting the mice with the GFP-tagged MMTV. If homing is disrupted in the mice with targeted deletion of E-cadherin, they will also be used to test whether MMTV infection of the mammary gland is dependent on lymphocytes that home using this ligand. We will also determine whether these mice ultimately develop mammary tumors.

As a result of these studies, we hope not only to better understand MMTV biology, but the interaction of lymphoid cells with mucosal tissues such as the mammary gland.