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Dissection of Immune Modulation by MUC1/sec or IEP, Which Enables Induction of an Effective Anti-Tumor Immune Response

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Many tumor antigens which have been targets for immunotherapy are expressed by normal cells, but over expressed or altered in structure or surface localization on transformed cells. One such protein which has been documented in breast cancer cells is Mucin1 (MUC1). Immune responses to MUC1 have been recognized in animal models and cancer patients, but have not led to successful tumor rejection. In our laboratory, isoforms of the MUC1 molecule were investigated, and it was found that expression of a secreted isoform (MUC1/sec) by an aggressive murine mammary tumor resulted in rejection of the tumor in vivo. In parallel, expression of a transmembrane form of the molecule which localized to the surface of the mammary tumor cell, resulted in tumor growth and metastasis, similar to the parental tumor. This rejection was determined to be immunologically mediated. Furthermore, mammary tumor cells secreting MUC1/sec offered protection against tumor growth of the parental cell line, and transmembrane MUC1 expressing tumors. It was also discovered that a unique peptide found in MUC1/sec and named Immuno Enhancing Peptide (IEP), was not seen in any known protein sequence, and also offered protection against tumor growth. Previously Obermair et al, reported that primary malignant ovarian cases were positive for the transmembrane forms of MUC1, but negative for the MUC1/sec variant. Expression of the MUC1/sec isoform was however found in all of the benign lesions, thus correlating the absence of MUC1/sec with malignancy.

In this proposal we plan to dissect immune modulation by MUC1/sec or IEP, which enables induction of an effective anti-tumor immune response. The first aim is to determine whether MUC1/sec modulates the expansion or maturation of dendritic cells (DC), which are potent stimulators of anti-tumor immune responses. Using BD Pharmingen antibodies to MHC class II, CD11b, CD11c, CD80, CD45-1, CD40, and CD86, we will analyze the levels of DC in Lymph nodes and spleens of mice implanted with tumors expressing either the transmembrane or secreted form of MUC1. These markers are expressed at low levels on immature DC and at high levels on mature DC. Analysis will be performed at our institutional flow cytometry facility carrying a BD LSR™, and BD LSR II. Using BD IMagnet™ cell separation and DC enrichment sets we will isolate DC from tumor bearers and test antigen presentation to T cells in vitro. T cell proliferation and cytokine secretion will be measured by BD OptEIA Elisa Sets (IL-2, IL-4, IFNg) and BD CBA Flex Sets and Kits (Mouse Th1/Th2).

In our preliminary studies we found that the parental tumor line and tumors expressing the transmembrane form of MUC1, express high levels of urokinase plasminogen activator (uPA), reported to be a prognostic marker of aggressive tumors and metastatic disease. However, in tumors expressing MUC1/sec the levels of uPA are not detected at the protein level. Furthermore, MUC1/sec expressing cells have a tremendous upregulation of the chemokine CCL2, known as Monocyte Chemoattractant Protein1. The second aim will be to evaluate whether uPA downregulation and CCL2 upregulation

by MUC1/sec leads to increased monocyte migration. Using the BD IMagnet™ and CD11b magnetic particles we will isolate macrophages from peritoneal cavities of animals implanted with the different tumors, and test whether MUC1/sec has enhanced their ability to migrate towards CCL2 in an in vitro migration system offered by BD Biocoat. We will also test the type of macrophages produced in tumor bearers and in the presence of uPA or CCL2 by stimulation of the macrophages in vitro using BD Pharmingen recombinant proteins, and analysis of cytokine production using BD CBA Flex Sets to interleukins and transcriptional activators.

Our third aim is to investigate whether MUC1/sec impedes the generation of immature myeloid suppressor cells (MSC). GR-1+CD11b+ MSC have been documented in different tumor models, playing a role in suppression of anti-tumor immune responses. We will analyze levels of MSC in different tumor bearers by flow cytometry using BD Pharmingen antibodies to GR-1 and CD11b. We will also separate out MSC using BD IMagnet™ and Ly6G/Ly6C (GR-1) magnetic particles. MSC will then be characterized by cell surface markers using BD Pharmingen antibodies. We will also separate out T cells using BD IMagnet™ and mouse T lymphocyte enrichment set, and test the ability of isolated MSC from different tumor bearers to suppress activation of normal T cells. Finally, we will culture isolated MSC with conditioned media from different tumor cell cultures, and test whether the cytokine profile of MSC changes using BD CBA Flex Sets or inflammation kits, and BD OptE1A Elisa Kits. To analyze further which inflammatory cells are recruited to the different tumor cells, we will mix the tumor cells with BD Puramatrix peptide hydrogel, and inject the mixture subcutaneously into mice. The extractable gel will aid in identification of cellular recruitment to tumor cells expressing MUC1/sec, which do not lead to an extractable tumor due to immune dependent rejection.

This research will help determine which immunological parameters in tumor bearers provide for better anti-tumor immune responses, and provide insight into immune modulation by MUC1/sec or the unique IEP present in the molecule, for their potential use as immunotherapeutic agents in breast cancer therapy.

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