Novel antigen-cloning technique may boost efforts to develop a melanoma vaccine

April 15 2007

In recent years, researchers have worked to develop a number of vaccines to help the immune system fight tumors. Cancer vaccines are not intended to prevent cancer; rather, they are used to boost immune responses to preexisting tumors. Unlike traditional chemotherapy, vaccines have relatively low toxicity and, potentially, a high degree of efficacy.

To date, these vaccines have rarely been designed to directly stimulate one of the body's most critical immune responders, the helper T cells. Though helper T cells contain receptors on their cell surfaces that are capable of recognizing and binding to tumor-related antigens, scientists have been stymied by the complex and time-consuming process required to isolate and clone the antigens for vaccine development.

In working to identify a key tumor antigen in melanoma and other cancers, scientists at The Wistar Institute have now developed a novel way to clone an antigen recognized by a helper T cell. Already, Herlyn's group has used the new cloning technique to identify a new tumor antigen called ribosomal protein L8, or RPL8. Findings on the new cloning method and the newly identified tumor antigen will be published as a Priority Report in the April 15 issue of Cancer Research.

The new antigen-cloning approach may allow scientists to design vaccines capable of directly stimulating helper T cells, aiding the development of vaccines not only for cancer but also for infectious diseases, says Dorothee Herlyn, D.V.M., senior author on the study and a

professor in the Molecular and Cellular Oncogenesis and Immunology programs at Wistar.

"Most of the melanoma vaccines currently in development work to activate a type of white blood cell called cytotoxic T-lymphocytes, or CTL," says Herlyn. "Though CTL have the ability to destroy cancer cells, they don't have the ability to call upon the full capabilities of the immune system, as do the helper T cells."

The new tumor antigen discovered in her lab, RPL8, is an ideal vaccine candidate because it has the potential for eliciting both helper T cells and CTL responses, Herlyn says. RPL8 is a protein involved in protein synthesis and is also expressed in normal cells. Herlyn's study shows that RPL8 is over-expressed not only in melanoma, but also in breast cancer cells and gliomas, the most common type of brain tumor, indicating that it has potential as a vaccine for patients with these tumors.

In their study, the researchers used their new cloning technique to clone copies of the RPL8 antigen from a melanoma. The scientists then showed that a peptide of RPL8 could stimulate a response in helper T cell clones and lymphocytes in four out of nine melanoma patients. The antigen created no response in cells taken from healthy donors.

The study also showed that RPL8 might contain multiple regions that are capable of eliciting an immune response, suggesting that RPL8 may be an ideal vaccine target for patients that display this antigen in tumors. According to Herlyn, these findings are important because there aren't many vaccines in development to trigger a helper T cell response for many types of cancer.

"Most of the cloned melanoma antigens that are known to target helper T cells are mutated and individual-specific, meaning that they may work in treating a single individual but will be ineffective in treating a large

percentage of patients," Herlyn says.

Herlyn notes that the new antigen-cloning technique developed for this study is a major advance over the cumbersome method previously required to accomplish the task, and she anticipates that experimental vaccine developers will find it of significant value in their work.

Both the new and the old methods begin with the same steps. In order to identify and clone an antigen, researchers start with a melanoma cell or other cell of interest likely to contain relevant antigens. Based on the genes in that cell, they then make a complementary DNA (cDNA) library. Using the resulting cDNA library, the investigators can then express sequences of individual genes in recipient cells to find the cell or cells with cDNA able to stimulate T cells.

The old approach to cloning antigens adds an additional layer of complexity to the process. It requires knowing which cellular marker, or class II human leukocyte antigen (HLA), serves as a restriction element for specific T cells. Researchers must clone this restriction element and other class II HLA and transfer them into recipient cells, along with the cDNA library.

With her new technique, Herlyn expresses the cDNA library in bacteriophages that are incubated with B cells from the same patient from whom the T cells were derived. Because B cells are antigen-presenting cells, once they have picked up, or "eaten," the phages, the B cells will express the peptides that are encoded by the cDNA library. The new process eliminates the need to transfer the HLA genes into recipient cells and match the genes to each T cell type as it permits the B cells to present the peptide to the T cells in context with their own HLA class II.

Herlyn and her group now plan to use the RPL8 antigen to develop a melanoma vaccine for patients with advanced disease. "And because the

RPL8 antigen is expressed by breast cancer and gliomas, we may be able to develop a vaccine that could be used to treat these types of cancer, as well."

Source: The Wistar Institute