

Progress Report

The last decade has seen an increasing interest in the immunotherapy of brain tumors. One reason for this is that immunotherapy has the potential to spare normal cells and target only tumor cells. To date, the main limiting factor of targeting tumors has been the lack of tumor-specific markers. This obstacle has been overcome with the identification of a novel glioma tumor marker, IL13 α 2R. This tumor marker is expressed only by malignant brain tumors and is, in fact, a marker of glial transformation, as some tumors progress from low grade astrocytomas to high grade or malignant tumors. We have identified a series of amino acid sequences on this marker which stimulate cytotoxic T cells of the immune system and can lead to tumor cell destruction. The goal of this project has been to perform further *in vitro* as well as *in vivo* studies in order to determine the potential of these amino acid sequences as a vaccine against malignant brain tumors.

We first analyzed a series of glioma cell line cultures for the expression of the IL13 α 2R receptor and were able to show that the majority of cell lines tested were positive for the tumor marker. We then generated a T cell specific against the amino acids within the IL13 α 2R and tested the ability of the T cell to destroy a variety of the tumor cell lines which express the receptor. Our results indicate that the T cell were extremely effective in destroying tumor cells expressing IL13 α 2R, and the degree of the effectiveness was proportional to the expression of the receptor. Having successfully established an *in vitro* model, we then turned our attention to animal studies. In the first preliminary experiments, mice were immunized with a peptide which elicits the T cell response and then challenged with intracranial tumors. Those mice which were immunized with the T cell specific peptide exhibited longer survival than animals immunized with non-specific peptides or control animals.

In the second phase of the project, we examined the ability of the peptide to induce an immune response against established intracranial tumors. This was accomplished by first introducing a tumor into a mouse brain and then immunizing the mouse with the T cell specific peptide. Mice treated with peptide immunizations did not exhibit an enhanced survival as compared to controls. In addition to assessing animal survival, we also isolated T cell from mice treated in this fashion and re-examining those T cells *in vitro*. The T cells were functional and specific against the peptide of interest. These results suggest that while peptide immunizations might be beneficial in the preventive setting, they do not offer a significant benefit in established tumors. Nevertheless, these results must be interpreted with caution as the difference in survival might be attributed to the dose of peptide vaccines or the timing of the vaccines. We are currently pursuing experiments aimed to further answer those questions. We are also attempting to examine whether augmenting the number of peptide specific T cells might improve the survival of animals with experimental brain tumors.