

Central Nervous System Tumor Immunity Generated by a Recombinant *Listeria monocytogenes* Vaccine Targeting Tyrosinase Related Protein-2 and Real-Time Imaging of Intracranial Tumor Burden.

Experimental Studies

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Abstract:

OBJECTIVE: Previously, we demonstrated that a recombinant *Listeria monocytogenes* (rLM) vector encoding the melanoma-associated antigen, tyrosinase related protein (TRP)-2, could successfully treat subcutaneous B16 melanomas. The purpose of the present study was twofold: 1) to test whether this rLM-nucleoprotein (NP)/TRP-2 could generate antitumor immunity to a B16 tumor challenge in the immunologically privileged central nervous system (CNS) and 2) to develop a noninvasive imaging modality to monitor tumor progression in the brain after immunotherapy.

METHODS: Mice were vaccinated with either a control rLM strain expressing only a viral antigen (rLM-NP) or a strain expressing both the viral epitope and TRP-2 (rLM-NP/TRP-2). These mice were then analyzed for their ability to mount tumor-specific T-cell responses, to generate protective antitumor immunity to a CNS tumor challenge, and for the localization of T cells at the tumor site. To noninvasively measure tumor growth within the CNS *in vivo*, we developed a B16 cell line expressing firefly luciferase that could be readily detected via bioluminescent imaging.

RESULTS: Vaccination with rLM-NP/TRP-2 induced a robust, tumor-specific CD8⁺ T-cell response to the dominant cytotoxic T lymphocyte epitope of TRP-2 and selective interferon- γ secretion when cocultured with B16 melanoma cells *in vitro*. Significant decreases in CNS tumor sizes were easily visualized in mice vaccinated with rLM-NP/TRP-2 compared with mice that received a control rLM expressing the NP epitope alone (rLM-NP). The subsequent decreased tumor size and extension of survival induced by rLM-NP/TRP-2 was similarly associated with an early increase of tumor infiltrating T cells.

CONCLUSION: The ability to treat tumors arising within the CNS is difficult because of the nature of the anatomic confines of the brain and a microenvironment that may not promote immune responsiveness. These studies describe an *in vivo* bioluminescent imaging system to monitor CNS tumor growth in mice, which we successfully used to document decreased intracranial tumor progression and size after vaccination with rLM-NP/TRP-2. The results suggest that metastatic tumors in the CNS can be targeted immunotherapeutically without overt autoimmune toxicity.