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Is the immune response a friend or foe for viral therapy of glioma?

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See articles in this issue by Hiraoka and Inagaki et al., pp. 918–929, and Mitchell et al., pp. 930–939.

There have been extensive investigations on the role of immune responses in viral therapy. In addition to direct cytolytic effects on cancer cells, viral therapy may facilitate release of danger signals, activation of innate inflammatory reactions, and induction of antitumor immunity. These effects are often optimized through expression of immune activating transgenes via a variety of viral therapies. However, we must be aware of the fact that the immune system will also counteract the viral therapy by directing adaptive immune responses to viral antigen epitopes, thereby leading to premature clearance of therapeutic viruses before they mediate antitumor effects,[1] which will become more significant after repeat viral treatments. Furthermore, many viruses are able to suppress both innate and adaptive immunity through molecular mimicry and competitive inhibition, including viral proteins that act as immunomodulatory decoys and signaling pathway inhibitors,[2] and may even actively induce recruitment of immunosuppressive cells into the tumor microenvironment.[3,4] Each virus may have different impacts on the immune system, and therefore, it is important to delineate specific mechanisms of immune-modulation caused by the virus of investigation.

In this issue of *Neuro-Oncology*, Mitchell et al evaluate antitumor immune responses induced by treatment with 5-fluorocytosine (5-FC) in Toca 511–expressing gliomas in the flanks of syngeneic mice.[5] Toca 511 is a tumor-selectively replicating retroviral vector encoding a yeast enzyme that converts 5-FC, an antifungal prodrug, into 5-fluorouracil (5-FU), a classic anticancer drug. Using flow cytometric analyses of subcutaneous tumors, the authors characterized the kinetics of immune cell infiltrates in relation to the treatment course. One of the most prominent changes they have observed is the reduction of tumor-associated macrophages, myeloid-derived suppressor cells, and tumor-associated monocytes over the course. It is also noteworthy that CD4+ T cells polarized away from T helper (Th)2, Th17, and CD8+ T cells showed an increase of interferon (IFN)-γ–producing populations. Mice that demonstrated complete response to the treatment mounted protective immunity against tumor rechallenge and could confer this immunity by adoptive transfer of T cells. These results are encouraging, as the viruses used contained no additional immune activating transgenes.

Few gene therapy studies have evaluated the time course of immune infiltrate changes in as much detail as is done in the current study. However, the study was conducted primarily using the subcutaneous Tu-2449 tumor model. It has been well described that there are remarkable differences of the microenvironment between subcutaneous and intracranial tumors, and the same cell line inoculated in the subcutaneous versus intracranial space demonstrates entirely different pathological features and responses to CD4+ or CD8+ T cell–mediated immunity.[6] Because the model cell line (ie, Tu-2449) is a glioma line and Toca 511 is being developed for treatment of glioma, it is highly desirable that detailed and well-designed analyses are to be conducted with intracranial models. There are many published studies evaluating immune cell infiltrates in mouse intracranial glioma models, so it should be feasible to do so. However, it is reassuring that the adoptive transfer experiment in this study did employ T cells isolated from the spleens of mice that had been previously cured of intracranial gliomas by Toca 511/5-FC; adoptive transfer of these T cells conferred the ability to reject established intracranial gliomas in treatment-naïve recipient mice, while T-depleted splenocytes did not confer such protective immunity.

In this regard, a companion paper jointly first-authored by Hiraoka and Inagaki et al in this issue provides further complementary data by evaluating Toca 511 in orthotopic brain tumor models using both human glioma xenografts and syngeneic murine gliomas, employing in vivo bioluminescence imaging to monitor tumor responses in individual animals over time.[7] Complete response with long-term survival, as well as

rejection of rechallenged tumor, required the intact host immune system, especially CD4+ T cells. It is noteworthy that, despite the expression of retroviral antigens in Toca 511–treated tumors, subsequent rechallenge of cured mice with uninfected parental Tu-2449 cells was completely rejected >300 days later, indicating development of protective immunity against endogenous tumor antigens. It is also especially noteworthy that CD4+ T cells mediated a critical role in the observed antitumor immunity, while Tu-2449 cells do not appear to express major histocompatibility complex (MHC) class II. The authors discuss possibilities of MHC-independent cytotoxic effects by CD4+ cells as well as cross-presentation of tumor antigens by CD4+ Th1 cell–dependent IFN-γ stimulated M1-like macrophages. Further investigations into these possible mechanisms are of particular importance, given recent identification and characterization of an MHC class II–binding epitope in mutant isocitrate dehydrogenase 1.[8]

However, a caveat in terms of deriving clinically relevant insights from these studies may be their choice of the Tu-2449 cell line for their tumor models in immunocompetent mice. This cell line was originally derived from spontaneous gliomas that developed in glial fibrillary acidic protein–v-src transgenic mice.9 The presence of the v-src oncogene may make the tumor cell line artificially immunogenic, perhaps due to point mutations or abnormal tertiary structure of the truncated Src protein.

Taken together, these 2 studies provide important information as to the immunological milieu induced by the Toca 511 and 5-FC treatment, resulting in elimination of immunosuppressive tumor stromal cells and activation of antitumor immunity. Notably, 5-FU has been reported to cause immunogenic cell death and elimination of myeloid-derived suppressor cells,[10] but conventional chemotherapy with systemic 5-FU also causes myelotoxicity and damages the immune system. In contrast, with retroviral prodrug activator gene therapy, 5-FU is locally generated directly within infected glioma cells, and the immune system remains intact. Further investigations as to the role of CD4+ T cell–mediated antiglioma immunity and refinement of the model system, in terms of the expanded use of intracranial tumor models and a less immunogenic cell line, would tremendously contribute to the advancement of this important field.

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