

Intra-tumoral dendritic cells increase efficacy of peripheral vaccination by modulation of glioma microenvironment

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Pilot data showed that adding intratumoral (IT) injection of dendritic cells (DCs) prolongs survival of patients affected by glioblastoma multiforme (GBM) treated by subcutaneous (SC) delivery of DCs. Using a murine model resembling GBM, we investigated the immunological mechanisms underlying this effect. C57BL/6/N mice received brain injections of GL261 glioma cells. Seven days later, mice were treated by 3 SC injections of DCs with or without 1 IT injection of DCs. DC maturation, induced by pulsing with GL261 lysates, was necessary to develop effective immune responses. IT injection of pulsed (pDC), but not unpulsed DCs (uDC), increased significantly the survival, either per se or in combination with SC-pDC ($P < .001$ vs controls). Mice treated by IT-pDC plus SC-pDC survived longer than mice treated by SC-pDC only ($P = .03$). Injected pDC were detectable in tumor parenchyma, but not in cervical lymph nodes. In gliomas injected with IT-pDC, CD8+ cells were significantly more abundant and Foxp3+ cells were significantly less abundant than in other groups. Using real-time polymerase chain reaction, we also found enhanced expression of IFN- γ and TNF- α and decreased expression of transforming growth factor-beta (TGF- β) and Foxp3 in mice treated with SC-pDC and IT-pDC. In vitro, pDC produced more TNF- α than uDC: addition of TNF- α to the medium decreased the proliferation of glioma cells. Overall, the results suggest that IT-pDC potentiates the anti-tumor immune response elicited by SC-pDC by pro-immune modulation of cytokines in the tumor

microenvironment, decrease of Treg cells, and direct inhibition of tumor proliferation by TNF- α .

Keywords: dendritic cells, glioma, intratumoral vaccination, tumor microenvironment

Strategies for cancer immunotherapy critically rely on the use of dendritic cells (DCs) for antigen presentation in peripheral lymph nodes, where CD8+ T lymphocytes are instructed to initiate a cytotoxic anti-tumor response.¹ Such a response, however, has to develop in a tumor microenvironment that is strongly characterized by the presence of diverse immunosuppressive factors, allowing the tumor to escape immune surveillance:² brain tumors provide an excellent example of this scenario.³ Several clinical experiences have been reported for DC immunotherapy of malignant gliomas and specifically of glioblastoma multiforme (GBM), the most frequent and aggressive of primary brain tumors.⁴ Results in terms of safety have been satisfactory but clinical efficacy, especially when treating relapses of GBM, can certainly be increased.⁵ The production of different immune suppressive cytokines, particularly transforming growth factor-beta (TGF- β), and the presence of CD4+ CD25+ Foxp3+ T cells (T-regulatory cells, Treg) has been correlated to evidence of low or decreased efficacy of DC immunotherapy in preclinical models and in patients.^{6–9} Thus, combined strategies linking peripheral immune responses to a modification of the tumor microenvironment are desirable in order to improve the clinical potential of DC immunotherapy.

Direct intratumoral (IT) injection of unpulsed DCs (uDC) has been attempted in different tumors, including GBM:^{10–17} the results may encourage further investigations on the molecular and cellular

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Supplementary Material

Supplementary material is available at *Neuro-Oncology* online.

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Conflict of interest statement. None declared.

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