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B Cells Are Critical to T-cell–Mediated Antitumor Immunity Induced by a Combined Immune-Stimulatory/ Conditionally Cytotoxic Therapy for Glioblastoma[1,2]

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Abstract

We have demonstrated that modifying the tumor microenvironment through intratumoral administration of adenoviral vectors (Ad) encoding the conditional cytotoxic molecule, i.e., HSV1-TK and the immune-stimulatory cytokine, i.e., *fms*-like tyrosine kinase 3 ligand (Flt3L) leads to T-cell–dependent tumor regression in rodent models of glioblastoma. We investigated the role of B cells during immune-mediated glioblastoma multiforme regression. Although treatment with Ad-TK+Ad-Flt3L induced tumor regression in 60% of wild-type (WT) mice, it completely failed in B-cell–deficient Igh6$^{-/-}$ mice. Tumor-specific T-cell precursors were detected in Ad-TK+Ad-Flt3L–treated WT mice but not in Igh6$^{-/-}$ mice. The treatment also failed in WT mice depleted of total B cells or marginal zone B cells. Because we could not detect circulating antibodies against tumor cells and the treatment was equally efficient in

Abbreviations: GBM, glioblastoma multiforme; TK, thymidine kinase; Flt3L, *fms*-like tyrosine kinase 3; WT, wild-type; APC, antigen-presenting cell; Ad, adenoviral vector; MZB, marginal zone B cell; LN, lymph node; MLR, mixed leukocyte reaction; GFP, green fluorescent protein; DC, dendritic cell

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[2]This article refers to supplementary materials, which are designated by Table W1 and Figures W1 to W4 and are available online at www.neoplasia.com.

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WT mice and in mice with B-cell–specific deletion of *Prdm 1* (encoding Blimp-1), in which B cells are present but unable to fully differentiate into antibody-secreting plasma cells, tumor regression in this model is not dependent on B cells' production of tumor antigen–specific immunoglobulins. Instead, B cells seem to play a role as antigen-presenting cells (APCs). Treatment with Ad-TK+Ad-Flt3L led to an increase in the number of B cells in the cervical lymph nodes, which stimulated the proliferation of syngeneic T cells and induced clonal expansion of antitumor T cells. Our data show that B cells act as APCs, playing a critical role in clonal expansion of tumor antigen–specific T cells and brain tumor regression.

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Introduction

Glioblastoma multiforme (GBM) is a malignant brain cancer, accounting for approximately 50% of newly diagnosed primary brain tumors in the United States. GBM has a dismal prognosis owing to the local infiltrative tumor growth that makes complete surgical resection virtually impossible, the intrinsic radiotherapy and chemotherapy resistance of glioma cells, and their high rate of mutation. Novel therapeutic strategies such as vaccination/immunotherapies have been developed to target GBM cells disseminated throughout the brain [1]. We developed an anti-GBM immunotherapeutic approach based on engineering the tumor microenvironment, which uses a combined conditional cytotoxic/immune–stimulatory gene therapeutic modality. It consists of an adenoviral vector (Ad) encoding herpes simplex virus type I–thymidine kinase (Ad-TK), which, in the presence of ganciclovir, kills proliferating cells, and a second Ad encoding *fms*-like tyrosine kinase 3 ligand (Ad-Flt3L), which recruits antigen-presenting cells (APCs) to the brain tumor microenvironment [2]. We have shown that this combination therapy induces an antitumor immune response and immunologic memory [2–4]. While the role of cytotoxic T cells in the clearance of peripheral and brain tumors has been well documented, the role of B cells in antitumor immunity has remained debatable. This is due to several reasons: 1) increased numbers of tumor-infiltrating B lymphocytes can correlate with poor prognosis of patients harboring metastatic carcinomas [5] or with improved survival of breast carcinoma patients [6,7]; 2) resting B cells have been implicated in promoting carcinogenesis by exacerbating inflammation [8]; 3) although B cells are relatively abundant, the frequency of B cells bearing a BcR specific for a particular antigen is very low (between 10^{-4} and 10^{-5}), potentially limiting the effectiveness of B cells as APCs during initial priming of immune responses [9]; and 4) studies in B-cell knockout mice revealed that B cells actually suppress the development of immune responses *in vivo* against lymphoma, colon cancer, and melanoma (but not sarcomas) [10,11] and depletion of B lymphocytes enhances melanoma vaccination efficacy [12], whereas in separate studies, B lymphocytes were implicated in promoting fibrosarcoma tumor regression [13].

Bone marrow–derived B cells develop into either follicular B cells or marginal zone B cells (MZB) in the spleen. Follicular B cells ($B220^+/CD23^{high}/CD21^{low}$), which account for most peripheral mature B cells, are found in the circulation, the germinal center of peripheral lymph nodes (LNs), and the white pulp of the spleen. They participate in T-cell–dependent immune responses and immunologic memory [14]. MZB cells ($B220^+/CD23^{low}/CD21^{high}$) are derived from circulating progenitors, but when they arrive to the spleen, they locate in the marginal zone and do not recirculate; they have been shown to capture blood-borne antigens and deliver them to dendritic cells (DCs) of the follicular areas [15]. Also, activated MZB cells can migrate to the T-B border and directly induce the expansion of antigen-specific T cells [16].

Prompted by the central role of B cells in autoimmune diseases [17–19] and by the successful induction of *in vitro* T-cell responses using tumor antigen–pulsed, CD40-activated B cells [20,21], we investigated the role of B cells in brain tumor regression induced by intratumoral treatment with Ad-TK+Ad-Flt3L. Using KO mice that lack B cells and specific antibodies that deplete total B cells or MZB cells, we found that, in the absence of B cells, Ad-TK+Ad-Flt3L fails to induce the regression of intracranial GBM. Tumor antigen–specific T-cell clonal expansion was also abolished in B-cell–deficient mice ($Igh6^{-/-}$), indicating that functional, mature B cells were required for mounting a systemic immune response against brain tumor antigens. The role of B cells in this antitumor immune response does not, however, seem to be mediated by the production of antitumor-specific antibodies because we could not detect evidence of humoral antitumor immunity and the treatment was still efficacious in mice deficient in plasma cells formation, $Prdm^{flox/flox}CD19^{Cre/+}$ mice. Although the most evident function of B cells in adaptive immune responses is the clonal differentiation of antigen-specific B cells into plasma cells and the subsequent secretion of antigen-specific immunoglobulin (Ig), B cells can also function as efficient APCs [9,17,20–22]. Ad-Flt3L/Ad-TK treatment induced an increase in the levels of B cells in the cervical LNs of WT mice. These B cells contained brain tumor remnants, increased expression of coactivation markers, and induced the clonal expansion of syngeneic tumor antigen–specific T lymphocytes. Taken together, our results imply that B cells may act as APCs to enhance clonal expansion of tumor antigen–specific T lymphocytes and T cell–dependent tumor regression within the central nervous system.

Materials and Methods

Ads

First-generation, E1/E3–deleted replication-deficient recombinant adenovirus serotype 5 was used in this study. We used Ad-Flt3L [3] and Ad-TK [3]; both transgenes are under the control of human CMV promoter. An Ad without a transgene was used as a control (Ad-0). All viral preparations were confirmed to be replication competent adenovirus and lipopolysaccharide (LPS) free. Viral titers were determined by an end-point dilution cytotoxic-effect assay. The methods for Ad generation, purification, characterization, and scale-up have been