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Regulatory T cells are redirected to kill glioblastoma by an EGFRvIII-targeted bispecific antibody

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Regulatory T cells (Tregs) play a central role in in tumor escape from immunosurveillance. We report that a bispecific T-cell engager (BiTE) targeting a mutated form of the epidermal growth factor receptor, i.e., EGFRvIII, potently redirects Tregs to kill glioblastoma through the granzyme-perforin pathway.

Glioblastoma multiforme (GBM) is the most common and aggressive primary malignant brain tumor. Despite multimodal therapy including surgical resection, radiation therapy and chemotherapy, GBM is uniformly lethal with a median survival of less than 15 months.[1] In addition, currently available treatments can cause collateral, toxic effects to surrounding, non-transformed, healthy cells. By contrast, immunological targeting of tumor-specific mutations can allow for eradication neoplastic cells while leaving otherwise eloquent tissues intact.

T cells in particular play a major role in mounting effective antitumor immune responses, in some instances eradicating bulky, invasive neoplasms. Still, the widespread use of T cell-based immunotherapy faces a number of challenges. First, non-specific activation of endogenous T cells, such as through global ligation with monoclonal antibodies, has resulted in disastrous autoimmune effects.[2] In addition, the development of tumor antigen specific T cells is laborious, often inconsistent, and further complicated by the need for adoptive transfer of lymphocytes and genetic modification through retroviral transduction. Lastly, regulatory T cells (Tregs) heavily infiltrate GBM and other solid tumor lesions, leading to potent suppression of anti-tumor immune responses and eventual tumor escape from immune-mediated rejection.

Addressing these barriers, an emerging immunotherapeutic approach is the use of bispecific antibodies designed to engage and activate circulating T cells, but only in the presence of a specific target antigen, thus affording potent and specific tumor cell lysis. One prominent subclass of the bispecific antibody format is the bispecific T-cell engager (BiTE). BiTEs consist of two single-chain variable fragments translated in tandem, with an effector-binding arm specific for the ε subunit of the CD3 activating complex expressed on the surface of T cells,[3] and a target-binding arm that can be directed against any number of epitopes that are differentially expressed on the surface of tumor cells.[4] A novel BiTE directed against a mutated form of the epidermal growth factor receptor (EGFRvIII)[5] holds great promise for improving the treatment of patients with GBM.[6,7] Upon peripheral administration in mice, the EGFRvIII BiTE localized to intracerebral tumors and recruited previously inactive T cells to eliminate EGFRvIII-expressing GBM, with complete response rates as high as 75%.[8]

We have recently demonstrated that the EGFRvIII-specific BiTE addresses another critical barrier that has traditionally impeded effective translation of immunotherapy, that is, the profound immunosuppressive state established by tumor-infiltrating Tregs.[9] One mechanism by which Tregs actively suppress and kill autologous immune cells is through elaboration of the granzyme-perforin pathway.[10] However, until our study it was unknown whether the cytotoxic mechanisms present in Tregs could be redirected to kill other types of cells, including tumors for example. Indeed, we found that not only did highly-purified Tregs express elevated levels of granzyme and perforin following BiTE-mediated activation, but that EGFRvIII-specific BiTE ultimately redirected Tregs to efficiently lyse EGFRvIII-expressing GBM. This activity was significantly abrogated in the presence of specific inhibitors of granzyme- and perforin-mediated cell death (**Fig. 1**). Of note, immunohistochemical analyses of human GBM revealed diffuse infiltration with

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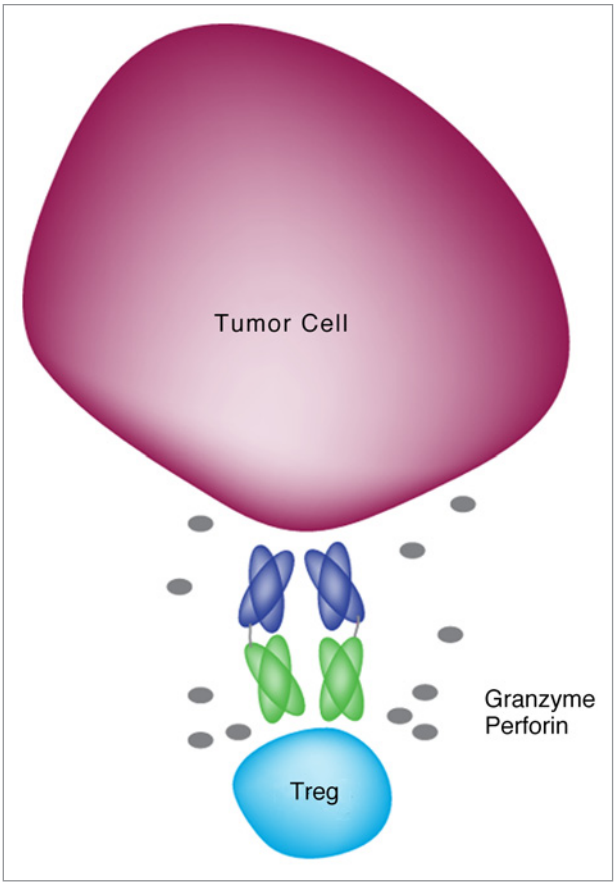

Figure 1. A bispecific T-cell engager specific for epidermal growth factor receptor variant III (EGFRvIII) redirects regulatory T cells to kill malignant brain tumor cells. EGFRvIII-specific BiTE harnesses the natural cytotoxic potential of regulatory T cells (Tregs), resulting in potent and efficient lysis of tumor cells via the granzyme-perforin pathway.

granzyme-expressing T cells also positive for the key Treg transcription factor, FoxP3.

In addition to being present in GBM, intratumoral Tregs are positively correlated with overall malignant behavior. When Tregs are depleted in vitro, autologous T-cell proliferation and cytokine secretion return to normal levels. Furthermore, in vivo Treg depletion in tumor-bearing mice prolongs survival.[11] Several investigators have attempted to translate these findings to enhance immune responses in human studies; however, strategies designed to deplete Tregs in the periphery do not efficiently eliminate the infiltrating, intratumoral population of Tregs, which may limit the therapeutic benefit of this approach. As a potential alternative, we have demonstrated that Tregs present in GBM may actually possess natural cytotoxic functions that can be reappropriated to directly kill tumors, and have provided data to support that such mechanisms can be manipulated advantageously through use of the BiTE therapeutic platform.

While these findings were obtained in the context of an EGFRvIII-specific BiTE, it is reasonable to believe that they can be extended to BiTEs targeting other tumor antigens. Further experiments are needed to elucidate the implications of our work with regard to the basic biology of Tregs, their cytotoxicity in the context of endogenous T cell receptor engagement, and the role of granzyme- and perforin-expressing Tregs that are naturally present in the tumor microenvironment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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