Splenectomy restores tumoricidal activity to promote elimination of intraocular tumors

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We recently demonstrated that splenectomy restores an interaction between CD8+ T cells and macrophages necessary for intraocular tumor elimination. Taking into consideration other studies indicating that intraocular tumor growth does not induce tumor-specific CD8+ T-cell tolerance, our data suggest that splenectomy may influence the phenotype of tumor-associated macrophages.

Over 30 years ago Streilein and Niederkorn1 revealed that the spleen contributes to immune escape of intraocular tumors by demonstrating that P815 tumors of DBA/2 origin, which grew progressively when transplanted in the anterior chamber of the eye of semi-allogeneic Balb/C mice, were rejected if the mice were splenectomized (SPLNX) prior to intraocular tumor challenge. Recently, we revisited and extended these observations using E.G7-OVA tumors in C57Bl/6 mice2 by characterizing the requirements for elimination of intraocular tumors in SPLNX mice as a first step toward understanding how to restore tumoricidal activity within the eye.

Intraocular tumor growth induces a unique form of systemic tolerance, termed anterior chamber-associated immune deviation (ACAID), in which CD4+ T-cell responses to tumor antigens are inhibited by antigen-specific CD8+ T suppressor cells.3 SPLNX abrogated the generation of T suppressor cells and restored tumor-specific CD4+ T cell responses, suggesting a critical tumoricidal effector.4 However, we demonstrated that CD4+ T cells were not required for rejection of intraocular tumors; rather, CD8+ T cells were indispensable.2

The simplest explanation for how the spleen limits immunosurveillance of intraocular tumors is that CD8+ T-cell tolerance to tumor antigens arises in the spleen, which is supported by our previous study indicating that introduction of soluble antigens into the anterior chamber induced expansion but functional inactivation of antigen-specific CD8+ T cells.5 However, we and others have shown that intraocular tumor growth does not induce systemic CD8+ T-cell tolerance.6-8 In fact, mice with intraocular tumors reject a subsequent tumor challenge in the skin or opposite eye via tumor-specific CD8+ T-cell responses.6 Therefore, if T-cell tolerance contributes to intraocular tumor growth it is confined to the primary tumor microenvironment.

In support of regional immune suppression within the eye, we have shown that E.G7-OVA eye tumors are resistant to adoptive cell transfer therapy (ACT) with in vitro generated tumor-specific OT-I CD8+ T-cell effectors whereas E.G7-OVA skin tumors are sensitive.8 Progressive intraocular tumor growth occurred despite CD8+ T-cell infiltration of intraocular tumors at higher T cell: tumor ratios than were observed in regressing E.G7-OVA skin tumors receiving the same treatment. CD8+ T cells infiltrating intraocular or skin tumors demonstrated comparable effector function, as measured by granule exocytosis and cytokine production (IFNγ and TNFα),8 indicating that T-cell tolerance could not explain the impaired immunosurveillance of intraocular tumors and suggesting that the interaction of CD8+ T cells with another immune cell population was critical for intraocular tumor elimination. In support of that notion, elimination of E.G7-OVA skin tumors required intratumoral macrophages that were induced to express tumoricidal concentrations of nitric oxide (NO) by IFNγ produced by CD8+ T cells.8-9 In contrast, macrophages within intraocular tumors expressed low non-tumoricidal concentrations of NO despite their expression of NO-synthase-2, which was also induced by IFNγ expressed by CD8+ T cells.8 Hence, impaired effector function in macrophages, rather than infiltrating CD8+ T cells, contributed to progressive intraocular tumor growth.

Rejection of intraocular tumors in SPLNX mice required CD8+ T cells, IFNγ, and FasL (Fig. 1) but not perforin or TNFα.2 IFNγ and FasL did not target tumor cells directly as SPLNX IFNγR1−/− mice failed to eliminate intraocular tumors that expressed FasL.3 Bone marrow chimeric mice indicated that rejection of intraocular tumors in SPLNX mice required IFNγR1 and Fas expression on immune cells, and SPLNX increased the frequency of activated macrophages within intraocular tumors in an IFNγ and Fas/L-dependent manner suggesting a cellular target for IFNγ and FasL. Intratumoral macrophages were necessary at the effector stage as the elimination of intraocular tumors in SPLNX mice deficient for CD8+ T cells given ACT therapy with activated OT-I CD8+ T-cell effectors was abrogated if intratumoral macrophages were eliminated by local administration of clodronate.
Hence, SPLNX restored intraocular tumor macrophage effector function that was associated with tumor rejection. It remains unclear how macrophages eliminate intraocular tumors in SPLNX mice as NO production is dispensable. An adjuvant effect of SPLNX is not limited to intraocular tumors because Ugel and coworkers recently demonstrated greater efficacy of ACT therapy with activated OT-I CD8+ T cells in SPLNX mice with established E.G7-OVA skin tumors. As tumor growth was associated with increased numbers of splenic CD11b+Ly6C+GR-1int cells that inhibited OT-I CD8+ T-cell responses in vitro, the authors concluded that tumor progression was the result of CD8+ T-cell tolerance induced by these myeloid derived suppressor cells (MDSCs). However, CD11b+Ly6C+GR-1int cells are also precursors of tumor-associated macrophages (TAMs).

As TAMs are required for elimination of E.G7-OVA skin and intraocular tumors, SPLNX may change the composition of the tumor microenvironment by increasing the frequency of TAMs that are sensitive to polarization by CD8+ T cells toward a tumoricidal phenotype. It is important to note that low-dose chemotherapy also reduced the number of splenic MDSCs and demonstrated a similar adjuvant effect for ACT therapy, suggesting a viable alternative to SPLNX in patients with malignancies.

In conclusion, it is clear that SPLNX restores tumoricidal CD8+ T-cell responses; however, the mechanism remains unclear. Our observations of intraocular tumor progression despite induction of tumor-specific CD8+ T-cell responses indicate that T-cell tolerance induced by MDSCs does not explain the impaired immunosurveillance of intraocular tumors. As the interaction between CD8+ T cells and macrophages within the tumor microenvironment is critical for tumor elimination, a careful comparison of macrophages in SPLNX and control mice is warranted to evaluate whether SPLNX alters TAM phenotypes.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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