Disruption of evasive immune cell microenvironment in tumors reflects immunity induced by radiation therapy

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ABSTRACT

Radiation administered to murine colon tumors induced durable remissions that were dependent on CD4+ and CD8+ T cell immunity, and antigen cross priming CD8+ dendritic cells (DCs). Remissions were associated with marked reductions in the infiltration of myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs) and Treg cells, and a marked increase in CD8+ T cells.

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Tumor cells are able to evade immune rejection by promoting a microenvironment in the tumor stroma that is immunosuppressive. Contributions to the suppressive state include the stromal infiltration of innate immune cells including myeloid-derived suppressor cells and tumor-associated macrophages that express PDL-1 and PDL-2 ligands for negative co-stimulatory receptors, and amino acid catabolic enzymes and peroxides that suppress T cell activation and survival. Infiltration of regulatory CD4+CD25+FoxP3+ T cells also block tumor immunity in combination with the tumor-cell-induced upregulation of negative co-stimulatory molecules such as PD-1 and Tim-3 on infiltrating conventional CD4+ and CD8+ T cells.

Evasion of immunity is most potent in tumors that have a balance favoring the infiltration of the suppressive myeloid cells and regulatory lymphocytes as compared to conventional T cells, and in which the majority of conventional T cells are impaired by the expression of the negative co-stimulatory receptors. In clinical studies of tumor immune cell microenvironments, the balance of suppressive cells versus conventional CD8+ T cells, and suppressive receptor/ligand expression on both tumor and immune cells provides an important prognostic indicator of outcome after cancer therapy.

The goal of our recent study was to determine whether a single high dose of radiation targeted to advanced CT26 and MC38 murine subcutaneous colon tumors can induce durable complete remissions. We also determined whether induction of antitumor immunity was required, and radiation induced immunity was reflected in changes in the tumor microenvironment. We found that increasing the single dose of radiation resulted in an increased incidence of durable remissions, and an incidence of about 90% was achieved with 30 Gy, the maximum dose of stereotactic body radiation therapy (SBRT) used clinically. Remissions were dependent on T cell immunity, since RAG-2−/− mice lacking adaptive immune cells and mice depleted of CD4+ or CD8+ T cells by treatment with mAbs failed to develop remissions after radiation treatment. Mice in remission resisted rechallenge with tumor cells, and their purified T cells transferred antitumor immunity to adoptive hosts.

We concluded that radiation therapy induced an immune response to the colon tumors that was required for their elimination. Previous studies of CT26 tumor by Berzofsky and coworkers showed that an evasive immune cell network was induced by the tumor cells growing in the lungs by activation of type II natural killer T (NKT) cells. The latter cells activated MDSCs to suppress CD8+ T cell killing of tumor cells. To obtain further insights into the role of the evasive immune networks in the growth of subcutaneous tumors and the response to radiation therapy, we systematically investigated the immune cell microenvironment of the tumors before and at serial time points after treatment. The percentage and absolute numbers of MDSCs and macrophages in untreated tumors was greater than that of the T cells. In addition, there was a marked increase in the percentage of CD4+ T cells that were Tregs in the tumors as compared to the spleen, and among all the CD4+ and CD8+ T cells in the tumors there was a markedly increased expression of PD-1. In the tumors, PD-1 expression was linked to Tim-3 expression among CD8+ T cells. The changes in the balance of suppressive immune cells and high frequency of expression of negative co-stimulatory receptors in the tumors as compared to the spleen highlighted the immune evasive changes induced by the CT26 tumors.

We followed serial changes in the immune cell microenvironment of CT26 tumors induced by radiation therapy of the
BALB/c mice to determine whether the evasive microenvironment was disrupted. There was a wave of infiltrating CD8+ T cells starting around day 6 that continued to increase in concert with a concomitant decrease in tumor infiltrating CD4+ Tregs and in MDSCs. By day 14 after radiation therapy, a massive CD8+ T cell infiltrate had occurred with a marked depletion of MDSCs. The decrease in MDSCs was a consequence of the CD8+ T cell infiltration, since the decrease did not occur in RAG-2-/- mice nor in mice depleted of CD8+ T cells with monoclonal antibodies (mAbs). The MDSC depletion and tumor cell eradication were dependent on CD8+ T cell production of IFNγ and both failed to occur in d-/- mice. We performed similar experiments in C57BL/6 mice bearing the MC38 colon tumor, and found similar results.

Tumor eradication and MC38 tumor infiltration by CD8+ T cells after radiation therapy was dependent on CD4+ T cell help, and the expression of CD40L on the CD4+ T cells. Lack of CD4+ and CD8+ T cells in C57BL/6RAG-2-/- mice abrogated tumor remissions. Addition of wild type but not CD40L-/-/CD8-/- T cells to wild type CD8+ T cells injected into RAG-2-/- mice restored remissions and T cell infiltration. In addition, remissions and CD8+ T cell infiltration were dependent on the presence of antigen cross priming CD8+ DCs, since BALB/c Batf3-/- mice that have a selective loss of the latter subset of DCs failed to show the benefits of radiation therapy. Add back of CD8+ DCs restored benefits.

In summary, the data showed that single high doses of radiation-induced immune-mediated remissions in the CT26 and MC38 tumors that involved changes in several different types of immune cells as outlined in the schematic diagram in Fig. 1. Previous reports indicated that radiation induced the release of tumor antigens, and also stimulated the activation and maturation of antigen-presenting cells. In our report, CD8+ DCs stimulated CD8+ T cells to make immune responses to the tumor antigens, and CD4+ T cells helped to amplify these responses via the CD40 and CD40L receptor/ligand interaction. The killing of tumor cells by the radiation and the depletion of the MDSCs by the infiltration of the CD8+ T cell removed the key cellular sources of suppressive PDL-1 and galectin-9 (Gal-9) ligands and suppressive arginase-1 (Arg-1) and nitric oxide (NO), thereby disrupting immune evasion.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

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