The role of radiotherapy in reversal of tumor-associated T cell anergy and functional exhaustion

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Background

The immunosuppressive tumor microenvironment and chronic T cell stimulation that occurs in the presence of cancer results in CD8 T cell dysfunction that has been difficult to reverse with immunotherapy (IT) alone. This dysfunction can be separated into two categories of anergy and exhaustion. Anergy is a lack of proliferative response to stimulation with cytokine or antigen resulting from inadequate costimulation or suboptimal T cell receptor (TCR) stimulation during T cell priming. Functional exhaustion is the inability of T cells to kill or produce effector cytokines in response to stimulation. We hypothesized the addition of ionizing radiotherapy (RT) to IT with agonist αOX40 & blocking αCTLA-4 antibodies would reverse tumor associated CD8 T cell anergy and exhaustion.

Materials & Methods

We utilized an established model of CD8 T cell anergy in which transgenic, OVA-specific, OT-I CD8 T cells are adoptively transferred into centrally tolerant POET-1 mice bearing OVA expressing tumors. The development of anergy/exhaustion was monitored within this T cell population and animals were treated with a single 20 Gy fraction of RT using a SARRP, OX40/αCTLA-4, or combined IT/RT 21 days after transfer. Animals in these experiments received 100 ug OX40 ip on days 0 & 1 and 200 ug αCTLA-4 on days 0, 2, & 4 following RT. To assess anergy/exhaustion in endogenous CD8 T cell responses CT26 colon carcinoma bearing BALB/c mice were treated with IT, RT, or combined IT/RT either 10 or 17 days after tumor implantation. Animals in these experiments received 250 ug OX40 ip on days 0 & 4 and 200 ug αCTLA-4 on days 0, 2, & 4 following RT. The frequency/function of tumor-specific (AH1 or OT-I) CD8 T cells was monitored within this T cell population and animals were treated transferred into centrally tolerant POET-1 mice bearing OVA expressing tumors.

Results

Figure 1: OT-I cells become anergic and functionally exhausted following adoptive transfer into MCA-205-mOVA tumor bearing mice. (A) Following adoptive transfer tumors initially regress and subsequently progress as OT-I cells become anergic. (B) OT-I cells become anergic and functionally exhausted following adoptive transfer to CT26 tumor bearing mice. OT-I T cell anergy and functional exhaustion in endogenous CD8 T cell responses CT26 colon carcinoma bearing BALB/c mice were treated with IT, RT, or combined IT/RT 21 days after transfer. Animals in these experiments received 100 ug OX40 ip on days 0 & 1 and 200 ug αCTLA-4 on days 0, 2, & 4 following RT. To assess anergy/exhaustion in endogenous CD8 T cell responses CT26 colon carcinoma bearing BALB/c mice were treated with IT, RT, or combined IT/RT either 10 or 17 days after tumor implantation. Animals in these experiments received 250 ug OX40 ip on days 0 & 4 and 200 ug αCTLA-4 on days 0, 2, & 4 following RT. The frequency/function of tumor-specific (AH1 or OT-I) CD8 T cells was monitored within this T cell population and animals were treated transferred into centrally tolerant POET-1 mice bearing OVA expressing tumors.

Figure 2: Following development of anergy and functional exhaustion OT-I spreads to OT-I and OT-I+ cells. Animals were treated with immunotherapy and vaccination or radiotherapy. The percentage of OT-I Thy1.1 cells in LN or TIL was measured following treatment (A&B). Proliferation of OT-I cells in LN or TIL following treatment was measured by Ki-67 expression (C&D) & IFNg expression (E). *P<0.05 vs control & IT alone

Figure 3: Anergy experiments were performed using OT-I-Nur77-GFP cells expressing GFP in relation to the strength of T cell receptor stimulation. Animals received immunotherapy and either vaccination with soluble OVA or radiotherapy with 20 Gy in a single fraction or 30 Gy spread over 3 fractions. Radiotherapy results in T cell receptor stimulation in this anergy model. *P<0.05 vs IT alone

Figure 4: CT26 dual flank tumor bearing mice were treated at day 10 (early) or day 17 (late) following tumor implantation. Combination therapy resulted in increased percentages and absolute counts of AH1-AS tetramer positive cells within TIL (A&B). TIL were stimulated using AH1-AS peptide and expression of IFNg was measured (C). Proliferation of CD8 T cells in TIL was measured by Ki-67 expression (D). *P<0.05 vs control & IT alone

Conclusions

Combined ablative radiation and immunotherapy with αOX40/αCTLA-4 results in increased CD8 TCR signaling, reversal of T cell anergy, and can prevent development of functional exhaustion. These novel results suggest the addition of radiotherapy to immunotherapy can effectively reverse some tumor-associated T cell dysfunction where IT alone is insufficient.

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