Suppression of the T cell Response and Enhancement of Detrimental Effects of Radiation on the Brain

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Introduction

- Cognitive impairments are common but poorly understood complications of cancer and cancer therapy involving chemotherapy and/or irradiation[1].
- The temporal lobe, and in particular the hippocampus, seems particularly sensitive to detrimental effects of chemotherapy and radiation on cognition[2].
- A pro-inflammatory profile in the brain has been associated with behavioral changes and cognitive impairments, including memory impairments and decreased executive function[3–5].
- Enhanced pro-inflammatory tumor environments are associated with increased survival and tumor regression, while anti-inflammatory tumor environments are correlated with severely limited survival[6].
- An improved immune environment in the tumor at the time of treatment also increases the efficacy of radiation therapy (Figure 1).
- Though immunotherapy enhances the immune response of patients with poor tumor infiltrate profiles, it is unclear how immunotherapy might modulate the effects of radiation on the brain[7].

Objectives

1. Test the hypothesis that activation of the T cell response using anti CTLA4 pretreatment will enhance the detrimental effects of radiation therapy on cognition.
2. Test the hypothesis that activation of the T cell response using anti CTLA4 pretreatment will enhance the detrimental effects of radiation therapy on markers of inflammation and hippocampal neurogenesis.
3. Test the hypothesis that inhibition of the T cell response using anti-CTLA4 pretreatment will enhance upregulation of inflammatory markers in the brain following radiation therapy.

Methods

Female, wild-type 2-4 month-old old BALB/c mice purchased from Jackson Laboratory were divided into four experimental groups of 10 mice each: 1) healthy mice, no treatment; 2) healthy mice, combined anti CTLA4 pretreatment plus radiation; 3) colorectal model, no treatment; 4) colorectal model, combined anti CTLA4 pretreatment plus radiation. The colorectal tumor model was prepared as follows: 1x10^4 CT26 cells were injected s.c. in the right flank. Radiation was delivered as shown in Figure 2. 250µg anti-CTLA4 antibody was delivered i.p at day 7, prior to radiation therapy, to groups 2 and 4.

Cognitive testing was performed during the 2 weeks following irradiation. All mice were tested first for hippocampus-dependent spatial learning and memory in the water maze over 5 days and for hippocampus-dependent contextual fear conditioning and hippocampus-independent cued fear conditioning in the subsequent 2 days.

For water maze testing, the mice were first trained to locate a visible platform for 2 days. Each day contained 2 sessions with 2 trials each. The next 2 days, the mice were trained to locate a first hidden platform location. To assess spatial memory retention for this location, a probe trial (no platform) was performed 24 h later. One hour following the probe trial, the mice were trained for fear conditioning.

In the fear conditioning tests that were used here, mice learned to associate a neutral stimulus, i.e. a tone (CS), with a foot shock (US) and thereby come to fear the previously neutral CS. Trained mice display this conditioned fear by ceasing all movement except for respiration in an attitude called 'freezing.' The neural circuitry underlying fear conditioning is well documented and so this type of learning can be used to assess the functioning of discrete brain regions in mouse models of neurological disease. The type of training determines the brain regions that are involved in the learning and memory processes. After a 2 min period, during which the mouse is allowed to explore the chamber, a tone was delivered followed after 30 sec by a foot shock (0.35 mA over 2 seconds). This tone-shock pairing was repeated at 4.5 min. Training took place in a light and sound attenuated chamber (termed the 'conditioning chamber') that is equipped with a video camera to record freezing behavior.

One day later the mice were first placed in the same context but no shock or tone was presented (contextual), and freezing behavior was recorded during a total period of 10 min. One hour later, the mice were placed in a new context (containing a different odor, cleaning solution, floor texture, walls and shape) allowed to explore for 3 minutes before being re-exposed to the fear conditioning tone and freezing was assessed for an additional 3 minutes. Freezing was measured using a Med Associates fear conditioning equipment and video tracking system.

Following behavioral testing, the mice were perfused for immunohistochemistry and confocal microscopy of synaptic and immune markers or fresh hippocampal and cortical tissues were dissected for tissue levels of molecular outcome measures.

Immune mediated control of CT26 colorectal tumors in mice

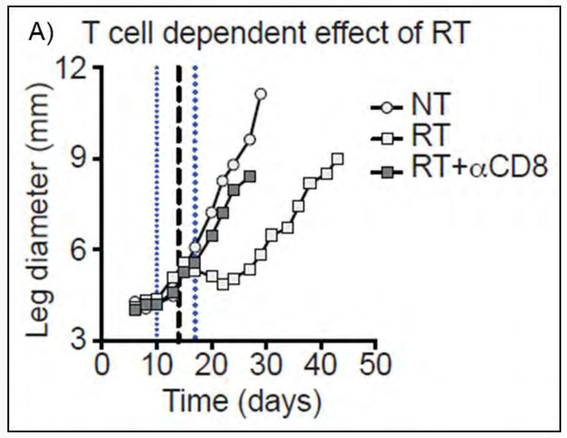

Figure 1. Use of anti-CTLA4 to inhibit the suppression of the anti-tumor T cell response increases responses to radiation therapy and improves survival outcomes, an effect that is T cell dependent. BALB/c mice were challenged with 1x10^4 CT26 subcutaneously then treated on d14 with 20Gy focal radiation. (A) Average tumor diameter in mice left untreated (NT) or treated (RT) with radiation alone or radiation combined with CD8 depletion on days 10 and 17. (B) Growth of individual CT26 tumors in mice (i) untreated, (ii) treated with 250µg anti-CTLA4 on d7, (iii) treated with 20Gy RT d14, (iv) treated with 250µg anti-CTLA4 on d7 and 20Gy RT d14.

CT-guided treatment of s.c. tumors using the SARRP

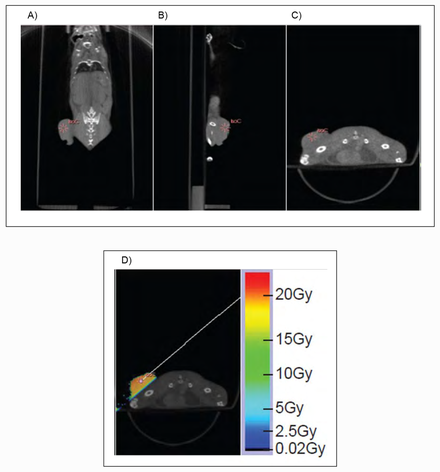

Figure 2. Radiation was delivered to treatment groups of healthy mice or mice bearing established 14 day subcutaneous CT26 tumors using the Small Animal Radiation Research Platform (SARRP). Using a cone-beam CT scan with 360 projections, the tumor was visualized and the isocenter was placed within the tumor (A-C). Treatment plans were prepared for 20Gy focal radiation using a 10mmx10mm collimator at a 50 degree angle to deliver dose to the isocenter with minimal dose to radiosensitive organs. Treatments were well tolerated with no evidence of toxicity to the overlying skin or underlying tissues.

Reduced measures of anxiety in mice receiving combination therapy

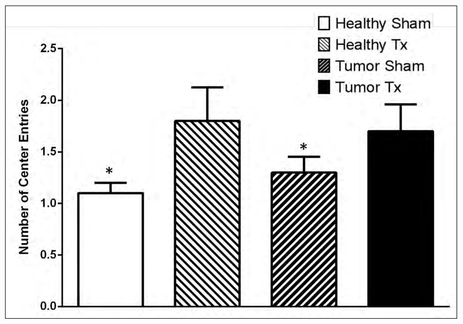

Figure 3. Mice treated with combination therapy show decreased measures of anxiety as compared to untreated mice as demonstrated by increased number of entries into the center in the open field. N = 10 mice/group. Error bars are S.E.M.

Effects of combination therapy on water maze performance

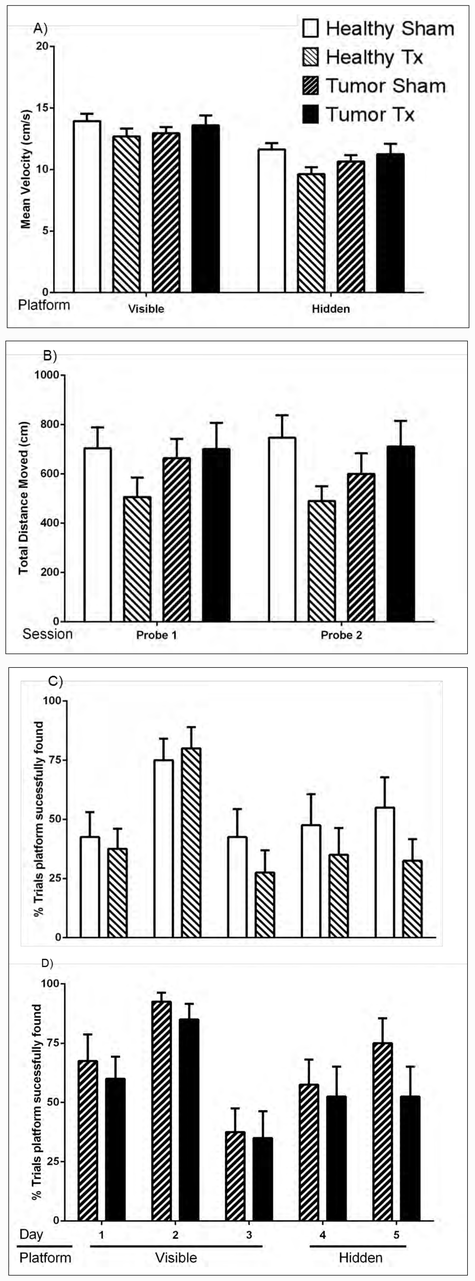

Figure 4. Healthy mice treated with combination therapy show reduced performance in the water maze. Mice were trained in the visible platform (non-spatial) and hidden platform (spatial) components, and spatial bias was tested using a probe trial 24 h following hidden platform training. Shown are (A) the average velocity during visible and hidden training sessions, (B) total distance moved during each probe trial, and percent of visible and hidden platform training sessions in which the platform was successfully found by day for (C) healthy and (D) CT26 mice. N = 10 mice/group. Error bars are S.E.M.

Treated mice show no impairment in hippocampus-dependent contextual fear conditioning

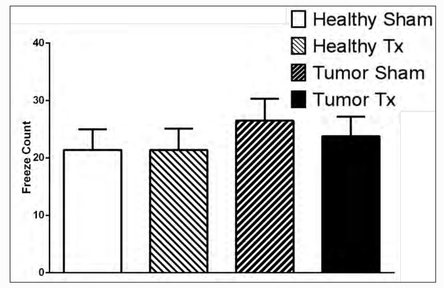

Figure 5. Mice treated with combination therapy do not show impaired hippocampus-dependent fear responses as demonstrated by freeze count in conditioned environment 24 h after training. Shown is the percent of time spent freezing in conditioned context on test day, 24 h after training. N = 10 mice/group. Error bars are S.E.M.

Treated mice show impaired fear response to hippocampus-independent cued fear conditioning

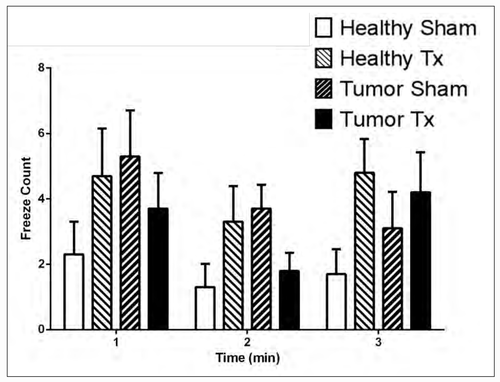

Figure 6. Healthy and CT26 tumor mice treated with combination therapy show impaired hippocampus-independent fear response as demonstrated by decreased freeze count in response to cued stimulus 24 h after training. Shown is the count of instances of freezing behavior during conditioned stimulus, 24 h after training. N = 10 mice/group. Error bars are S.E.M.

Discussion

- Combination treatment of anti-CTLA4 immunotherapy and radiation therapy:
  - Decreases measures of anxiety.
  - Reduces performance in the water maze.
  - Impairs hippocampus-independent cued fear memory.
- Some of these effects are more pronounced in healthy mice than mice with CT26 colorectal tumors.
- These results indicate the importance of including healthy mice (without tumors) in assessing the effects of combination therapy on the brain.

Future Perspective

- Planned immunohistochemistry and multiplex cytokine assays using tissues from these mice may elucidate the mechanisms underlying these effects.
- As BALB/c mice are relatively poor learners in hippocampus-dependent cognitive tests, experiments with strains that perform well on such tests will be used in future studies.

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