Combining tumor-targeted superantigens with anti-CTLA-4 results in synergistic anti-tumor effects in B16 tumor bearing mice

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Introduction

Tumor-targeted superantigens (TTSs) utilize the powerful T cell activating property of superantigens such as staphylococcal enterotoxin A (SEA) in fusion with anti-tumor Fab-fragments to target T cells against tumor cells. TTSs show anti-tumor activity in a number of experimental tumor models including the B16 mouse melanoma transfected with the human tumor-associated antigen EpCam recognized by the C215 monoclonal antibody.

The TTS C215Fab-SEA activated T cells with certain TCR Vβ expression to produce predominantly TH1 cytokines resulting in expansion and differentiation of these cells into T effector cells such as cytotoxic T lymphocytes (CTLs). Treatment with C215Fab-SEA resulted in potent tumor inhibition and prolonged survival of C57Bl/6 mice with B16-EpCam lung metastases. EpCam expressing tumors were infiltrated and targeted by $CD4^+$ and $CD8^+$ T cells within 48 h after initiating C215Fab-SEA treatment. In parallel $Foxp3^+$ Treg cells infiltrated the tumors as potential modulators of the superantigen induced TH1 response. The TTS induced T cell activity was dampened and short term reactivation was inhibited possibly due to the suppressive effects by the Treg cells.

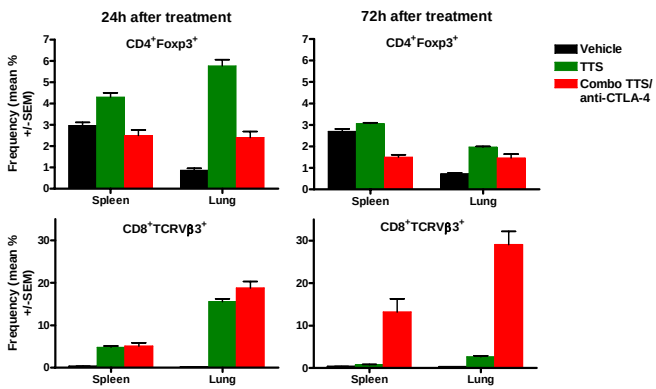
It has been well established that anti-CTLA-4 antibodies can interfere with the activation of Treg cells. Here we have studied the expansion of Treg cells, the CTL activity and anti-tumor effects of combining C215Fab-SEA with anti-CTLA-4 treatment.

Materials and Methods

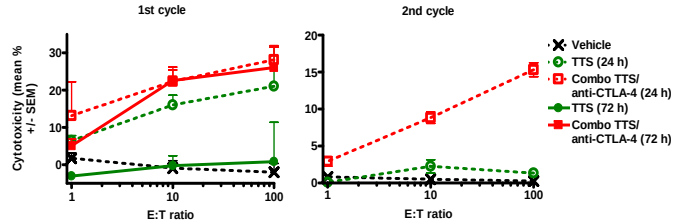
- Tumor model: C57Bl/6 mice were inoculated i.v. with EpCam-tranfected murine B16-F10 (B16-EpCam) melanoma cells into the tail vein to induce lung tumors.
- Treatment: Recombinant C215Fab-SEA was expressed in E.coli. Mice were routinely given cycles of 3 or 4 daily injections with C215Fab-SEA (0.5 or 10 μg/mouse) i.v. Anti-CTLA-4 antibody was administered i.p. at 200 μg/mouse.
- Flow cytometric analysis of splenocytes and lung cells was performed 24 and 72 hours after the last TTS treatment according to standard settings on a FACSCanto II™ flow cytometer.
- Cytotoxicity of spleen cells from treated mice against SEA-coated A20 tumor cells was measured at various effector to target (E:T) ratios in a standard 4-hours ^{51}Cr-release assay. Spleens were removed 24 and 72 hours after the last TTS treatment.
- On day 21 mice were sacrificed and the lungs were removed. After fixation in Bouins solution, the numbers of lung tumors were counted.
- In the survival experiments, animals were sacrificed when showing signs of morbidity or otherwise 90 days after tumor inoculation.

Results

TTS and anti-CTLA-4 in combination reduces the frequency of Treg cells and increases the frequency of $CD8^+$ CTLs in the spleen and lungs of B16-EpCam lung metastases bearing mice.



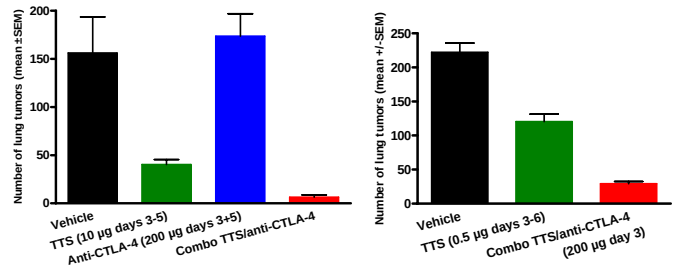
TTS and anti-CTLA-4 in combination prolongs the splenocyte cytotoxic activity in the first treatment cycle and maintains it in the second.



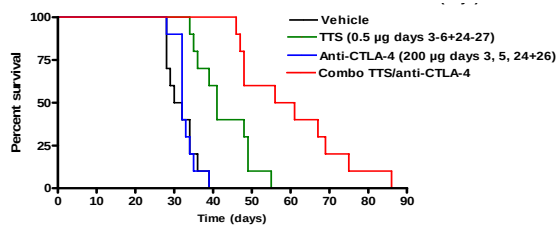
TTS and anti-CTLA-4 in combination synergistically reduces the number of lung metastases in B16-EpCam tumor bearing mice.

2 doses of 200 μg anti-CTLA-4 i.p. have no anti-tumor effect. 3 daily 10 μg C215Fab-SEA (TTS) i.v. doses reduce the number of tumor metastases. The combo shows anti-tumor synergy.

4 daily 0.5 μg C215Fab-SEA (TTS) i.v. doses reduce the number of tumor metastases. The combo shows enhanced anti-tumor effect.



TTS and anti-CTLA-4 in combination synergistically prolongs long-term survival in B16-EpCam tumor bearing mice.



Conclusion

The combination of TTS and anti-CTLA-4 for treatment of B16-EpCam tumors showed synergy as compared to the monotherapies. It can therefore be concluded that the potent anti-tumor effects by TTS therapy can be further enhanced by interfering with Treg cells in this tumor model. The expansion of Treg cells was inhibited and the CTL activity was enhanced.

References

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