A population of ectoenzyme expressing T-cells is associated with immunotherapy resistance in metastatic melanoma patients

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Abstract

Therapies targeting T-cell checkpoints have resulted in durable antitumor responses leading to FDA approval of immunotherapies for metastatic melanoma and in extending the list of other tumors for which PD-1/PD-L1 inhibition is approved. Despite the striking clinical responses to anti-CTLA-4 and anti-PD-1/PD-L1 antibodies, patients with advanced-stage disease are rarely cured, with a median survival of $<2$ years. Here, we hypothesize that the presence of ectoenzyme expressing T-cells may predict response to immunotherapy and that these T-cells are associated with resistance to these treatments.

Figure 1

Figure 2. Tmem frequencies correlate with other peripheral blood immunosuppressive populations. Peripheral blood samples from melanoma patients treated with sequential nivolumab-ipilimumab or ipilimumab-nivolumab were evaluated by flow cytometry. The frequency of Tmem was determined in the CD3+/CD8+ T-cell population of the CD28-/+CD127-/+CD39-/+CD57- population (A) and compared to the frequency of the Tmem population of the CD28-/+CD127-/+CD39-/+CD45RO- population (B). Tmem frequencies were determined by an unpaired t-test. (C) Paired T-tests were used to determine significance of the frequencies of Tmem in patients with and without disease progression. Significance was determined by an unpaired t-test.

Figure 3. Phenotypic analysis of Tmem in metastatic melanoma patients PBMC. Metastatic melanoma patients PBMC were evaluated by flow cytometry. (A) CD3+CD4+CD127-/+CD39-/+CD57- T-cells were gated and the representative graph of CD138 vs. CD28 is shown. (B) Patient T-cells were activated via CD3/CD28 Dynabeads and the four CD28/39 quadrants of the CD28/39/CD57/CD127-/+CD39-/+CD57- population evaluated by intracellular flow cytometry for TGFβ expression. A representative histogram of expression is shown in each left panel, and analysis in 30 patient samples shown in the right panels. Significance was determined by repeated measures ANOVA with Tukey’s post-hoc tests comparing all groups.

Figure 4. Tmem suppress autologous TSSL. (A) CD3+/CD4+CD127-/+CD39-/+CD57- T-cells were sorted from patient samples and cultured with TGFβ. Patient-derived TGFβ labeled CD3+ T-cells were cocultured with autologous Tmem, and the frequencies of Tmem were determined by an unpaired t-test. The Tmem population of the TGFβ CD3+T-cells was compared to the TGFβ population of the TGFβ CD3+T-cells. Significance was determined by an unpaired t-test.

Take-Aways

- Relative high peripheral blood frequencies of CD3+/CD4+CD127-/+CD39-/+CD57- T-cells are associated with immunotherapy resistance in melanoma patients.
- Tmem cells correlate with increased frequencies of immunosuppressive populations in patients.
- Tmem cells have a phenotype associated with immunosuppression including high expression of adenosine generating ectoenzymes, PD1L, TGFβ, and IL-10.
- Tmem cells suppress the function of autologous T-cells
- Tmem cells are present in tumor-bearing mice and negatively associated with overall immune infiltrate into the tumor.

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