Emlentar Tumor Infiltrating Lymphocytes (TIL) Profoundly Differ from Remnant T cells

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ABSTRACT

Adoptive T cell therapy with autologous tumor infiltrating lymphocytes (TIL) provides an attractive objective response rate and a complex response in 24% of patients with metastatic melanomas. The process of generating TIL from resected tumor involves manipulating the tumor into 1-3 mm fragments and expanding TIL in the presence of IL-2 (c) and a pre-Rapid Expansion Protocol (pre-REP). During the pre-REP, tumor-resident immune cells are enriched (eTIL) and proliferate. The length of the pre-REP typically varies between 1-2 days, depending on cell growth. Resident tumor fragments (remnants) are discarded and the expanded TILs are subjected to a Rapid Expansion Protocol (REP) with irradiated PBMC feeder, anti-CD3 and IL-2. Viable cells remaining in the tumor remnants (rTIL) following the pre-REP were investigated to assess their function and phenotype. We evaluated and compared the TIL and eTIL in melanomas, breast, renal, pancreatic, lung and colorectal tumors (n=9). Tumor resident T cells are consistently phenotypically distinct from circulating T cells and are determined by differential expression of various markers (Table 1). The fundamental differences in TIL were: Increased CD69 (7 fold MFI in CD4+) (p<0.001), deviated LAG3 (low MFI in CD8+) (p=0.02), TIM3 (3 and 2 fold MFI in CD8 and CD4 respectively) (p=0.01), CD34 (3 fold MFI in CD8+) (p<0.001) and CD3 (p<0.05). A REP of rTIL and eTIL resulted in comparable expression. The phenotypic signature of TIL was sustained post REP and included expression of Tim3, PD-1, and CD28. These studies have identified notable differences in the biology of self-perpetuating tumor-resident T cells and the signals associated with exhaustion and retention. These data provide additional insight into the biological TIL populations that should be used for adoptive T cell therapy in patients and raise important questions about the nature of tissue-resident T cells in sites of chronic inflammation such as tumor.

RESULTS

TIL have reduced NK cells and phenotypically resemble a tissue-resident memory T cell

Table 1. TIL or TILs from melanoma tumors were isolated from patients (n=9). T cell phenotypes were assessed by flow cytometry (n=9). p values represent the difference between rTIL and eTIL using student’s unpaired T test.

Tumor resident remnant T-cells are phenotypically distinct from emigrating T-cells

rTIL demonstrate a less exhausted phenotype compared to eTIL

rTIL have greater metabolic capacity than eTIL

rTIL expand and remain phenotypically distinct from eTIL during the REP

Figure 1. The tumor is excised from the patient and transported to the GMP Manufacturing facility. Upon arrival the tumor is fragmented and placed in G-Rex flasks with 0.2 % TIL expansion (pre-REP expansion). TIL cells that emigrate out of the tumor in response to IL-2. The TIL are tumor resident cells that are isolated from a syngeneic aliquot of tumor remnants. The eTIL and rTIL are cultured with feeders and OKT3 for REP expansion.