NON-FUNCTIONAL T CELL RESPONSES IN NON-SMALL CELL LUNG CANCER ARE INDUCED DURING TUMOR ANTIGEN-SPECIFIC T CELL PRIMING IN THE MEDIASTINAL LYMPH NODE

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Background In non-small cell lung cancer (NSCLC), response to checkpoint blockade therapy (CBT) is associated with tumor-infiltrating CD8+ T cells, but not all T cell-infiltrated tumors respond to CBT. The subgroup of T cell-infiltrated but CBT-resistant tumors has been clinically described as containing "non-functional" T cell responses. Mechanisms governing the generation of non-functional T cell responses remain poorly understood, and treatment options for this subgroup are limited.

Methods We utilized a transplantable, syngeneic murine NSCLC cell line derived from an autochthonous NSCLC driven by Kras^{G12D} expression and p53 deletion (KP cell line) to model non-functional T cell responses. To study antigen-specific responses, we engineered KP cells to express the model CD8+ T cell antigen SIY for certain experiments. CBT consisted of combined anti-CTLA-4 and anti-PD-L1 therapy.

Results Orthotopic KP lung tumors failed to respond to CBT, but KP flank tumors were controlled by CBT. Lung and flank tumors contained activated CD8+ T cells, providing a platform to compare functional and non-functional CD8+ T cell responses in NSCLC. Single-cell RNA sequencing revealed that lung tumor-infiltrating CD8+ T cells lacked effector and exhaustion molecules despite clonal expansion. Analysis of antigen-specific CD8+ T cells revealed that this lung cancer-specific T cell dysfunction was established during priming in lung-draining mediastinal lymph nodes (mLN) despite robust T cell proliferation. RNA sequencing and flow cytometry of antigen-specific CD8+ T cells found that T cells primed in the mLN underwent blunted effector differentiation characterized by a lack of effector molecules CD25, Granzyme B, and TIM-3, but retention of TCF-1. This phenotype persisted in lung tumors, consistent with our initial observations of absent effector and exhaustion molecule expression. Many CD8+ T cells from NSCLC patients expressed an analogous gene expression program distinct from T cell exhaustion. TCF-1+ CD8+ T cells in lung tumors did not mediate tumor control and failed to differentiate into effector cells after CBT. To investigate alternative therapeutic strategies of reinvigorating lung tumor-reactive T cells, we focused on IL-2 and IL-12, as expression of their receptors was reduced in mLN-primed T cells. Administering recombinant IL-2 and IL-12 was sufficient to restore effector T cell differentiation, induce lung tumor control, and significantly extend survival of lung tumor-bearing mice.

Conclusions Our results suggest that non-functional CD8+ T cell responses in NSCLC arise from failed effector T cell differentiation during priming. Transient combination therapy with IL-2 and IL-12 overcomes this dysfunctional state to induce protective T cell responses in CBT-resistant tumors.

Ethics Approval All mouse experiments were approved by MIT's Committee on Animal Care (CAC) - DHHS Animal Welfare Assurance # D16-00078

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.303>