

1087 **ADDITION OF IL-2 OVERCOMES LUNG TUMOR
RESISTANCE TO IL-12 BY COORDINATING CYTOTOXIC
AND REGULATORY T CELL RESPONSES**

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Background IL-12 is a pleiotropic cytokine with potent T cell stimulatory ability that can induce tumor regression in pre-clinical models. Translation of IL-12 has been hampered by high toxicity and a lack of efficacy. While many efforts have been made to engineer IL-12 to be less toxic and more effective, our understanding of resistance to IL-12 is limited due to a lack of appropriate model systems. We have identified a model of IL-12-resistant lung cancer that allows us to probe mechanisms of IL-12 response and resistance.

Methods We utilized the syngeneic, transplantable murine lung cancer KP cell line to analyze the response to extended half-life IL-12 or IL-2 fused to murine serum albumin (IL12-MSA, MSA-IL2). Expression of the CD8⁺ T cell antigen SIY in KP cells (KPSIY) allowed the tracking of antigen-specific anti-tumor immune responses.

Results IL12-MSA induced significant tumor control and improved survival of mice with subcutaneous KP flank tumors. However, mice inoculated intravenously with KP cells to form metastatic lung tumors derived no survival benefit from IL12-MSA. Using KPSIY cells, we determined that successful IL12-MSA immunotherapy in the flank setting involved significant expansion and effector differentiation of SIY-reactive CD8⁺ T cells in the tumor-draining lymph node (TdLN), and the depletion of CD4⁺ regulatory T cells (T_{reg}) from the tumor microenvironment. In the lung tumor setting, however, T cells were unresponsive to IL12-MSA, as SIY-reactive T cells did not expand in lung TdLNs and did not upregulate the effector molecules CD25, GzmB, and TIM-3. Using fluorescently labeled IL12-MSA, we found significantly reduced IL-12 binding by SIY-reactive CD8⁺ T cells and T_{reg} in the lung tumor setting, explaining the lack of IL12-MSA efficacy against lung tumors. However, combined MSA-IL2 and IL12-MSA improved lung tumor control and synergistically enhanced CD8⁺ T cell activation. In lung tumors and lung TdLNs, the addition of MSA-IL2 increased the binding of IL12-MSA by T_{reg}, suggesting that IL-2 sensitized lung tumors to IL-12 through a coordinated enhancement of tumor-reactive CD8⁺ T cell activation with a concomitant inhibition of T_{reg}.

Conclusions Resistance to IL-12 can be mediated by tumor-reactive T cells with low IL-12 receptor expression, therefore IL-12 receptor expression should be considered when administering IL-12-based therapies. IL-2 can sensitize T cell responses to IL-12 through a feed-forward mechanism of CD8⁺ T cell activation and concurrent T_{reg} inhibition.

Ethics Approval The experiments in this abstract were approved by the Massachusetts Institute of Technology Committee on Animal Care, Protocol Number 0220-006-23, NIH Assurance Number D16-00078.

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