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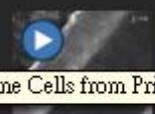
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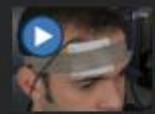
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Stephanie K. Watkins¹, Ziqiang Zhu¹, Keith E. Watkins², Arthur A. Hurwitz¹

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Abstract

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Isolation of Immune Cells from Primary Tumors

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Abstract

Tumors create a unique immunosuppressive microenvironment (tumor microenvironment, TME) whereby leukocytes are recruited into the tumor by various chemokines and growth factors^{1,2}. However, once in the TME, these cells lose the ability to promote anti-tumor immunity and begin to support tumor growth and down-regulate anti-tumor immune responses^{3,4}. Studies on tumor-associated leukocytes have mainly focused on cells isolated from tumor-draining lymph nodes or spleen due to the inherent difficulties in obtaining sufficient cell numbers and purity from the primary tumor. While identifying the mechanisms of cell activation and trafficking through the lymphatic system of tumor bearing mice is important and may give insight to the kinetics of immune responses to cancer, in our experience, many leukocytes, including dendritic cells (DCs), in tumor-draining lymph nodes have a different phenotype than those that infiltrate tumors^{5,6}. Furthermore, we have previously demonstrated that adoptively-transferred T cells isolated from the tumor-draining lymph nodes are not tolerized and are capable of responding to secondary stimulation in vitro unlike T cells isolated from the TME, which are tolerized and incapable of proliferation or cytokine production^{7,8}. Interestingly, we have shown that changing the tumor microenvironment, such as providing CD4⁺ T helper cells via adoptive transfer, promotes CD8⁺ T cells to maintain pro-inflammatory effector functions⁵. The results from each of the previously mentioned studies demonstrate the importance of measuring cellular responses from TME-infiltrating immune cells as opposed to cells that remain in the periphery. To study the function of immune cells which infiltrate tumors using the Miltenyi Biotec isolation system⁹, we have modified and optimized this antibody-based isolation procedure to obtain highly enriched populations of antigen presenting cells and tumor antigen-specific cytotoxic T lymphocytes. The protocol includes a detailed description of various methods to derive a comprehensive panel of CD8⁺ tumor-infiltrating lymphocytes from the mouse.

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