Online Proceedings

Poster Session 2

Commons East, Easel 47

1:00 PM to 2:30 PM

Characterization of Necroptotic Tumor Cell Signals and Contribution to Tumor Control

Kristy Chiang, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Andrew Oberst, Immunology
Mentor: Annelise Snyder, Immunology

Necroptosis is a lytic and immunogenic form of programmed cell death (PCD), characterized by pro-inflammatory cytokine production and the release of intracellular molecules, including cell-associated antigens. This form of death occurs after RIPK3, Receptor-Interacting Protein Kinase 3, activation. RIPK3 is a cell death regulator that promotes necroptosis. Previous studies have shown that necroptotic cells can promote maturation of antigen presenting cells (APCs) and are capable of priming antigen-specific CD8+ T cell responses. Understanding the downstream immunogenic effects of necroptosis allows for implicative applications within the field of tumor immunology. Previous work has shown that necroptotic NIH-3T3 fibroblasts produce inflammatory cytokines and chemokines, contributing to their immunogenicity. To recapitulate the immunogenic nature of 3T3s, we evaluated inflammatory chemokine and cytokine production via ELISA (enzyme-linked immunosorbent assay), quantifying these productions through antibodies and color-change, from necroptotic tumor cell line supernatants, including B16.F10 melanoma and LL/2 adenocarcinoma cells. Surprisingly, our results did not show significant chemokine or cytokine production from necroptotic tumor cells, suggesting that anti-tumor effects are not from signals derived from the tumor micro-environment (TME). This suggests that other forms of signaling downstream of RIPK3 activation, such as up-regulation of non-chemokine transcriptional programs, could be the source of inflammatory signal production by necroptotic tumor cells. Further actions include RNA sequencing of necroptotic cancer cell lines to differentiate global changes between apoptotic and necroptotic forms of cell death, to provide unbiased analysis of transcriptional changes. With evidence of T cell-mediated immunity and the immunogenic nature of necroptosis, induction of this form of cell death within the TME will activate anti-tumor immune responses, potentially controlling tumor growth and contributing to the field of immunotherapy.

Session 2B

Enhancing Immune Responses Targeting Infection, Injury and Cancer

Session Moderator: Kristin Anderson, Immunology

MGH 228

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Immunotherapy for Ovarian Cancer: Combining Engineered T Cell Therapy with Immune Checkpoint Blockade

Maddie Grace Burnett, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Kristin Anderson, Immunology, Fred Hutchinson Cancer Research Center
Mentor: Philip Greenberg, Medicine and Immunology

Ovarian cancer is the deadliest of gynecological malignancies, with approximately 70% of patients diagnosed at an advanced stage of disease. With the current standard of care, cytoreductive surgery plus chemotherapy, the relapse rate is about 70% and the 5-year survival rate is less than 50%. Innovative therapeutic interventions are greatly needed, and one promising new strategy mimics the body's endogenous anti-cancer immune response. This immunotherapy approach uses donor T cells engineered to express a T cell receptor (TCR) that is specific for a tumor-expressed antigen. Mesothelin (MSLN) is a validated antigen target, frequently over-expressed in cancer cells compared to healthy tissues. Using the mouse ID8 ovarian tumor model, we found that T cells engineered to express a high-affinity MSLN-specific TCR can kill ovarian cancer cells. However, the T cells lose effector function over time, which correlates with increased expression of the T cell inhibitory receptors PD-1, Tim-3 and Lag-3, markers of T cell exhaustion. Signaling through inhibitory receptors is stimulated by respective ligands that we showed are present in the tumor microenvironment (TME). Blocking these "immune checkpoint" signals can improve T cell proliferation and anti-tumor function within various TMEs. By applying checkpoint blockade, we hypothesized that both

donor and endogenous T cells will have prolonged longevity and increased effector function in our ovarian cancer model. In ongoing studies, ID8 ovarian tumor-bearing mice received MSLN-specific T cells plus antibodies that block PD-1, Tim-3 and Lag-3 checkpoints; T cell phenotype and function were characterized at early and late time-points. We will discuss our findings and the potential relevance of this combination immunotherapy as a clinical treatment of ovarian cancer.

POSTER SESSION 3

Commons East, Easel 68

2:30 PM to 4:00 PM

STAT1 Activation Following Interferon Sensing Bifurcates Antiviral and Inflammatory Responses

Chia Heng Lee, Recent Graduate,
Mentor: Ram Savan, Immunology
Mentor: Adriana Forero

Type I and type III interferons (IFNs) are the key modulators in innate immunity that mediate antiviral responses to clear infections. It is known that the two families of IFNs signal through the same JAK/STAT signaling pathway to induce a similar antiviral response despite engaging different receptors. By utilizing the same signaling pathway, the two IFN systems converge upon a common transcription activation complex known as Interferon Stimulated Gene Factor (ISGF3), which induces gene expression once it is fully assembled. Intriguingly, their downstream responses differ greatly. While type I IFN induces a robust but pro-inflammatory response, type III IFN induces a more delayed but sustained response without triggering inflammation. The molecular mechanism governing this differential outcome remains poorly understood. We proposed that such outcome is due to a differential ISGF3 recruitment, which in turn governs the induction of pro-inflammatory chemokines. Since ISGF3 complex consists of STAT1, STAT2 and IRF9 subunits, and its gene transactivation ability is dependent on STAT1 and STAT2 activation, it is possible that differential phosphorylation of these transcription factors accounts for the initiation of the inflammatory responses. To investigate the functional implication of STAT1 and STAT2 activation, we stimulated STAT1-deficient U3A and STAT2-deficient U6A cells with type I IFN, and measured the expression of downstream genes that are known to be pro-inflammatory. Our results show that STAT1 is the crucial factor that accounts for the different outcome between the two IFN systems. Considering that type I IFN are used extensively to treat chronic viral infections, but its pro-inflammatory nature results in poor clinical outcomes, such understanding may elucidate a new therapeutic target that can be manipulated to minimize inflammation associated with IFN treatment.