Investigating Potential Mechanisms for Negative Regulation of T Cell Activation

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***Short Abstract* — Anti-tumor T cells quickly reach a state of unresponsiveness to antigenic stimuli in the tumor microenvironment. Inhibitory receptors, also known as immune checkpoints, are critically linked to this anergy. Yet, a definitive model of T cell activation that incorporates the effects of these molecules has not been established. Here, we have devised a minimal model of T cell activation that captures the spatial effects of LAG3, a negative regulator of TCR signaling. We show that our model successfully replicates experimental observations and provides insights into the mechanism by which LAG3 functions to limit T cell activity.**

***Keywords* — T Cell Activation, Immunological Synapse, LAG3, Exhaustion, Immunotherapy, Mathematical Modeling.**

T lymphocytes are a major class of immune cells responsible for the recognition of pathogens and other potentially harmful substances that may enter the body. Upon activation T cells proliferate, a process that is required for a successful defense against infections and other diseases, most importantly, cancer. T cell proliferation is tightly regulated because excessive proliferation can lead to systemic damage, such as occurs in auto-immune disease [1]. Inhibitory receptors, like PD1, CTLA4, LAG3, play a crucial role in regulating T cell activation. Persistent exposure to antigen in the tumor microenvironment can lead to a phenomenon called "exhaustion" [2], a state of immune cell dysfunction marked by sustained expression of inhibitory receptors. Exhaustion is thought to be an important reason anti-tumor T cells fail to contain tumors, making these inhibitors a potential target for cancer immunotherapy treatments. Here, we have developed a minimal spatial model, calibrated against experimental data, to investigate potential mechanisms for negative regulation of T cell activation by LAG3.

T cells are activated following successful recognition of a foreign antigen on the surface of antigen-presenting cells (APCs) by T cell receptors (TCRs). Triggering of TCRs leads to phosphorylation of tyrosine residues on the cytoplasmic portions of the receptor subunits by the tyrosine kinases LCK and FYN. Considerable phosphorylation of these immunoreceptor tyrosine-based activation motifs (ITAMs) occurs only when several TCR-containing complexes aggregate into a condensed structure known as the immunological synapse [3]. Another tyrosine kinase, ZAP70, is recruited to phosphorylated ITAMs and is also phosphorylated by LCK. Phosphorylated ZAP70 (pZAP70) then activates a downstream signaling cascade that leads to T cell proliferation [4].

LAG3 is an activation-induced cell surface molecule [5] that inhibits T cell activation in a currently unknown manner. Our experimental data shows that LAG3 rapidly localizes to the synapse by associating with TCRs upon antibody stimulation. To understand the spatial effects of this reorganization, we have formulated a compartmental model of early T cell signaling events that models the immune synapse as a partition on the cell surface. We hypothesize that TCR-mediated aggregation of LAG3 can result in significant CD4-LCK dissociation in the synapse. LCK separated from CD4 may not be suitably positioned to phosphorylate the ITAMs limiting downstream signal transduction [6]. Furthermore, the synapse excludes the phosphatase CD45, allowing sustained phosphorylation of ITAMs only in this region [7]. We simulated the model for both WT and LAG3-deficient cells and used pZAP70 and CD4-LCK association levels to calibrate our model. We also performed sensitivity analysis to determine the impact of various model parameters on pZAP70 levels.

Our model replicated trends in pZAP70 levels across experimental timepoints while maintaining the appropriate ratio between WT and LAG3 deficient regimes. While LAG3 is expected to cause dissociation of CD4-LCK, experimental findings showed that the ratio of CD4-LCK association increased in favor of WT cells upon stimulation. This counterintuitive behavior could be explained by trafficking of a majority of LAG3 into the synapse allowing CD4 and LCK, largely present outside, to form a stable association. Another surprising prediction of the model is that pZAP70 levels show a biphasic response to variation in the affinity of CD4-LCK binding. This behavior arises in the model because of the interplay between ZAP70 phosphorylation, which is activating on the one hand and targets it for degradation on the other. It is our hope that further refinement and analysis of the model will lead to a better understanding of the working of LAG3 and may lead to novel strategies for targeting it to boost immune responses to cancers of various types.

References

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