Class I HDAC's regulate T cell inhibitory checkpoint expression and exhaustion

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Background: Chronic antigen stimulation, a common feature of both cancer and persistent viral infection, leads to the development of a dysfunctional T cell state called exhaustion. Exhausted T cells are characterized by a loss of effector function and expression of inhibitory receptors like PD-1. Although there are multiple inhibitory receptors, little is known about the mechanisms that control patterns of expression. We hypothesized that chromatin accessibility and architecture plays an important role in dictating patterns of inhibitory molecule expression and regulating T cell exhaustion and that inhibitory receptor expression can be therapeutically targeted with epigenetic modifying drugs.

Methods: Human $CD8^+$ T cells from healthy donors were activated *in vitro* in the presence of cytokines and/or epigenetic modifying drugs. *In vivo*, mice were infected with Lymphocytic Choriomeningitis Virus (LCMV), and treated with either entinostat, a class I HDAC inhibitor or with vehicle control. Antigen-specific responses were analyzed using tetramer staining and multi-color flow cytometry, and *in vivo* cytotoxic T cell function was analyzed using an *in vivo* CTL assay.

Results: Inhibition of class I HDAC molecules differentially affected checkpoint molecule expression in antigen-specific $CD8^+$ T cells; TIM-3, CTLA-4, and LAG-3 expression was significantly increased whilst PD-1 expression was not affected. Surprisingly, this increase was associated with production of IFNγ and granzyme B. Using nanostring gene expression analysis, we found that at least two clusters of differential regulation of inhibitory molecules exist in $CD8^+$ T cells. Interestingly, PD-1, PD-L1, B7-H3, and ICOS expression were unaffected by class I HDAC inhibition. Conversely, a cluster defined by TIM-3, CTLA-4, TIGIT, LAG-3, and BTLA, was significantly upregulated by class I HDAC inhibition. To understand the functional implications of these results, experiments were carried out in the LCMV chronic infection system that induces profoundly exhausted, hypofunctional T cells. Mice treated with entinostat during the initial priming phase of the T cell response only went on to have high expression of TIM-3, CTLA-4, and LAG-3 long term. They also produced significantly more granzyme B and were capable of greater target cell lysis in an *in vivo* CTL assay. $CD8^+$ T cells from entinostat treated mice also showed a greater proportion of CD127+ memory precursors and a lower proportion of KLRG1+ short-lived effector cells.

Conclusions: In both human and mouse CD8+ T cells there are two clusters of differential regulation of inhibitory molecule expression. These patterns are regulated by class I HDACs. Inhibiting class I HDACs during T cell activation programs these cells for different patterns of checkpoint expression and is associated with significantly increased cytolytic function. These results suggest that combinations of checkpoint inhibitors and epigenetic modifying agents may be able to reprogram exhausted T cells in cancers where checkpoint inhibitors alone have been unsuccessful.

Conflicts of Interest: The authors declare no relevant conflicts of interest

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