**Introduction**

T cells develop and differentiate in the thymus. Cytokines and signals from antigen presenting cells cause thymocytes to differentiate into various functional classes, each with a unique gene expression pattern.

Naive helper (CD4+) and cytotoxic (CD8+) T cells further differentiate into central memory, effector memory, or terminally differentiated T cells. Central memory cells survive and proliferate for an extended period, allowing for subsequent immune responses. Effector memory cells release cytokines and die within a few days. Terminally differentiated cells have immediate inflammatory effects and low proliferative capacity.

Chimeric antigen receptor modified T cells (CAR-T cells) are effective antitumor agents when treating chronic lymphocytic leukemia and acute lymphoblastic leukemia. However, attempts to treat other types of leukemia, as well as solid tumors, with CAR-T cells have been less successful. One of the major barriers to widespread use of CAR-T cells is their toxicity.

CAR-T cells cause cytokine release syndrome (CRS), on-target off-tumor effects, and neurological toxicities. CRS occurs due to buildup of cytokines following the activation of immune cells, which leads to inflammation and disrupts the normal function of cells. CAR-T cells cause on-target off-tumor effects when they target antigens which present on both healthy and tumor cells. Several deaths in clinical trials have been attributed to these.

The addition of a “suicide gene” encoding the caspase-9 protein to CAR-T cells has been proposed as a method to decrease their toxicity. This strategy uses rimiducid (AP1903), a drug that causes dimerization of the caspase-9 protein. Dimerization of caspase-9 domains leads to CAR-T cell death and thereby prevents inflammation and off-tumor effects.

Early trials have found that the “suicide gene” and rimiducid are able to decrease the toxicity of CAR-T cells. However, the effect of this drug on the native population of T cells is unknown.

**Objective**

To determine if rimiducid influences the development and differentiation of human T cells. If this drug is to be used clinically, it is important to understand any effects it may have on the immune system.

**Methods**

Human peripheral blood mononuclear cells (PBMCs) were isolated from buffy coat by layering with Ficoll-HISTOPAQUE-1077 and centrifugation. PBMCs were divided into three time points (days four, six, and seven) and three treatments. The control group was not treated. The stimulation group was treated with CD3 and CD28. The rimiducid group was treated with CD3 and CD28 as well as 2μL of 1mM rimiducid. Cells were mixed with antibodies for various markers of T cell development and differentiation and were analyzed using flow cytometry.

**Results**

**Figure 1: Differentiation of thymocytes into helper and cytotoxic T cells.** A. Flow cytometry data showing fluorescence of single T cells for markers of CD4 and CD8. B. Average percentage of CD4+ cells. Error bars represent one standard deviation. C. Average percentage of CD8+ cells. Error bars represent one standard deviation. Rimiducid appears to increase the percentage of helper T cells (p=.0159) and not have an effect on the abundance of cytotoxic T cells.

**Figure 2: Development of helper T cells.** A. Flow cytometry data showing fluorescence of helper T cells for markers of CD45RA and CD27. B. Average percentage of cell types on day six. Error bars represent one standard deviation. C. Average percentage of cell types on day four. Error bars represent one standard deviation. Rimiducid does not appear to affect the development of CD8 T cells.

**Figure 3: Development of cytotoxic T cells.** A. Flow cytometry data showing fluorescence of cytotoxic T cells for markers of CD45RA and CD27. B. Average percentage of cell types on day seven. Error bars represent one standard deviation. Rimiducid does not appear to affect the development of CD8 T cells.

**Conclusions**

Treatment with rimiducid increases the prevalence of helper T cells (CD4) in vitro. It also increases the prevalence of naive helper T cells while decreasing the prevalence of central memory and terminally differentiated helper T cells.

Rimiducid does not appear to affect the prevalence of cytotoxic (CD8) T cells or the differentiation of these cells in vitro.

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