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Optimization of Fluorescent Phagocytic Assay Using Apoptotic Cells

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Sjögren's Syndrome is a systemic autoimmune disease that primarily affects the exocrine glands and is characterized by severe dry eyes and mouth. Previous studies have shown that there are elevated levels of the microRNA miR-146a in Sjögren's patients. Mir-146a is a microRNA that has been found to be involved in down regulating inflammation. Yet, in patients with Sjögren's Syndrome, there is a large upregulation of miR-146a that exists alongside chronic inflammation. This led us to investigate the role of miR-146a in Sjögren's Syndrome. We found that miR-146a upregulates phagocytosis of E. coli by human macrophages. Therefore, we hypothesized that this upregulation of phagocytosis should also apply to apoptotic cells. In order to test this hypothesis, we had to optimize the induction of apoptosis in Jurkat cells and fluorescently label them for the phagocytosis assay. We induced apoptosis using a topoisomerase inhibitor etoposide and performed a dose response curve to determine the optimal etoposide concentration. We then assessed Jurkat viability using trypan blue exclusion and Annexin V staining. We then fluorescently labeled the apoptotic cells with phrodo staining. Phrodo is a pH-sensitive fluorophore that only fluoresces in acidic pH. Finally we co-incubated the fluorescently labeled apoptotic jurkats with human macrophages (THP-1 cells) at a 4:1 ratio for the phagocytosis assay. We tested 10 to 100 micromolar concentrations of etoposide and found the 40 micromolar concentration yielded optimal levels of apoptosis in the Jurkat cells. The phrodo staining procedure was developed to fluorescently label the apoptotic cells. Lastly, we performed the phagocytosis assay by incubating the fluorescently labeled apoptotic jurkat cells with THP-1 human macrophages which resulted in phagocytosis of the apoptotic Jurkats. The conditions for the assay were optimized, and we plan to continue further research on miR-146a to investigate its effect on the phagocytosis of apoptotic cells.