Increased T Cell Turnover in Sickle Cell Disease

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Introduction

People living with Sickle Cell Disease (SCD) are known to have higher levels of inflammation which play a prominent roll in disease pathogenesis. When isolating PBMCs from these individuals we noticed a proportion of their lymphocytes were smaller in size compared to healthy controls. This led us to ask if lymphocytes turnover faster in SCD. To characterize their turnover we first focused on CD4+ T cells, the longest lived lymphocytes using an ex vivo model. Our ex vivo culture system includes fibroblastic reticular cells (FRCs) which are stromal cells that promote T-cell survival and maintenance of phenotype.

Methods

Red cell waste products from consented individuals with sickle cell disease were used to isolate PBMCs by Ficoll gradient. Control PBMCs were similarly isolated from apheresis products of healthcare workers. In three healthy donors and three donors with SCD, CD4s and CD8s were purified and labeled with eFluor 670 proliferation dye and cultured in media with 30% FCS with and without FRCs for seven days. Samples were collected daily and stained with naïve and memory T cell markers. CountBright beads were added to obtain absolute cell counts and eFluor 780 live/dead dye was used to identify cell death. Flow cytometric analysis was performed to track cell division and death. B cells and NK cells were similarly tracked.

Results

Higher rates of division and death were observed in CD4 and CD8 T cells isolated from donors living with SCD on and off FRCs. Consistent with literature, FRCs promoted survival, the original cellular phenotype and reduced the frequency of cell division in both cohorts. Notably, T lymphocytes from donors with SCD were observed to undergo more proliferation compared to healthy donors. Immunophenotyping performed on the CD4 and CD8 T-lymphocytes showed a propensity for memory cells to undergo proliferation.

Conclusion

Our data suggests that T lymphocytes undergo faster turnover in individuals living with SCD when compared to healthy donors. This suggests that the hyperinflammatory state in SCD has a large impact on lymphocyte dynamics which may have important implications in the era of gene therapy.

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