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Understanding lymphopenia through the role of activation and death cell pathway of T lymphocytes in multiple sclerosis patients treated with dimethyl fumarate

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Abstract

Introduction

The dimethyl fumarate (DMF) is a drug indicated to patients with form remitting/relapsing of the MS, being administrated orally and daily. DMF can cause side effects such as gastrointestinal disorders and lymphopenia. The studies of lymphopenia of the patients are extremely important, thus the reduction of lymphocytes count could result in immunosuppression and predispose the MS patients to opportunistic infections. Therefore, the reason to undesirable reaction could be understood through IL-2 pathway in T lymphocyte activation and death cell pathway to same lymphocytes, besides T lymphocyte activation mediated by monocytes and dendritic cells, which these innate cells perform the induction by IL-27 cytokine. The DMF contribute with treatment acting in nuclear factor (erythroid-derived 2)-like 2 (NRF2) pathway that results in anti-oxidant responses and may to inhibit of NF-KB causing

downregulation pro-inflammatory cytokines that drives proliferation and activation prohibition in T lymphocytes. Although of the benefits effects, there are a lot study to understand DMF action to comprehend the side effects.

Objective

To understand the contribution of the IL-2 pathway in T lymphocyte activation to appearance of lymphopenia. To evaluated difference cellular proliferation of T lymphocytes from PBMC culture between patients treated with DMF and heathy individuals. To analyze intracellular and extracellular metabolic system of the IL-2 and IL-27, evaluating the genetic expression as well as the presence of those cytokines in extracellular environment. Finally, to evaluated expression of IL-2 receptor, and death receptors from TRAILs and FAS pathway on cellular membrane from T lymphocytes.

Methodology

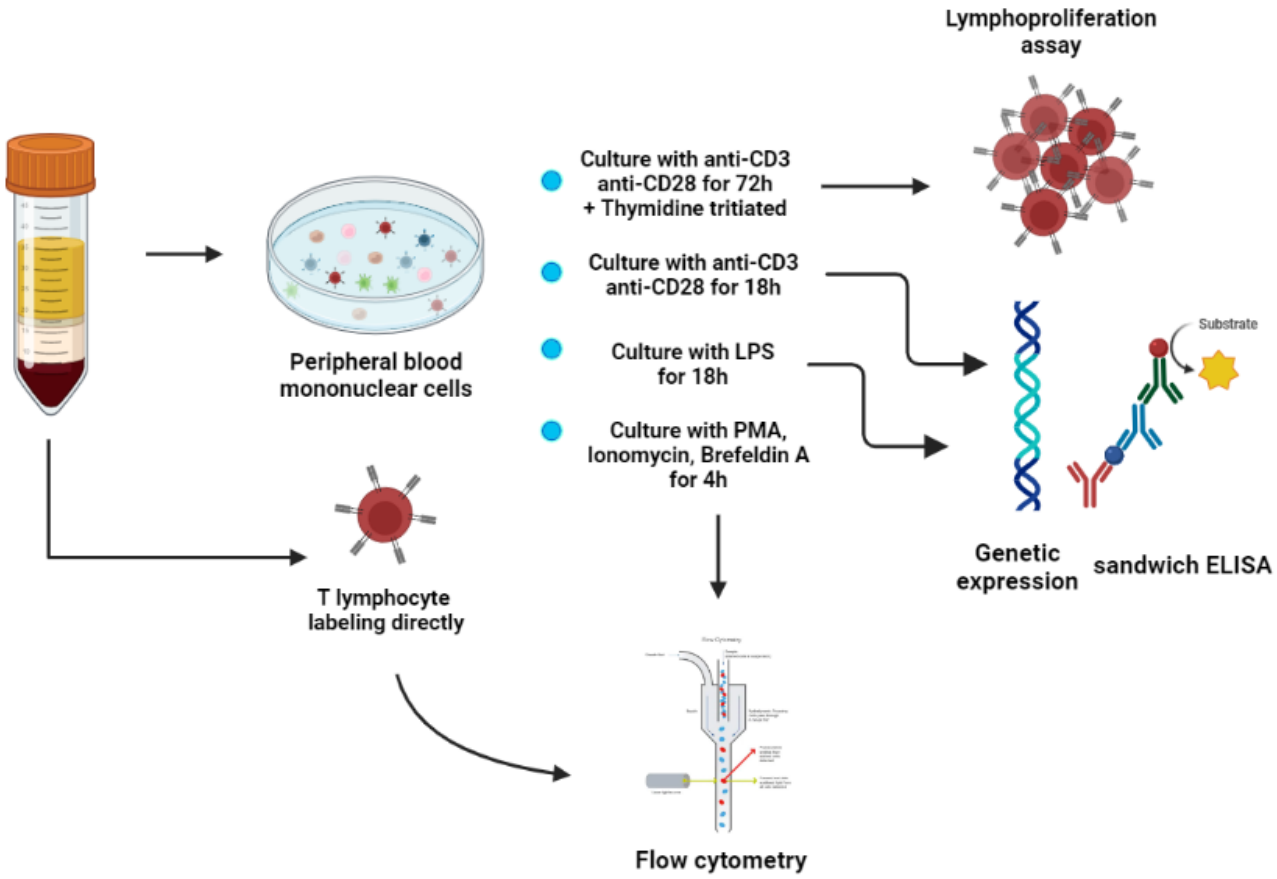

Figure 01 – General methodology. Created with BioRender.com.

Blood was collected and separated through the gradient separation technique to obtain the peripheral blood mononuclear cells (PBMC). As indicated in figure 01, once we got PBMC, part of them were directed to culture cell with anti-CD3 and anti-CD28 for 72h with tritiated thymidine added before 18h to 72h of culture, to evaluated lymphoproliferation of PBMCs, especially T lymphocytes. Another part we directed to two types of culture cells, one with anti-CD3 and antI-CD28 to T lymphocyte stimulation and another one with LPS to monocytes e dendritic cells stimulation, both in culture for 18 hours to evaluate expression of IL-2 and IL-27 by Real-Time PCR and production by sandwich ELISA. Also, from PBMC part of them were directed to flow cytometry directly with T lymphocyte labels to $CD4^+$ and $CD8^+$, being labeled all subunits of the IL-2 receptor (CD122, CD132 and CD25) some death receptors (CD95, CD262 and CD263).

Results and discussions

The study shows that there is no significant reduction of T lymphocytes activation in MS patients treated with DMF. Also, the results about genetic expression shows high expression of the IL-2, IL-27 and ZFP36 and the extracellular environment shows less expression of the IL-2 and IL-27 that's could indicate deficient production. Besides that, there is discrete reduction in CD95 (FAS receptor) from CD8 and significative reduction from CD4 T lymphocytes in patients, and TRAIL pathway is showed small reduction of CD292 in CD4 T lymphocytes and small increase of CD292 in CD8 T lymphocytes and in regarding TRAIL pathway, the CD293 appearance bigger in both cell in patients treated DMF. This study shows that DMF could be inductor to apoptosis on T cell. Taking in consideration the small range of samples, these results bring the necessity of a bigger cohort analysis to validate the variances and tendencies.