Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: The effect of tofacitinib on peripheral blood leukocytes from early arthritis patients

Synopsis:

Rheumatoid arthritis (RA) is a chronic, systemic immune mediated disease that mainly affects joints, characterized by synovial inflammation, bone erosion and cartilage destruction. Disturbances in both innate and adaptive immune systems have been described in RA patients. Janus kinase (JAK) inhibitors or JAKi are a new class of oral medications recently approved for the treatment of RA. JAK inhibitors suppress the activity of one or more of the JAK family of tyrosine kinases [JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2)], thus interfering with the JAK-Signal Transducer and Activator of Transcription (STAT) signaling pathway. JAK-STAT signaling pathway is critical for immune cell proliferation, survival and differentiation and its inhibition leads to multi-cytokine blockade and abrogated inflammation. Tofacitinib, an oral JAK1 and JAK3 inhibitor, was recently approved for the treatment of RA patients who have had an inadequate response or intolerance to conventional synthetic or biologic disease modifying anti-rheumatic drugs (DMARDs). However, preliminary clinical evidence indicate that patients treated in an earlier phase of the disease have a better response. Furthermore, our group has previously demonstrated that early treatment with tofacitinib in animal models of arthritis can abrogate disease and completely prevent bone and cartilage damage. Therefore, we hypothesize that JAK-STAT signaling pathway is key to chronic arthritis onset and its early inhibition with tofacitinib might have a major effect on the immune cascade, allowing lasting disease control. The main goal of this study is to analyze the in vitro effect of tofacitinib on peripheral blood leukocytes (B cells, T cells, monocytes and dendritic cells) from untreated early arthritis patients. For that, blood samples will be collected from untreated early arthritis patients (<1 year of disease duration) followed up at the Rheumatology Department, Hospital de Santa Maria, Lisbon Academic Medical Centre, Portugal. A group of age and gender-matched healthy volunteers will be also included as controls. Peripheral blood mononuclear cells (PBMC) will be isolated by density-gradient centrifugation and cell viability will be estimated with Trypan Blue dye exclusion. Cells will be cultured during 24h-72 hours at 37ºC, 5% CO2 in complete medium, in the presence or in the absence of tofacitinib and appropriate cell stimuli. After culture, cells will be collected and JAK-STAT signaling pathway activation will be evaluated on peripheral blood leukocytes (B cells, T cells, monocytes and dendritic cells) by flow cytometry. In addition, supernatants will be harvested and stored at -80ºC to analyze antibody and cytokine production by enzyme-linked immunosorbent assay (ELISA) and/ or multiplex assay. All samples will be used for research purposes only. All the experimental work will be developed at Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa, Portugal.

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