Phenotypic Transition of Periodontitis-derived Macrophages

Cristina C. Villar*[1]; Mariane C. Sloniak[1]; Ana Paula Lepique[2]; Josiane B. Assis[2]; Luciana Saraiva[1]; Giuseppe A. Romito[1]

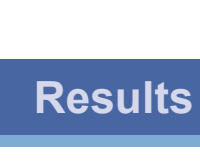
[1]Division of Periodontology, School of Dentistry, University of São Paulo, Brazil;
[2]Institute of Biomedical Sciences, University of São Paulo, Brazil

Objective

The aim of this ongoing study is to evaluate the ability of periodontitis-derived macrophages to polarize towards pro-inflammatory M1 and pro-healing M2 phenotypes and undergo phenotypic transition from M1 to M2 phenotype.

Methods

Study population



Systemically healthy subjects with history of generalized grade B periodontitis (n=10), generalized grade C periodontitis (n=10) and periodontally healthy subjects with no history of periodontitis (n=10) will be enrolled.

IRB # 1.981.738

Sample collection and processing

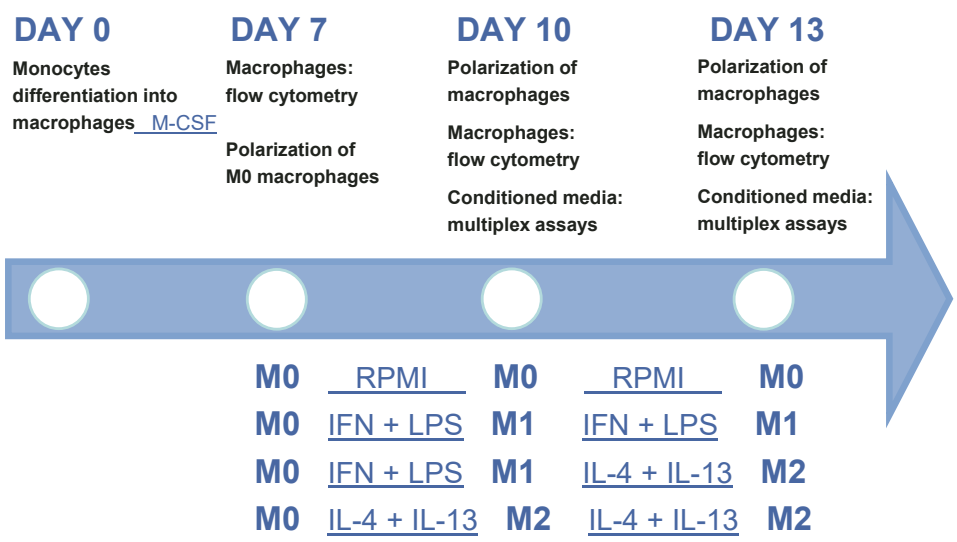
Thirty milliliters of peripheral blood will be drawn from all research subjects.

Freshly draw peripheral blood mononuclear cells (PBMC) will be isolated using Ficoll-Plaque.

CD14+ monocytes will be isolated by negative magnetic sorting.

Monocytes will be incubated in RPMI + macrophage colony-stimulating factor (M-CSF) for 7 days

Macrophage Differentiation and Polarization



Outcomes Variables

Characterization of macrophage phenotype:

M1: HLA-DR and CD197

M2: CD163 and CD206

Quantification of secreted cytokines, chemokines and growth factors

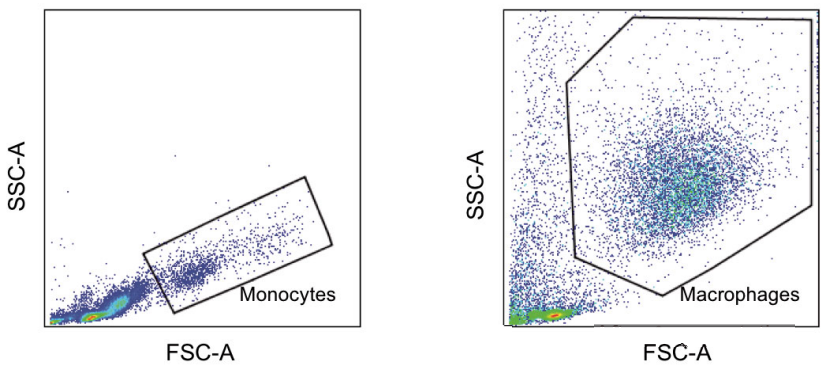
M1: interleukin (IL-)1β, IL-2, IL-6, IL-12, TNF-α, RANTES and vascular endothelial growth factor (VEGF).

M2: IL-1ra, IL-4, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), platelet-derived growth factor (PDGF)-BB, monocyte chemoattractant protein-1 (MCP-1) and IL-10.

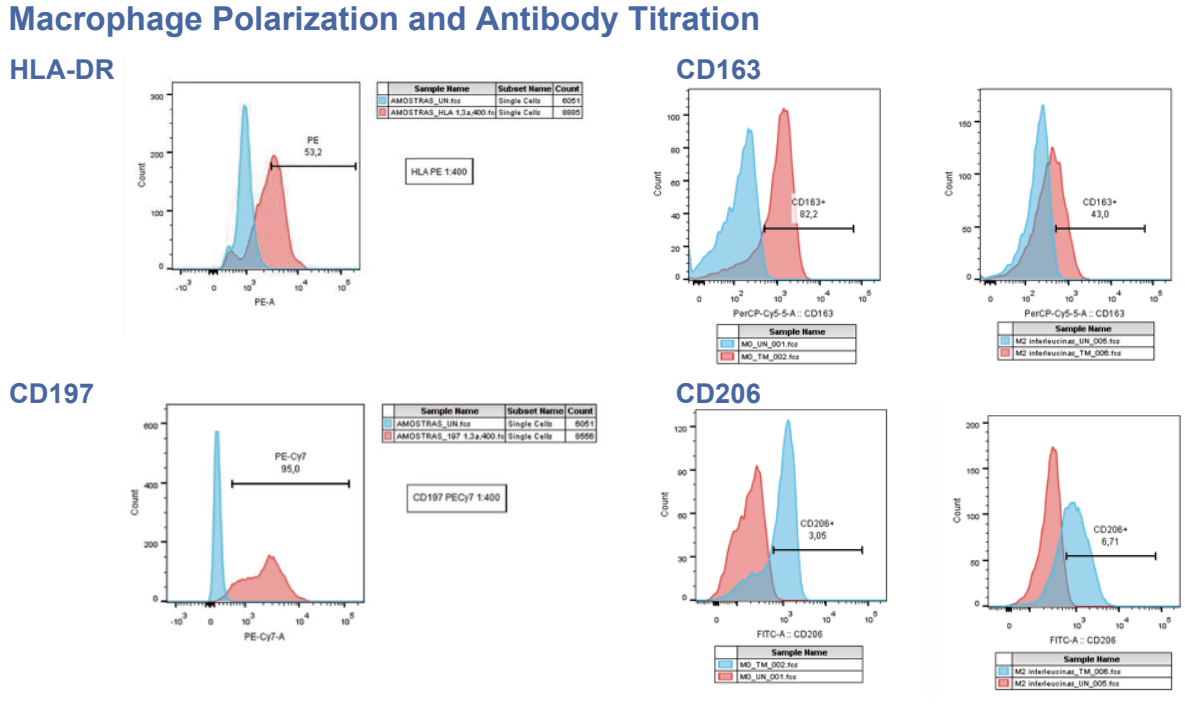
Results

Average number of cells	mean ± S. D. (10e6)
PBMC	35.60 ± 10.65
CD14+ monocytes	2.34 ± 1.01
Macrophages (day 7)	1.98 ± 0.71

Monocyte-macrophage Differentiation



Macrophage Polarization and Antibody Titration



Conclusions

Ideal methods for PBMC isolation, PBMC differentiation into macrophages, M0 macrophage polarization into M1 and M2 macrophages were established.

Osteology Foundation
Grant # 16/159

*Cristina Cunha Villar
villar@usp.br