Abstract: P1374

Title: RAMAN SPECTROSCOPY STUDIES OF IMMUNE RESPONSE ELEMENTS

Abstract Type: Poster Presentation

Session Title: Gene therapy, cellular immunotherapy and vaccination - Biology & Translational Research

Background:

With fast development and growing demand for cell-based immunotherapies and other immunomodulatory strategies e.g. microenvironment targeting, there is an urgent need to develop less invasive methods for cell analysis and quality control. Ideal tools serving these purposes should facilitate non-destructive, label-free analyses, retaining the analysed cell intact and unchanged. Raman spectroscopy (RS), measuring inelastic scattering corresponding to molecular vibration frequencies of different molecules, enables label-free subcellular imaging with high specificity and sensitivity. Through measuring metabolic state of the cells, RS may convey additional information e.g. regarding cell activation status, that can be utilized for diagnostic and research purposes.

Recent success of chimeric antigen receptor (CAR) T cells and CAR-macrophages immunotherapies created a need for cell analysis methods allowing to identify activation status without affecting their therapeutic potential of cells. Since co-stimulated recognition of the antigen through the T cell receptor results in metabolic changes, clonal expansion, and cytokine production, T cell activation status is an ideal target for RS analysis. Similarly, macrophage phenotypes, namely pro-inflammatory M1 and immunosuppressive M2, are characterized by divergent metabolic status and potentially can be differentiated by means of RS.

Aims:

Tracking subtle metabolic changes in T-cells and macrophages upon activation using Raman spectroscopy.

Methods:

Naïve T cells were isolated from healthy donors and stimulated with human T-cell activator anti-CD3/anti-CD28 Dynabeads. THP macrophages were PMA-differentiated THP-1 monocytic cell line while CD14 macrophages were derived from donors using CSF1 stimulation. Macrophages were subsequently polarized into M1 and M2 correspondingly with IL-4 and IL-13 or IFN-gamma and LPS. M0 macrophages or naïve T cells were used as a reference and control to M1 and M2 macrophages and activated T cells, respectively. Cell phenotypes were confirmed using flow cytometry and q-PCR. RS imaging was performed on the WITec Alpha 300 system. Raman maps were collected and analysed with K-means cluster analysis (KMCA) followed by Partial Least Squares–Discrimination Analysis (PLS-DA) and Principal Component Analysis (PCA).

Results:

Analysis of the Raman spectra revealed characteristic bands for activated T cells that mainly correspond to lipids (1440, 1240, 1660 and 2850 cm^{-1}) while spectra of naïve T cells manifest itself by relatively higher contribution of proteins (1008, 1041 and 2930 cm^{-1}) and nucleic acids (733, 790, 1583 cm^{-1}). Moreover, the accumulation of carotenoids found exclusively in naïve T cells, but not in activated. RS analysis of polarized macrophages showed the increased signal from hemoproteins (740-760 cm^{-1}) in all three subtypes but different hemoproteins to lipids ratio between M0, M1 and M2. The degree of lipid unsaturation can be considered an important parameter in the identification of the M1 phenotype of macrophages.

Summary/Conclusion:

Our results indicate that Raman spectroscopy is a potent, non-invasive, label-free tool to monitor changes in immune cells activity that can complement current diagnostic methods. Spectroscopic markers can be used to

build discrimination models that can be implemented for machine-learning leading further to automatization of cell analysis.

Keywords: Immune response, T lymphocyte, Macrophage