BioMed Research Journal
BMRJ, 3(S1): 04
www.scitcentral.com

ISSN: 2578-8892

Abstract: Open Access

Extracellular Traps and Mitochondrial Dynamic in Stimulated Autologous Leukocyte Cultures from Healthy Human Blood Samples

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Published November 01, 2019

ABSTRACT

Extracellular Traps (ETs) are structures of chromatin and intracellular proteins which are extruded in leukocytes under inflammatory conditions. The presence of beta-tubulin and HLA-DR has not been reported in the traps. Not all ETs are created equal; this depends on the source of stimulation. On the other hand, phenotypes adopted by immune cells in their responses are influenced by "mitochondrial dynamic" (MD). MD is the set of characteristics of shape, position and size of mitochondria. These organelles are currently considered as regulating functions of innate and adaptive immunity. Mitochondrial damage was reported in dendritic cells and macrophages exposed to NETs. Objectives: to generate ETs in cultures of leukocytes challenged with LPS or fMLP and to carry out the beta-tubulin, HLA-DR labeling, on the other hand, to observe the morphological characteristics of the mitochondria in lymphocytes in the LPS, fMLP or OVA assays. Methods: Autologous cultures from healthy human blood samples (n=10) with ethical consent (HNC, FCM), anti-coagulated with heparin were stimulated with 25 ng/ml LPS or 0.25 ng/mL fMLP or 100 ug/mL OVA, 30 min. Immunofluorescence technique with anti-beta tubulin and anti HLA-DR antibodies, DNA staining with DAPI. Paired blood samples provided the controls. Cells of the cultures were studied with transmission electron microscopy.

Results: Beta-tubulin and HLA-DR molecules were localized in the ETs. Alterations of mitochondrial morphology of lymphocytes were observed in the samples with LPS with increase in size and complexity of the cristae with electrolucid images (t-test for paired samples, $p<0.0001$). Differences of mitochondrial areas were observed in OVA assay ($p<0.005$) and in fMLP assay were not observed differences.

Conclusion: The expression of beta-tubulin and HLA-DR contributes to the better understanding of the composition of the ETs generated by different stimulators and may have significance as a therapeutic target. HLA-DR release in ETs may influence environment contributing to antigen presentation class II pathway. The ETs would affect the DM of the surrounding cells, influencing cellular function.

Keywords: Extracellular traps, Mitochondrial dynamics, Human leukocytes, Lymphocytes, Beta tubulin, HLA-DR

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Citation: Rinero R, Reyna MV, Rodríguez FM, Carabajal Miotti CL, de Frattari SR, et al. (2019) Extracellular Traps and Mitochondrial Dynamic in Stimulated Autologous Leukocyte Cultures from Healthy Human Blood Samples. BioMed Res J, 3(S1): 04.