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Cellular Interactions in Autologous Culture of Human Leukocytes: Mitochondrial Dynamics, Cellular Activation and Extracellular Traps

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Abstract

In this work of scientific research of the Mitochondrial Dynamics (MD) in lymphocytes in autologous cultures of human total leukocytes from peripheral blood stimulated with the Lypopolisaccharide (LPS) endotoxin and the Ovalbumin (OVA) heterologous antigen was carried out. In addition, the activated phenotype (effector cells and memory cells) of T lymphocytes was marked for CD45RO and MD was studied in lymphocytes in relation to the presence of B7 costimulatory molecules (CD80 and CD86) in antigen presenting cells (APCs) in autologous cultures. Extracellular traps (ETs) generated in cultures stimulated with LPSwere visualized with fluorescence microscopy and were observed like diffuse staining or fibrillar appearance with DAPI. In paired control samples without stimulation, the occurrence of ETs was in average value: 3.4% of the cells. A higher percentage of cells that release ETs was observed in cultures stimulated with LPS versus the paired control group ($p < 0.0001$). In ultrastructural observations differences were registered in the size of the lymphocyte cytoplasm. On the other hand, MD in stimulated lymphocytes shows changes with respect to the paired controlled samples: in the size (area) mitochondrial and the position of the mitochondria: polarization. No statistically significant differences were observed in number of mitochondria per cell at 30 minutes of culture. In multiple trials stimulated with LPS, lymphocytes with conventional mitochondria and others with morphologically altered mitochondria with electrolucent ridges were observed. After stimulation, in paired samples cell activation in lymphocytes was selected for CD45RO positivity. The changes in MD in stimulated lymphocytes correspond to the state of cellular activation in parallel detected by immunofluorescence (IF) with anti-CD45RO. The increase in the expression of B7 molecules in the APCs given by the stimuli contributed to the state of lymphocyte activation.

Keywords: *human leukocytes; mitochondrial dynamics; cellular activation; extracellular traps*

Introduction

Mitochondria are double membrane organelles that provide the cell with metabolic functions that include energy production through oxidative phosphorylation. They are responsible for aerobic respiration. Mitochondrial morphologies vary widely from one cell type to another [1] and they are dynamic organelles that are subject to a constant cycle of division (fission) and fusion to maintain their function [2]. The mitochondria seen with the transmission electron microscope (TEM) have a smooth outer contour membrane and an internal membrane parallel to the first one that forms folds into the organelle and these are called "mitochondrial ridges". The ATP released by the mitochondria towards the cytoplasm is a universal source of energy that is used in cellular work: transport through membranes, synthesis and motor activity of the cell [3]. It is noteworthy that mitochondria are also involved in very important cellular processes such as apoptosis, aging, calcium homeostasis and cell signaling [4]. Mitochondria ensure cellular function and they are determinants

in the phenotypes that immune cells adopt in their responses. It is defined as "mitochondrial dynamics" (MD) to the set of characteristics of shape, position and size of mitochondria[5]. It is considered which includes three processes: a) the remodeling of the mitochondrial reticulum by fusion/fission, closely linked to the cellular metabolic state; b) motility subcellular mitochondrial, particularly relevant in polarized cells and what ensures the local supply of ATP, and c) the remodeling of the ultrastructure mitochondrial and condensation of its matrix [6]. The fusion allows them to mix their contents ensuring the complementation of the protein components, the repair of the mitochondrial DNA (mtDNA), and the distribution of metabolic intermediates. Fission, on the other hand, allows mitochondria to be increased in number and capacity and to separate mitochondria for lysosomal autophagy (mitophagy). The alteration of these protein quality control mechanisms has been identified in multiple cardiac diseases such as cardiac hypertrophy, dilated cardiomyopathy, ischemic heart disease, heart failure and is closely related in the mitochondrial control of apoptosis [2].In neurodegenerative diseases mitochondrial alterations have been described, such as aberrant fragmentation [7]. It has been linked to mitochondrial morphology with the survival and metabolic adaptation of cells to stress and aberrant mitochondrial fissions have been observed in many diseases [8].

Mitochondria play a key role not only in the generation of ATP, but also in the production of reactive oxygen species (ROS) and in the induction of apoptosis[9]. Mitochondria with regulatory functions of the innate and adaptive immunitysupport cellular function and they are determinants in the phenotypes that immune cells adopt in their responses [10]. An example of this is the polarization of macrophage activation that includes changes in the MD. M2 macrophages have higher oxygen consumption and conversely the M1 exhibit a robust anaerobic glycolysis even in the presence of high oxygen levels[10].

Lymphocytes undergo metabolic changes during activation, they must adapt the energy and biosynthetic requirements on demand for cell proliferation in clonal expansion and for its effector functions[11].In the case of naive T cells a first signal is required, to antigen recognitionthrough the interaction of the antigenic peptide in the context of the major histocompatibility complex (MHC) with the T cell receptor (TCR), and a second signal, the "costimulatory signal" through molecules expressed in the antigen presenting cell (APC)[12, 13]. Memory cells act faster and immune responses are stronger. In the case of CD4 T cells describe modifications in MD in memory cells with regarding naive cells. Mitochondria are more complex, elongated, branched and more abundant in effector memory cells[14]. MD controls the fate of T cells through metabolic programming, effector activated T cells increase anabolic pathways of metabolism such as aerobic glycolysis, while memory T cells accentuate catabolic pathways such as fatty acid oxidation. Fused mitochondrial networks are maintained in memory T cells while fission predominates in effector cells [15]. T cell activation involves mitochondrial biogenesis that leads to the growth and division of preexisting mitochondria[16].

Activated memory and effector T cells express on their surface the CD45RO marker which is a tyrosine phosphatase regulating lymphocyte activation.[12, 13]. An Immune Synapse(IS) may occur in different circumstances, for a variety of functions. At present, the IS denomination is used generically to describe the contact surface between a T, B, or NK cell and an APC or a "target" cell [17]. The position of the mitochondria controls the influence of Calcium towards IS in T cells. [18, 19].T cell activation requires mitochondrial translocation to IS [20].

The activation of T cells requires a first signal, the interaction of the antigenic peptide in the context of the MHC with the TCR, and a second signal, the "costimulatory signal"[12, 13]. B7 costimulatory molecules are expressed in professional APCs, such as dendritic cells, macrophages and B lymphocytes [12, 13]. In polymorphonuclear (PMN) neutrophils, such B7 molecules stored in their cytoplasmic granules have been observed and under certain stimuli they are expressed on the cell surface [21]. The absence of costimulation in naive T cells results in "anergy" or lack of specific antigen response[22, 23]. The second signal can be stimulatory or inhibitory, this depends on the CD28 or CTLA4 receptor, respectively [23, 24]. B7-1 / B7-2: CD28 / CTLA-4 is the best characterized of costimulation pathways [25, 26]. CD28 is constitutively expressed in naive T cells, instead CTLA4 is induced by activation and has higher binding affinity with B7 molecules [27].Regulatory T cells (Treg) constitutively express CTLA4 and play an important role in maintaining self-tolerance [28].

In previous works of this laboratory it has been observed the colocalization of costimulatory molecules B7 in neutrophil extracellular traps (NETs) in autologous cultures of total leukocytes from blood samples of healthy humans [29]. This has implications for the possibility of breaking immune tolerance. The importance of this finding lies in the functions of neutrophils, the possibility of acquiring competence to be a professional APC and play a role in immunomodulation. The presence of costimulatory molecules in the NETs influences the environment of the cell, the activation of the B7-1 / B7-2: CD28 / CTLA-4 pathway can lead to stimulation or inhibition of other cells, such as naive T cells, the various effector T cells or Treg cells [29].

Extracellular traps (ETs) are structures composed of chromatin, histones and granular proteins, being initially described in PMN neutrophil leukocytes by Brinkmann et al (2004) [30] calling them NETs. NETs consist of a mesh of decondensed DNA fibers, cytotoxic histones and antimicrobial peptides (AMPs) with the ability to trap and make invading pathogens harmless. NETs are associated with numerous pathologies since they contain several biomolecules which are also cytotoxic for the body's own cells, in addition to their desired antimicrobial effects. Therefore, an exaggerated release of NETs should be avoided, leading to the need for control mechanisms to regulate their formation [31]. It is currently known that other cell types besides PMN neutrophils that are capable of generating ETs, for example, mast cells, monocytes, tissue macrophages and eosinophils [32-34]. ETs constitute a new defense mechanism of the immune system in response to various microorganisms and other stimuli [30]. ETs released to the extracellular environment can affect the various surrounding cells, especially T lymphocytes and CPAs along with their interactions. The decrease in T lymphocyte activation threshold by NETs has been described [35].

Recently, we published the finding of beta tubulin in ETs and altered MD in autologous cultures of human leukocytes stimulated with lipopolysaccharide (LPS) [36].

For all the above, a study of MD in relation to expression of costimulatory molecules (B7) and activation molecules (CD45RO) in human leukocyte interactions in autologous culture stimulated with an endotoxin LPS or heterologous antigen (Ovalbumin, OVA) was performed.

Materials and Methods

Human Blood Samples

Samples of human blood, anticoagulated with heparin, of healthy people (n = 10) with informed consent donated by the Blood Bank, Institute of Hematology and Hemotherapy (IHH) of the Universidad Nacional de Córdoba(UNC), were used, in anonymity, with serology data. Ethical approved by the Ethics Committee of the Hospital Nacional de Clinicas, Facultad de Ciencias Médicas, UNC, Argentina. HNC, FCM, UNC. RePIS 3381 and RePIS 3412.

Autologous Cultures of Total Leukocytes

Cultures of total leukocytes of human blood were made. The blood samples were subjected to the following tests at the IHH, UNC: Hudleson (Wiener), VDRL (Wiener), Chagas HAI (Wiener), Chagas EIE (BioMerieux), HBs EIE (BioMerieux), HBc (BioMerieux), HCV EIE (Murex), HIV Ac EIE (BioMerieux), HIV Ag EIE (BioMerieux), and HTLV EIE (Murex). Cells were cultured in suspension, in 24-well sterile culture dishes (some samples with sterile coverslips in the bottom) in gassed incubator at 37°C in TC199 medium (with Earle and L-glutamine salts) (SIGMA, St. Louis, MO) added with serum from the same donor. The classic 0.5% Tripan Blue exclusion test were used for cell viability. All cell cultures were prepared under sterile conditions under hood equipped with ultraviolet light and laminar flow. Paired samples, control without stimulation, or with stimulation were taken at 30 min.

Stimulation with LPS

Total leukocytes in autologous cultures were stimulated 30 min with LPS (Lipopolysaccharides from *Escherichia coli* Sigma-Aldrich) 25 ng/ml from time zero at 37°C.

Stimulation with OVA

Total leukocytes in autologous cultures were stimulated 30 min with OVA 100 µg/ml (Ovalbumin, Difco, Detroit, Michigan) from time zero at 37°C.

Generation of ETs

Cells grown in medium with serum from the same donor were stimulated: with LPS (LPS from *E. coli*, SigmaAldrich) 25 ng/ml, to form ETs at 37°C in a gassed incubator. Culture samples were taken at 30 min. The ETs were visualized with fluorescence microscopy using DAPI (Sigma, St. Louis, MO). The

released ETs were visualized by immunofluorescence microscopy and the percentage of cells releasing ETs was calculated as the average of six fields (400x) normalized to the total number of cells.

Immunofluorescence (IF) Technique

IF was performed with cell signaling technology. Cultured cells after stimulation with LPS for 30 minutes were washed briefly in PBS (phosphate buffered saline), fixation was performed with 4% paraformaldehyde for 10 minutes and washed in three changes in PBS. It was incubated with 5% blocking serum albumin in PBS to prevent non-specific staining for 20 minutes. It was washed with PBS. It was incubated with anti-CD45RO (FITC; Santa Cruz Biotechnology) or with anti-CD80 (FITC; Santa Cruz Biotechnology); anti-CD86 (PE; Santa Cruz Biotechnology), at 4 ° C overnight, It was washed with PBS and nuclear staining with DAPI (4,6'-diamidino-2-phenylindole) (Sigma, St Louis, MO). It was mounted with medium 90% glycerol in PBS. Observation of preparations were performed in videomicroscope Axioscop 20, MC80, trinocular, Carl Zeiss. Paired blood samples provided controls.

Cytopreparations for Transmission Electron Microscopy (TEM)

Cell sediments were prepared with aliquots of samples of the cultures carried out. They were fixed in 1% glutaraldehyde in 0.1 M cacodylate buffer for 1 h and post-fixed in 1% OsO_4 in the same buffer, for 1 h. Then, the materials were dehydrated in ketones of increasing graduation and included in epoxy resin (Araldite) at 60°C, for 24–48 h. Subsequently, ultrafine cuts of 60–80 nm thickness (silver/gold interference color) were made, whiches were collected in copper grids of 250 bars per inch, contrasted with uranyl acetate and lead citrate, and studied with MET Zeiss LEO-906E

MD Parameters

The MD parameters observed in TEM related to 1. Shape and size were studied: area [16, 37] 2. Position and 3. Number of mitochondria per cell. FIJI software was used to record the parameters under study [38, 39].

Statistical Treatment of the Data

The *t*-test was used for paired samples. Infostat statistical program was used for its analysis [40].

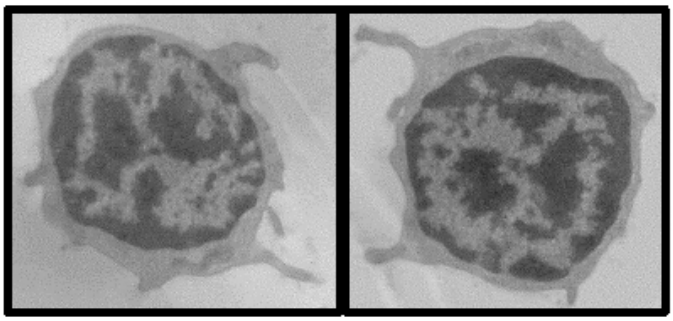
Results and Discussion

The MD in lymphocytes in autologous cell culture samples of human total leukocytes stimulated with LPS and with OVA showed changes with respect to the paired control samples. Ultrastructural observations were made with TEM of lymphocytes in paired samples of total autologous leukocyte cultures without stimulation and stimulated with LPS or OVA for 30 minutes. The typical mononuclear aspect and the poor cytoplasm of the lymphocytes were visualized in the paired control samples without stimulation, and in the stimulated samples in both cases cytoplasmic enlargement was observed (Figure 1). Regarding the position of the mitochondria, in both samples stimulated, cellular polarization was observed in the lymphocytes, with redistribution of mitochondria located in a sector. (Figure 1).

In total autologous leukocyte culture samples in multiple LPS-stimulated assays, lymphocytes with large-sized conventional mitochondria were observed (Figure 1) and other lymphocytes with morphologically altered mitochondria in their appearance to the TEM with electrolucent ridges were visualized (Figure 2).

The mitochondrial area in lymphocytes stimulated with either LPS or OVA showed changes with respect to the paired control samples. Increased mitochondrial area in lymphocytes was observed ($p < 0.0001$ and $p < 0.005$, respectively) (Figures 3, 4).

A



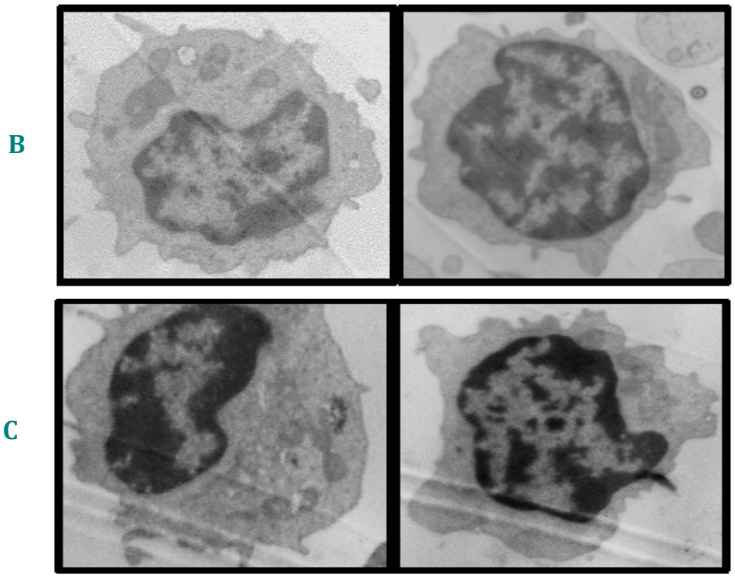


Figure 1. *Representative microphotographs of lymphocyte TEM in total autologous leukocyte cultures of healthy humans.* **A.** *Microphotographs of paired lymphocyte samples in total autologous leukocyte culture without stimulation, 30 minutes, 6000x.* **B.** *Microphotographs of lymphocytes in total autologous leukocyte culture, stimulation with 25 ng / ml of LPS, 30 minutes. 6000x* **C.** *Microphotographs of lymphocytes in total autologous leukocyte culture, stimulation with 100 ug / ml of OVA, 30 minutes. 6000x*

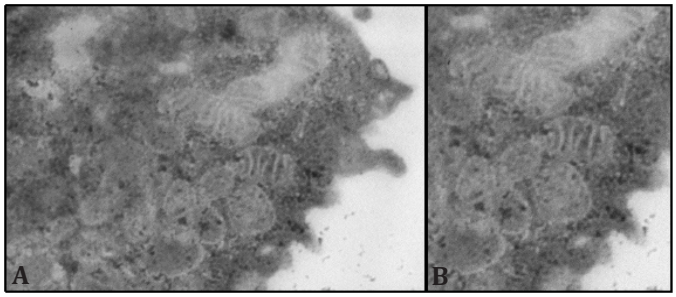


Figure 2. A. *Representative photomicrograph of TEM corresponding to part of the cytoplasm of a lymphocyte in a sample of total human leukocytes in autologous culture, stimulated with 25ng / ml of LPS, 30 min. Numerous mitochondria with membranes and electrolucent ridges are observed.* **B.** *Extension of A. 21500x.*

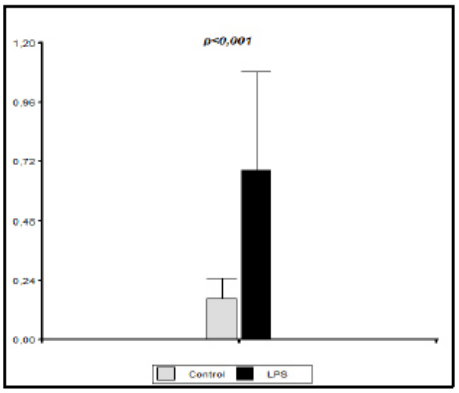


Figure 3. *Mitochondrial area in μm^2 in lymphocytes of total autologous leukocyte cultures, 30 minutes, paired controls and stimulated with LPS (from Escherichia coli Sigma-Aldrich) 25ng / ml Student t test for paired samples. The mean mitochondrial area in lymphocytes of paired control samples showed a significant difference p <0.0001, compared to samples stimulated with LPS.*

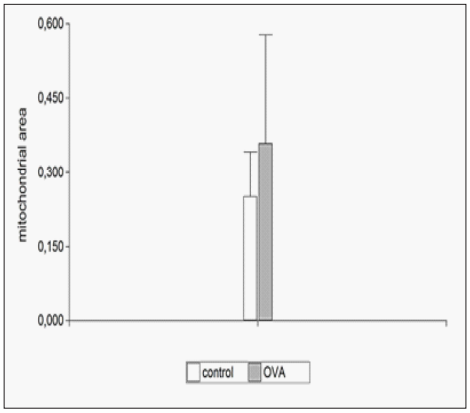


Figure 4. *Mitochondrial area in μm² in lymphocytes of total autologous leukocyte cultures, 30 minutes, paired and stimulated controls with 100 ug / ml of OVA (Ovalbumin, Difco, Detroit, Michigan) Student t test for paired samples. The mean mitochondrial area in lymphocytes of paired control samples showed a significant difference p <0.005, compared to samples stimulated with OVA.*

There were no significant differences in the number of mitochondria per cell between the various paired control and stimulated groups in the culture time of 30 minutes. (Figure 5).

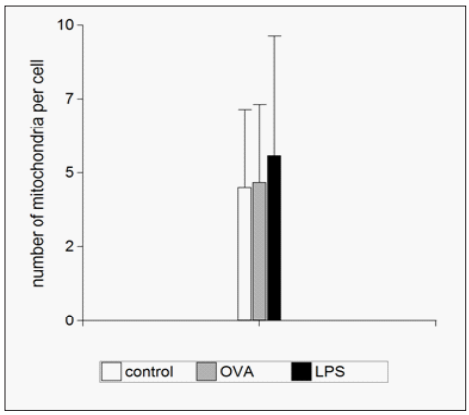


Figure 5. *Number of mitochondria per cell in human lymphocytes from total autologous leukocyte cultures, 30 minutes, paired and stimulated controls with 100μg/ml of OVA or with 25ng/ml of LPS. There are no statistically significant differences, t test for paired samples.*

The results of the ultrastructural study of MD in lymphocytes recorded an increase in mitochondrial area in samples of autologous cultures of human leukocytes stimulated with the heterologous OVA protein antigen and with the LPS endotoxin (Figures 3, 4), like ultrastructure of activated cells as described in the bibliography. The images of the stimulated samples allow us to observe the typical appearance of activated cells, with abundant cytoplasm compared to resting cells. In addition, cell polarization was observed, with the redistribution of mitochondria located in a sector (Figure 1).

The cell activation status in lymphocytes was indicated by positivity for CD45RO in autologous cell cultures of total leukocytes, from blood of healthy people after stimulation with LPS or with OVA, in paired samples. (Figure 6).

In paired control sample of total autologous leukocyte cultures of Figure 6A: numerous neutrophil PMN nuclei and a lymphocyte nucleus are stained with DAPI and positivity for CD45RO (green) is observed in the lymphocyte. In B: in the paired sample of autologous culture stimulated with LPS, nuclei of four lymphocytes stained with DAPI and all CD45RO positive cells (green) are observed. In C: in the paired sample of autologous culture stimulated with OVA, nuclei of numerous PMNs and lymphocyte leukocytes stained with DAPI are observed. Expression of the CD45RO marker (green) was observed in numerous cells. (Figure 6).

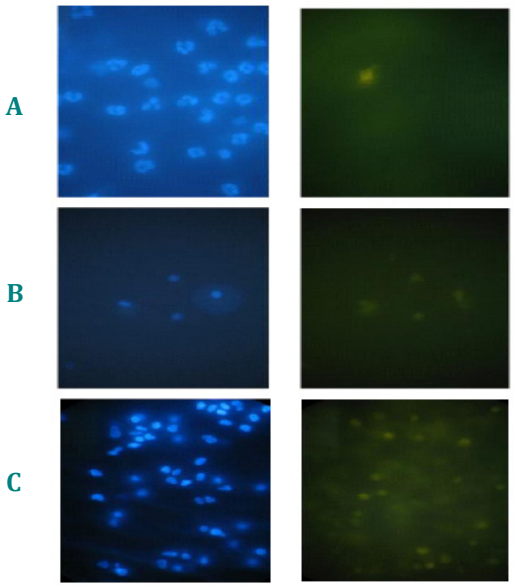


Figure 6. *CD45RO expression. Representative microscopy images of fluorescence from three independent donor experiments, after 30 minutes of culture showing CD45RO (green). CD45RO in total autologous leukocyte cultures stimulated with LPS or OVA. Nuclear staining with DAPI (blue). The scale bar represents 10μm. A. Without stimulation, paired control samples. B. With stimulation with 25ng / ml LPS. C. With stimulation with 100μg / ml OVA.*

The expression of costimulatory molecules B7 (CD80 and CD86) in leukocytes, after stimulation with LPS and OVA in total autologous leukocyte cell cultures was recorded (Figure 7).

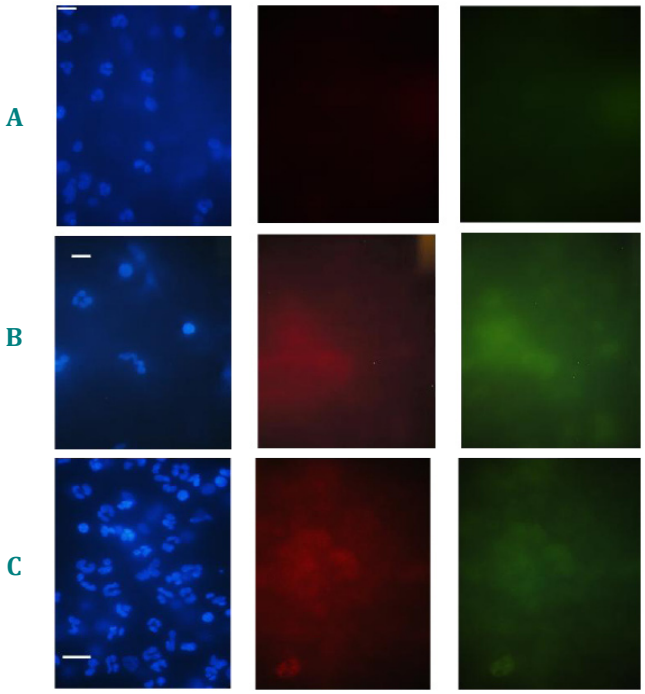


Figure 7. *Expression of costimulatory molecules B7 (CD80 and CD86). Images representative of fluorescence microscopy of three independent experiments of donors, after 30 minutes of total autologous leukocyte culture. A.paired controls. B. With stimulation with 25 ng / ml LPS. C. With stimulation with 100μg / ml OVA. Nuclear staining with DAPI (blue), anti-CD86 (red) (PE; Santa Cruz Biotechnology), anti-CD80 (green) (FITC; Santa Cruz Biotechnology). The scale bar represents 10 μm.*

Expression of costimulatory molecules B7: CD80 (green) and CD86 (red) was observed in stimulated leukocyte samples (Figure 7). In total autologous leukocyte culture: A. In paired control samples no expression of CD80 or CD86 was observed. In B. and C. In both samples stimulated with LPS and OVA, costimulatory molecules were expressed and positive staining was observed for CD80 and CD86.

As mentioned above, the increase in mitochondrial area (Figures 3, 4) in lymphocytes in autologous cultures of human leukocytes stimulated with OVA and with LPS was recorded in an ultrastructural study with TEM. Lymphocytes with abundant cytoplasm and polarization with redistribution of mitochondria that correspond to the activated lymphocyte phenotype were observed, compared to resting cells.

Autologous cultures of total blood leukocytes were performed and were stimulated to generate ETs with LPS.The generated ETs were visualized with fluorescence microscopy using DAPI for DNA staining. ETs were observed with DAPI as diffuse staining or fibrillar appearance (Figure 8).

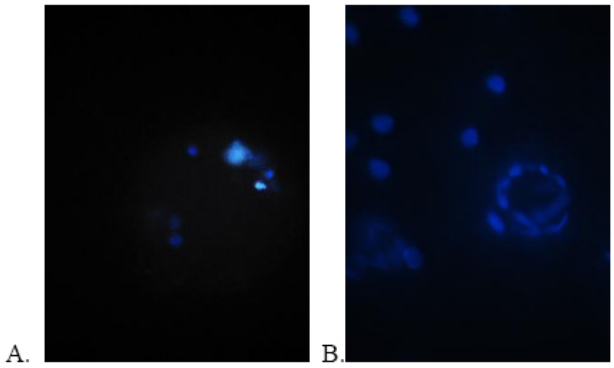


Figure 8. *ETs in autologous cultures of human leukocytes with and without stimulation. Representative images of immunofluorescence microscopy. 30 minutes of culture. DNA (blue) staining is observed with 4,6'-diamidino-2-phenylindoleDAPI, arrows: ETs. A. Paired control samples of total leukocyte culture without stimulation. 1000x B. Samples of autologous cultures of total leukocytes stimulated with 25 ng / ml LPS, 1000x.*

In paired samples controls without stimulation the formation of ETs was observed in average value: 3.4% of the cells (Figure 9), in coincidence with the bibliography [41]. Stimulation with LPS increased the baseline level of leukocyte ETs derived from healthy donors. A higher percentage of cells that release ETs was observed in cultures stimulated with LPS (Mean: 19.4%) (Figure 9).

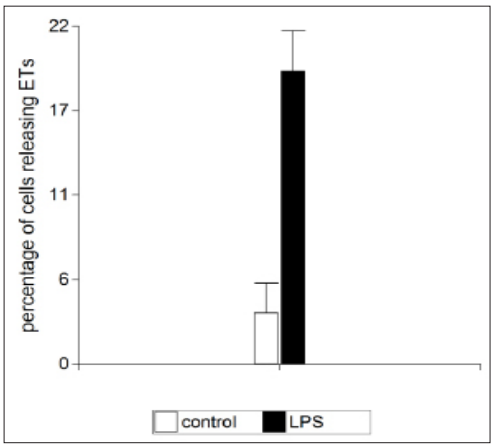


Figure 9. *Percentage of cells that release ETs. Human leukocytes stimulated with 25 ng / ml in autologous cultures, 30 minutes, controls: paired samples without stimulation. The ETs were visualized by immunofluorescence microscopy and the percentage of cells that release ETs was calculated as the average of five fields (400x) normalized by the total number of cells. Data are presented as mean ± SD of n = 10 and represented at least three independent donor experiments. *** p <0.0001, Student's t-test for paired samples.*

It is currently accepted that MD in immune cells is important in both their metabolic and immune functions [5]. Mitochondria ensure cellular function and are determinants in phenotypes that adopt immune cells in their responses and have regulatory functions of innate and adaptive immunity [10].

The MD in the ultrastructural study with MET in lymphocytes in autologous cell cultures samples of human total leukocytes stimulated with LPS and with OVA presented changes with respect to the paired control samples. The typical mononuclear aspect and the low cytoplasm of the lymphocytes were visualized in the paired control samples without stimulation, and on the contrary in the samples stimulated from zero time to 30 minutes of culture in both cases (with LPS or with OVA) it was observed cytoplasmic enlargement and cell polarization, with redistribution of mitochondria (Figure 1). Increase in mitochondrial area in lymphocytes ($p < 0.0001$ and $p < 0.005$) (Figures 3,4) with respect to paired control samples was observed, with no differences between the two stimulators.

The changes in position and size (area) in the MD are consistent with the lymphocyte phenotype in activation status, since mitochondria, as mentioned above, play a key role in cell function and are determinants in the phenotypes of the Immune cells in effector responses [10,14]. T cell activation involves mitochondrial biogenesis [16]. Regarding polarization, which ensures the local supply of ATP [6], the change in position of the mitochondria in the cells that was observed is consistent with the mitochondrial redistribution described in the cells that approximate a possible IS between the lymphocyte and the APC, although in our records there was no contact cell interaction at 30 min.

The activation of T cells involves mitochondrial biogenesis that leads to the growth and division of pre-existing mitochondria [16]and although there were no statistically significant differences in the number of mitochondria per cell between the various control and stimulated groups (Figure 5). This may be due to the short culture time (30 min). In immune responses, in vitro assays for proliferation in clonal expansion require at least 72 hours of culture for registration [13].

The lymphocyte cell activation status was indicated by positivity for CD45RO in autologous cell cultures of whole blood leukocytes from healthy people after stimulation with LPS and OVA in paired samples. The CD45RO molecule is a marker of activation status in effector and memory T cells [12,13], but it has also been observed in some PMN neutrophil phenotypes [42,43]. It is known that the isoform of the CD45RO molecule is expressed when the T cell is activated, and will be associated with the TCR and the co-receptor (CD4 or CD8) making the T cell more sensitive to stimulation by low concentrations of antigenic peptide complexes-MHC. When the cells differentiate into effectors and express on the surface the CD45RO isoform it lacks the exon A present in the CD45RA isoform that is expressed on the surface of the naive cells [13]. In autologous leukocyte culture samples stimulated with OVA, expression of the CD45RO marker was observed for activated T lymphocytes in numerous cells as expected, considering that OVA is a heterologous peptide antigen that can be recognized by those specific T lymphocytes. However, an increase in the percentage of activated cells labeled with CD45RO was also observed in the LPS endotoxin test compared to the paired control group without stimulation (data not shown). This could be explained taking into account that LPS is an activator of PMN neutrophils and monocytes that express costimulatory molecules in the surfaceswith their challenge and thus contribute to the second activation signal required for T cells.On the other hands the ETs generated with LPS stimulation may contribute to activation signal, also.

It is known that T and B cells also recognize LPS through pattern recognition receptors (PRR): TLR4, but in the case of T lymphocytes this only affects adhesion, chemotaxis and migration, it does not affect cytokine secretion or proliferation [44]. B7 costimulatory molecules are expressed at low intensity in resting APCs but can be induced by inflammatory cytokines and pathogen associated molecular patterns (PAMP) such as LPS, and recognized through PRR, such as TLRs [24]. The expression of costimulatory molecules B7 (CD80 and CD86) in leukocytes, after stimulation with LPS and OVA in total autologous leukocyte cell cultures was recorded after 30 minutes of culture (Figure 7). Lymphocytes with abundant cytoplasm and polarization with redistribution of mitochondria, and increased mitochondrial area correspond to the phenotype of activated lymphocytes, compared to resting cells. (Figure 1).

The increase of the mitochondrial area in lymphocytes and the expression of costimulatory molecules B7 (CD80 and CD86) in leukocytes are related because the stimulators caused the expression of B7 molecules in the CPAs that contribute to give the second activation signal required for T cells. After this, changes in MD are a consequence of the functional requirements of immune cells in responses, such as effector and memory cells that show phenotypic differences with respect to naive cells [14, 16, 45].

As menctioned earlier, an important role of MD has been described in various infectious, neurodegenerative, cerebrovascular and metabolic diseases with inflammatory components. The endotoxemia produced by the effects of endotoxins such as LPS in the blood leads to inflammation of multiple organs. On the other hand, research works have been published using different doses of LPS, and this results in mitochondrial biogenesis effects [46] in some cases and mitochondrial degeneration in others [47]. The findings of this work provide new data in relation to the characteristics of MD in response to challenges with this endotoxin. In the multiple trials stimulated with LPS, lymphocytes with conventional mitochondria and other lymphocytes with morphologically altered mitochondria in appearance with electrolucent ridges were observed (Figures 1, 2). It is inferred that the differences may be in the different donors from which autologous cultures are performed, because although all are selected for their negativity to the serological tests performed at the IHH, UNC, these only refer to infectious pathologies. No differences in other parameters could be ruled out.

On the other hand, in studies on aging and immune cells with challenge with OVA, it has been reported that in elderly individuals an association was observed between mitochondrial dysfunction and loss of ability to cross-present antigens in dendritic cells [48]. In this work, MD and the activation status of lymphocytes were related to the expression of costimulatory molecules in APCs. It should be noted that we worked with total autologous leukocyte cultures (without T-cell purification) where both B lymphocytes and monocytes and APCs are included.Total autologous cells were worked to resemble what happens in vivo, where all white blood cells are present with their interactions. In the OVA stimulation assays, expression of the CD45RO marker was observed for activated T lymphocytes in numerous cells since OVA is a heterologous peptide antigen that can be recognized by specific T lymphocytes and in addition the MD presented changes in these activated lymphocytes (increased area mitochondrial and polarization).

In Conclusion, it is considered to have contributed new data to expand the knowledge in this area of study, of medical interest.

Acknowledgment

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