THE ANTI-APOPTOTIC EFFECT OF LEUKOTRIENE B_4 IN NEUTROPHILS: A ROLE FOR NADPH OXIDASE-DERIVED ROS AND NF-κB

Barcellos-de-Souza, P.[1]; Lima-Resende, A; Canetti C.[2]; Barja-Fidalgo C.[1]; Arruda, M.A.[1]

[1]Laboratório de Farmacologia Bioquímica e Celular, UERJ, Brasil. [2]Instituto de Biofísica Carlos Chagas Filho, UFRJ, Brasil.

Introduction: Leukotriene B4 (LTB_4), an arachidonic acid-derived lipid mediator, is a known proinflammatory agent released in many inflammatory situations and it is able to activate biological responses in human neutrophils (PMN) as well as reactive oxygen species (ROS) generation by the NADPH oxidase complex. LTB_4 delays neutrophils spontaneous apoptosis through the activation of classical pro-survival signaling, which in turn may corroborate to the onset of a chronic inflammatory condition. Recently, ROS has emerged as second-messengers, coordinating intracellular signaling cascades, and thus modulating several biological phenomena, including apoptosis. In this study, we aim to elucidate the putative role of NADPH oxidase-derived ROS in LTB_4-induced antiapoptotic effect.

Methodology: PMN were isolated from whole blood of healthy volunteers by Ficoll-Paque™ density. ROS production was evaluated by cytochrome *c* reduction, lucigenin and luminol enhanced-chemiluminescence. Apoptosis was determined by cell morphology and annexin V-phosphatidylserine binding. Mitochondrial membrane potential was assessed by flow cytometry of JC-1-stained cells. Protein expression was evaluated by western blot analysis of total or nuclear extracts.

Results and conclusion: Our data show that NADPH oxidase-derived ROS are critical to LTB_4 pro-survival effect on neutrophils. This event depends on redox modulation of NF-κB translocation and IκB-α phosphorylation/degradation. We have also observed that LTB_4-induced Bad degradation and mitochondrial stability requires NADPH oxidase activity. Our results strongly indicate that LTB_4-induced antiapoptotic effect in neutrophils occurs via ROS-dependent signaling routes and we do believe that a better knowledge of molecular mechanisms underlying neutrophils spontaneous apoptosis may contribute to design better strategies to control chronic inflammation.

Financial Support: CNPq, FAPERJ, SR-2/UERJ, ABC/UNESCO/L'Oreal.