NITROXIDE-BASED DETECTION OF GLUTATHIONYL RADICALS DURING MACROPHAGE RESPIRATORY BURST

Maísa R.P.L. Brigagão[1], Pio Colepicolo[2], Ohara Augusto[2]

[1]Departamento de Ciências Exatas, UNIFAL-MG, Alfenas, MG; [2]Departamento de Bioquímica, IQ, USP, São Paulo, SP, Brazil

During stimulation, macrophages release reactive oxygen species (ROS) through NADPH oxidase complex activation. Thiols are required to maintain cellular functions, in particular glutathione (GSH) that acts as an important reductant to repair oxidized thiol proteins involved in cell signaling, and as the major low molecular weight target for a range of bioradicals to produce the glutathionyl radical (GS•). To investigate GS• participation in macrophage respiratory burst, we employed the nitroxide 4-9((-acridinecarbonyl)-amino)-2,2,6,6-tetramethylpiperidine-1-oxyl (Ac-Tempo) whose interaction with GS• switches off Ac-Tempo EPR signal while switching on acridine moiety fluorescence (λ_{exc} 361 nm, λ_{emi} 440 nm) (Borisenko et al JACS 126, 9221, 2004). Thus, inflammatory macrophages (10^6 cells/ml) were incubated with Ac-Tempo (50 µM, 5 min), and PMA (100 ng). Ac-Tempo fluorescence response was determined in parallel to superoxide anion (O_2•-) release through nitrobluetetrazolium reduction. Decrease on fluorescence was dependent on GSH levels as demonstrated by pre-treatment of cells with buthionine sulfoximine (an inhibitor of GSH synthesis) (~51%) and *N*-ethylmaleimide (an alkylating thiol reagent) (~75%). Fluorescence decrease matched the inhibition of O_2•- release (~48%). These results suggest a role of thiyl radicals in the maintenance of the respiratory burst of macrophages and may provide a therapeutic target for inflammation management.

Supported by CNPq, Instituto do Milênio Redoxoma.