PROGRAMMED CELL CLEARANCE: S-NITROSYLATION OF AMINOPHOSPHOLIPID TRANSLOCASE REGULATES MACROPHAGE ENGULFMENT OF TARGET CELLS

Tyurina, [1,2] Y. Y., Tyurin[1,2], V. A., Konduru[1,2], N. V., Basova[1,2], L., Cai[1,2], P., Potapovich[1,2], A. I., Bayir[1,3], H., Stoyanovsky[4], D., Shvedova[6], A. A., Quinn[7], P., Xue[8], D., Fadeel[9], B., *Kagan*[1,2,5], *V. E.*.

[1]Center for Free Radical and Antioxidant Health, Departments of [2]Environmental and Occupational Health, [3]Critical Care Medicine, [4]Surgery, and [5]Cancer Institute, University of Pittsburgh, Pittsburgh, PA, USA; [6]Physiology/Pathology Research Branch, Health Effects Laboratory Division, NIOSH, Morgantown, WV, USA; [7]Department of Life Sciences, King's College London, London, United Kingdom; [8]Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO, USA; [9]Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

Aminophospholipid translocase (APT) is responsible for the asymmetric distribution of phosphatidylserine (PS) across the plasma membrane, thus preventing PS externalization. The enzyme can be S-nitrosylated resulting in the loss of its activity. We hypothesized that nitrosative stress – acting through APT S-nitrosylation - enhances PS externalization in cells by inhibiting APT activity. This pathway should be particularly important during inflammation whereby the oxidative/nitrosative burst generated by macrophages may cause direct nitrosylation or trans-nitrosylation of APT in target cells. To experimentally address this hypothesis we utilized HL-60 cells that express high APT activity. S-nitroso-L-cysteine-ethyl ester (SNCEE) and S-nitroso-glutathione (GSNO) were used as prototypical cell-permeable and cell-impermeable trans-nitrosylating reagents. HL-60 cells externalized PS in response to SNCEE or GSNO treatment as evidenced by annexin V binding assay and fluorescence microscopy. No cytotoxic effects were induced by either of the trans-nitrosating agents. RAW 264.7 macrophages elicited enhanced phagocytosis towards "nitrosylated" HL-60 cells. Assessments of APT activity confirmed that S-nitrosylation is associated with an altered activity of the enzyme. We thus speculate that macrophage-induced nitrosative stress contributes to effective clearance of apoptotic cells and the regulation of inflammatory responses.

HFSP Program Grant Team (Award Year 2005)
Principal Investigator: FADEEL, Bengt (Sweden)
Co-Investigators: KAGAN, Valerian (USA), QUINN, Peter (UK), XUE, Ding (USA)

- **Sixth Human Frontier Science Program Awardees Annual Meeting**
- **Institut Pasteur, Paris, France – July 3-5, 2006**
- **Programme and abstracts**