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Role and Action Mechanism of Secretory phospholipase A_2 in Macrophage Activation

Suk-Hwan Baek, Ph.D. & professor

Department of Biochemistry & Molecular Biology, College of Medicine, Yeungnam University, Kyeongsan, Korea

The phospholipase A_2 (PLA_2) family represents a diverse group of enzymes that hydrolyze *sn*-2 fatty acid from the cell membrane. Several mammalian cytosolic PLA_2 and secretory PLA_2 ($sPLA_2$) have been characterized and classified into different families. At present, 12 distinct $sPLA_2$s have been identified in mammals and classified into different groups, depending on their primary structures as characterized by the number and position of cysteine residues. The $sPLA_2$s have the potential to mediate a wide range of biological activities for example; they are key components of phospholipid digestion, effectors of anti-bacterial activity, potential regulators of severe illness and cancer markers. There is no doubt that these *sn*-2 acylhydrolases are key players in normal biology and in pathophysiological events. Although $sPLA_2$s have been studied extensively in mammals, the physiological and pathophysiological functions of these enzymes remain unclear. Therefore, the aim of this study was to investigate the functional mechanism of $sPLA_2$.

The increasing number of mammalian $sPLA_2$ and the identification of different membrane proteins that bind $sPLA_2$s makes it likely that these enzymes also behave as ligands for receptors, and that their physiological function is not limited to their catalytic activity. To date, two main types (M and N) of $sPLA_2$ receptors have been identified. Although $sPLA_2$ receptors have been studied extensively in M-type receptor, the physiological functions of these receptors also remain unclear. Therefore, the another aim of this study was to investigate the function of $sPLA_2$ receptor.

Macrophages exert key functions during the innate immune response, which is vital for recognizing and eliminating invasive microbial pathogens. When microbial products bind to its

specific receptors, macrophage become activated and release a broad array of cytokines that orchestrate the host innate immune response. However, under circumstances, macrophages have deleterious effects. This is the case of septic shock, which is a severe systemic inflammatory response triggered by the interaction of lipopolysaccharide and some bacterial components with macrophages and other host cells. Sepsis may be regarded as a constellation of signs and symptoms representing the host's response to infection. Many pathogenic mediators of sepsis have been described, including cytokines, NO, and PLA_2.

In this study, we demonstrate that group IIA PLA_2 up-regulates the expression of inducible nitric oxide synthase (iNOS) through a novel pathway that includes M-type $sPLA_2$ receptor ($sPLA_2R$), phosphatidylinositol 3-kinase (PI 3-K), and Akt. Group IIA PLA_2 stimulated iNOS expression and promoted nitrite production in a dose- and time dependent manner in Raw264.7 cells. Upon treating with group IIA PLA_2, Akt is phosphorylated in a PI 3-K-dependent manner. Pretreatment with LY294002, a PI3K inhibitor, strongly suppressed group IIA PLA_2-induced iNOS expression and PI 3-K/Akt activation. The promoter activity of iNOS was stimulated by group IIA PLA_2, and this was suppressed by LY294002. Transfection with Akt cDNA resulted in Akt protein overexpression in Raw264.7 cells and effectively enhanced the group IIA PLA_2-induced reporter activity of the iNOS promoter. M-type $sPLA_2R$ was highly expressed in Raw264.7 cells. Transient expression of M-type $sPLA_2R$ enhanced group IIA PLA_2-induced promoter activity and iNOS protein expression and these effects were abolished by LY294002. Furthermore, consistent with this, we found that group IIA PLA_2 enhanced nitrite production and iNOS expression in stable expressing cells of M-type $sPLA_2R$. These results suggest that group IIA PLA_2 induce nitrite production by binding to M-type $sPLA_2R$, which then mediates signal transduction events that lead to PI 3-K/Akt activation.