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Review

Activation of human inflammatory cells by secreted phospholipases A_2 ☆

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Abstract

Secreted phospholipases A_2 (sPLA$_2$s) are enzymes detected in serum and biological fluids of patients with various inflammatory, autoimmune and allergic disorders. Different isoforms of sPLA$_2$s are expressed and released by human inflammatory cells, such as neutrophils, eosinophils, T cells, monocytes, macrophages and mast cells. sPLA$_2$s generate arachidonic acid and lysophospholipids thus contributing to the production of bioactive lipid mediators in inflammatory cells. However, sPLA$_2$s also activate human inflammatory cells by mechanisms unrelated to their enzymatic activity. Several human and non-human sPLA$_2$s induce degranulation of mast cells, neutrophils and eosinophils and activate exocytosis in macrophages. In addition some, but not all, sPLA$_2$ isoforms promote cytokine and chemokine production from macrophages, neutrophils, eosinophils, monocytes and endothelial cells. These effects are primarily mediated by binding of sPLA$_2$s to specific membrane targets (heparan sulfate proteoglycans, M-type, N-type or mannose receptors) expressed on effector cells. Thus, sPLA$_2$s may play an important role in the initiation and amplification of inflammatory reactions by at least two mechanisms: production of lipid mediators and direct activation of inflammatory cells. Selective inhibitors of sPLA$_2$-enzymatic activity and specific antagonists of sPLA$_2$ receptors are current being tested for pharmacological treatment of inflammatory and autoimmune diseases.

Keywords: Secreted PLA$_2$; Inflammation; Cytokine; Chemokine; Signal transduction

1. Introduction

Secreted phospholipases A_2 (sPLA$_2$) are low molecular weight enzymes that hydrolyze the ester bond at the sn-2 position of phospholipids generating free fatty acids and lysophospholipids [1]. Secreted PLA$_2$s are expressed and stored within several inflammatory cells and are released in the extracellular environment upon appropriate cell activation. The capacity of these molecules of being released accounts for the detection of several sPLA$_2$ isoenzymes in biological fluids and tissues. sPLA$_2$s are currently classified into major groups and several subgroups according to criteria of identifiable sequence homology [2].

Secreted PLA$_2$s have been initially described in snake (group I and II) and bee (group III) venoms [3]. These sPLA$_2$ isoforms induce rapid necrosis of skeletal muscle fibers as well as extensive neural damage and thus they have been referred to as myotoxic and neurotoxic sPLA$_2$s [4]. The first human sPLA$_2$ to

Abbreviations: PLA$_2$, phospholipases A_2^*; BAL, bronchoalveolar lavage; ARDS, adult respiratory distress syndrome; AA, arachidonic acid; MAPKs, mitogen-activated protein kinases; SMC, smooth muscle cells; HSPG, heparan sulphate proteoglycans; CRD, carbohydrate recognition domains; MR, mannose receptor; PAF, Platelet-activating factor; NO, nitric oxide; iNOS, inducible nitric oxide synthase; MMP, matrix metalloproteinase; TIMP-2, tissue inhibitor of metalloproteinase-2; ECM, extracellular matrix; SPI, sPLA$_2$ inhibitor; p38, MAPK p38; ERK1/2, extracellular-regulated kinase 1/2; ATF-2, activating transcription factor-2; JNK, c-jun N-terminal kinase; PI3K, phosphatidyl-inositol 3 kinase; Akt, protein kinase B; NF-κB, nuclear factor-kappa B; AP-1, activator protein-1; NFAT, nuclear factor of activated T cells; SPAN, snake presynaptic sPLA$_2$ neurotoxin

☆ The Roman number after the letter G indicates the group, the letter in caps after the number indicates the subgroup (e.g., GIB indicates group IB PLA$_2$).

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be identified was the pancreatic isoform (group IB, GIB), an enzyme that has a primary role in dietary lipid digestion [5]. The types of $sPLA_2$s identified in mammals have greatly increased and include now twelve isoforms referred to as GIB, GII (subgroups A–F), GIII, GV, GX and GXII (subgroups A–B) [6]. Interestingly, the profile of expression of $sPLA_2$s profoundly changes from cell to cell. This is a major difference with cytosolic PLA_2s whose profile of expression is rather constant within mammalian cells. Moreover, in the same cell or tissue the expression of the various $sPLA_2$ isoforms may be differentially regulated by specific events such as cell differentiation or inflammation.

A major input in the research field of $sPLA_2$s came from the observation that increased levels of $sPLA_2$s were detected either at sites of inflammation or in the blood of patients with inflammatory and autoimmune diseases. Early studies showed that GIIA accumulated in large quantities in the synovial fluid of patients with rheumatoid arthritis [7,8]. Similarly, increased $sPLA_2$ levels were detected in the plasma or serum of patients with acute pancreatitis [9], septic shock [10], rheumatoid arthritis [11], Crohn's disease and ulcerative colitis [12,13]. Furthermore, $sPLA_2$ activity was increased in the bronchoalveolar lavage (BAL) of patients with adult respiratory distress syndrome (ARDS) [14] or bronchial asthma [15,16], and in the nasal lavage of patients with allergic rhinitis [17]. These observations imply that local or systemic inflammation is associated with the release of $sPLA_2$s in vivo and raise the question of the role of $sPLA_2$s in inflammatory reactions.

The hypothesis that $sPLA_2$s may have a role in inflammation was initially supported by the observation that administration of $sPLA_2$s in vivo induced an inflammatory reaction. For example, intraarticular or intradermal injection of $sPLA_2$s in the rabbit caused acute inflammatory changes and tissue damage of the joints and the skin, respectively [18,19]. In addition, intratracheal instillation of $sPLA_2$s promoted neutrophilic pneumonia and lung tissue damage closely resembling those found in ARDS [20,21] whereas a septic shock-like syndrome occurred after intravenous administration of $sPLA_2$s in dogs, cats and rats [22].

The hydrolysis of arachidonic acid (AA) by a PLA_2 enzyme is the initial step for the biosynthesis of eicosanoids (prostaglandins, leukotrienes and lipoxins) and Platelet-Activating Factor (PAF). Therefore, it was initially thought that $sPLA_2$s' main activity was to provide substrates for the biosynthesis of proinflammatory lipid mediators. Consequently, it was proposed that the main mechanism of the proinflammatory action of $sPLA_2$s was related to their catalytic activity. However, studies in the last decades identified alternative mechanisms of action of $sPLA_2$s linked to their capacity to activate inflammatory cells and to induce the production of proinflammatory mediators other than eicosanoids [6]. These effects are mostly unrelated to $sPLA_2$ enzymatic activity and are rather due to the interaction of $sPLA_2$s with specific or promiscuous receptors and/or to other surface molecules expressed on target cells [23]. Further studies have also shown that $sPLA_2$s activate intracellular signaling events in inflammatory cells leading to generation of second messengers (e.g., increase of Ca^{2+}), phosphorylation of major kinases (e.g., mitogen-activated protein kinases, MAPKs) and activation of transcription factors (e.g., nuclear factor kappa B, NF-κB). These additional properties of $sPLA_2$s shed new light on the biological effects relevant to inflammation and immunoregulation exerted by this emerging family of extracellular mediators.

2. Expression of $sPLA_2$s in inflammatory cells

The concomitant presence of $sPLA_2$ activity and the occurrence of biological activities at sites of inflammation have suggested a causal connection between these enzymes and the inflammatory setting. However, the biochemical characterization of the $sPLA_2$ isoforms and their cellular sources remained largely unknown. Early characterization of the $sPLA_2$s released in biological fluids indicated that GIB and GIIA were associated with human diseases such as pancreatitis and rheumatoid arthritis [9,11]. However, for many years the identification of $sPLA_2$s in inflammatory cells and biological fluids has been hampered by the lack of techniques able to discriminate between the various isoforms. The development of specific and sensitive antibodies against the different isoforms allowed the detection of several other $sPLA_2$s (GIID, GIIE, GIIF, GV, GX, GXIIA and GXIIB) as being released during inflammatory processes [24].

Several attempts have been made to identify the cellular sources of $sPLA_2$. The presence of $sPLA_2$ has been demonstrated by various experimental strategies (i.e., functional activity, mRNA expression and protein detection) in platelets [25] and in various leukocyte types, i.e., neutrophils [26], eosinophils [27], basophils [28], monocytes [29] and T cells [30]. Other cells resident in tissues, such as mast cells [31], macrophages [32], fibroblasts [33], epithelial cells [34] and smooth muscle cells (SMC) [35], are also capable of synthesizing $sPLA_2$s.

When the different $sPLA_2$ isoforms were identified, it became clear that the $sPLA_2$ expression profile was different from cell to cell, even though the different techniques used to detect the various $sPLA_2$s were not directly comparable [1]. Most of the studies performed in humans examined the expression of $sPLA_2$s by immunohistochemistry in biopsies obtained from several tissues. Synovial and gut mast cells stained positive for GIIA [36,37] whereas fibroblasts from the same tissues mainly expressed GV [38,39]. Epithelial cells express GV in all tissues so far examined (gastrointestinal tract, kidney and lung) [39–43]. However, they also express additional tissue-specific $sPLA_2$ isoforms, i.e., GIIA, GIID, GIIE, GIIF and GX. Similarly, vascular SMC obtained from heart [39] and lung [43] were found to produce GIIA, but SMC isolated from the synovia [36] or gastric mucosa [39] also express GIIE. Interestingly, liver [40], synovial [36] and intestinal [37] macrophages produce GIIA whereas lung macrophages express GIID, GV and GX, but not GIIA [43]. Together these observations confirm that the expression of $sPLA_2$ isoforms in humans is quite different from cell to cell and, more importantly, that the