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Human group X secreted phospholipase A_2 induces dendritic cell maturation through lipoprotein-dependent and -independent mechanisms

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ABSTRACT

Objective: Increased secreted phospholipase A_2 (sPLA$_2$) activity has been documented in several inflammatory disorders. Among sPLA$_2$s, the human group X (hGX)-sPLA$_2$ has the highest catalytic activity towards phosphatidylcholine (PC), the major phospholipid of cell membranes and blood lipoproteins. hGX-sPLA$_2$ has been detected in human atherosclerotic lesions, indicating that sPLA$_2$s are an important link between lipids and inflammation, both involved in atherosclerosis. The presence of dendritic cells (DC), the most potent antigen presenting cells, in atherosclerotic lesions has raised the question about their role in disease progression.
Methods and results: In this study, we show that hGX-sPLA$_2$-treated LDL induces human monocyte-derived DC maturation, resulting in a characteristic mature DC phenotype and enhanced DC ability to activate IFNγ secretion from T cells. hGX-sPLA$_2$ phospholipolysis of LDL produces high levels of lipid mediators, such as lysophosphatidylcholine (LPC) and free fatty acids (FFAs), which also modulate DC maturation. The major molecular species of LPC containing a palmitic or stearic acid esterified in the *sn*-1 position induce DC maturation, whereas the FFAs can positively or negatively modulate DC maturation depending on their nature. hGX-sPLA$_2$ added alone can also activate DC *in vitro* through the hydrolysis of the DC membrane phospholipids leading, however, to a different cytokine profile secretion pattern than the one observed with hGX-sPLA$_2$-phospholipolysed LDL.
Conclusion: hGX-sPLA$_2$ secreted in inflamed tissues can contribute to local DC maturation, resulting in pro-Th1 cells, through the production of various lipid mediators from hydrolysis of either LDL and/or cell plasma membrane.

1. Introduction

Atherosclerosis is a chronic disease of the arterial wall in which inflammation and immunity interact to define plaque evolution [1]. In this process lipids and lipoproteins are known to play a key role. Among the immune cells detected in atherosclerotic plaques, dendritic cells (DCs) and T helper 1 (Th1) lymphocytes producing IFNγ have been shown to participate in disease progression [2]. DCs which are specialized antigen presenting cells need to be activated, in order to efficiently stimulate naïve T cells and induce their differentiation into polarized effector cells. This polarization depends on the signals T cells receive from DCs by cell to cell contact and by soluble secreted mediators such as cytokines and chemokines. DC activation can be triggered by various signals, including pathogen-associated molecular patterns and endogenous alarm signals associated with tissue damage and/or inflammation. We have previously shown that oxidized low density lipoprotein (oxLDL), a key player in atherosclerosis can promote mature DC generation *in vitro* [3], mainly through the production of the immunostimulating molecule lysophosphatidylcholine (LPC) [4,5]. This suggests that oxLDL plays an active role in the interface between immunity and inflammation. Native LDL prevents DC maturation induced by oxLDL or LPC, suggesting that the balance between native and oxidized LDL regulates the activation threshold of DC [3]. Moreover, native LDL and HDL inhibit the functional

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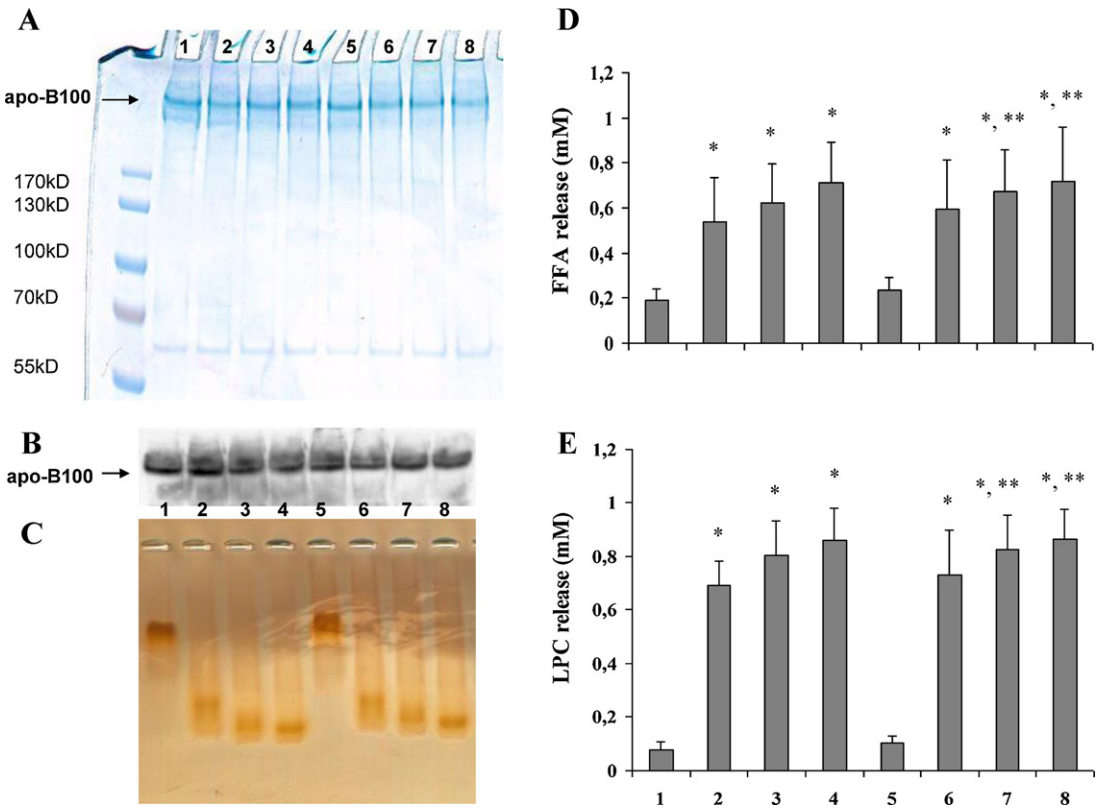

Fig. 1. Biochemical characterization of phospholipolyzed LDL (LDL-X). LDL (1 mg/ml) was treated for 3 h at 37 °C (1–4) in the absence (1) or in the presence of 100, 200, 500 nM hGX-PLA_2 (2, 3, 4 respectively), or for 24 h at RT (5–8), in the absence (5) or in the presence of 100, 200, 500 nM hGX-PLA_2 (6, 7, 8 respectively). The proteins of the resulting lipoproteins were then separated by 7% SDS-PAGE followed by (A) Coomassie Blue staining or (B) Western Blot analysis of apoB-100. Representative of 2 gels with similar results. (C) Agarose gel electrophoresis of lipoproteins followed by Sudan Red staining. Representative of 6 gels with similar results. (D) FFA release and (E) LPC release from the above treated lipoproteins. Results represent the mean ± SE of at least 5 experiments in duplicate; $^{*}p < 0.05$ when compared to LDL, $^{**}p < 0.05$ when compared to 100 nM hGX-$sPLA_2$-treated LDL.

maturation of DCs triggered by some toll-like receptor (TLR) stimulations [6]. These observations underline the role of lipoproteins and lipids in the modulation of the immune system indicating that biochemical modifications of lipoproteins during pathological conditions may reflect an abnormal situation and provide alarm signals to DCs, regulating their immune function.

Secreted phospholipases A_2 ($sPLA_2$s) can also be considered as endogenous alarm signals since their secretion is enhanced in inflamed tissues [7]. $sPLA_2$s constitute a family of structurally related, disulfide-rich, calcium-dependent enzymes, which hydrolyze glycerophospholipids to produce LPC and free fatty acids (FFAs) [8]. These enzymes exhibit major differences in their catalytic activities, substrate preferences and receptor binding properties [8]. Others and we have shown that human group IIA, III and V $sPLA_2$s can induce or regulate DC maturation and function [9–11]. Furthermore, group IIA, III, V and X $sPLA_2$s have been detected in human and/or mouse atherosclerotic lesions [12–14]. Among the $sPLA_2$s, the human group X $sPLA_2$ (hGX-$sPLA_2$) has unique enzymatic properties and binds with high affinity to zwitterionic phospholipids, especially to phosphatidylcholine (PC), the major phospholipid of cell membranes and lipoproteins [15]. Hydrolysis of PC on LDL by hGX-$sPLA_2$ preferentially releases arachidonic or oleic acid and LPC [16]. hGX-$sPLA_2$ is produced as a proenzyme and its activation is likely to be tightly controlled in different cells and various physiopathological conditions [7,17].

We previously showed that LDL phospholipolyzed by hGX-$sPLA_2$ (LDL-X) has a smaller diameter than LDL. LDL-X induces foam cell formation when added to human monocyte-derived macrophages [12] and initiates endothelial cell perturbation through ER stress signaling, providing an LDL oxidation-independent mechanism for the initiation of vascular inflammation in atherosclerosis [18,19]. Given the high activity of hGX-$sPLA_2$ on PC present in cell membranes and lipoproteins and its presence in the atherosclerotic lesions, along with LDL and DCs, we undertook the present study to examine the impact of phospholipolyzed LDL on DC maturation *in vitro*.

2. Materials and Methods

2.1. LDL isolation and treatment with hGX-$sPLA_2$

LDL (d = 1.019–1.063 g/ml) was isolated from frozen plasma by density gradient ultracentrifugation and was free of HDL [12]. Its protein content was determined by the bicinchoninic acid (BCA) method (Thermo Fisher Scientific, France). Freshly prepared filtered (0.45 μm) sterile LDL, 1 mg/mL protein in buffer containing 1 mM $CaCl_2$, 12.5 mM Tris–HCl (pH 8.0), 0.25 M NaCl and 0.0125% BSA was incubated with 100 nM, 200 nM, or 500 nM recombinant hGX-$sPLA_2$ [20] for 3 h at 37 °C or 24 h at room temperature (RT) and denoted as LDL-X. LDL treated as above but in the absence of hGX-$sPLA_2$ is denoted as LDL. In selected experiments, the activity of hGX-$sPLA_2$ was blocked by 10 μM $sPLA_2$ specific inhibitor LY329722. Conjugated dienes, a typical index of oxidation, were measured at 234 nm.

2.2. Non-esterified fatty acid (NEFA) and LPC measurement

The extend of LDL hydrolysis by hGX-$sPLA_2$ was determined in an aliquot of hGX-$sPLA_2$-treated and mock-treated-LDL using