Dendritic-cell activation by secretory phospholipase A2

Reinhold Ramoner, Thomas Putz, Hubert Gander, Andrea Rahm, Georg Bartsch, Claudia Schaber, and Martin Thurnher

Dendritic cells (DCs), also referred to as the sentinels of the immune system, induce and coordinate important functions of immune surveillance. DCs acquire immunity-initiating capacity only after a process of maturation usually induced by ligands that bind to members of the tumor necrosis factor (TNF) or toll-like receptor families. Secretory phospholipase A2 (sPLA2), which hydrolyzes the sn-2 ester bond of glycerophospholipids, regulates a variety of cellular functions including migration of endothelial cells and neurite outgrowth. In the present study we investigated the role of sPLA2 in DC biology. We report that human monocyte-derived DC cultures lack sPLA2 activity but respond to exogenous sPLA2. sPLA2 alone and in cooperation with TNF-α and interleukin 1 β (IL-1β) induced fatty acid release from DC membranes, which was accompanied by up-regulation of surface markers and by an increase in the migratory and immunostimulatory capacity of the DCs. Our findings indicate that secreted enzymes such as sPLA2 can contribute to DC maturation and emphasize the role of lipid mediators in the regulation of immune responses. This observation may also have implications for DC-based vaccine development. (BLOOD. 2005;105:3583-3587)

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Introduction

Phospholipases A2 (PLA2s; phosphatidylcholine-2-acylhydrolase, EC 3.1.1.4) represent a growing family of enzymes that catalyze the hydrolysis of the sn-2 fatty acyl ester bond of membrane glycerophospholipids to release free fatty acids and lysophospholipids such as arachidonic acid (AA) and lysophosphatidylcholine (lyso-PC). PLAs have been assigned to several groups and classified according to cellular localization, amino acid sequence, molecular mass, and calcium (Ca2+) requirement for enzymatic activity. The extracellular or secreted PLA2s (sPLA2s) are characterized by high disulfide bridge content, low molecular mass (~14 kDa), the requirement of millimolar concentration of Ca2+ for catalysis, and wide fatty acid selectivity in vitro. sPLA2 products such as AA and lyso-PC are themselves potent mediators that have been implicated in the regulation of cellular functions. In addition, AA and lyso-PC are subject to further metabolism. While AA can be converted into eicosanoids including the prostaglandins, leukotrienes, thromboxanes, and lipoxins, lyso-PC can be metabolized to generate lysophosphatidic acid (LPA) or platelet-activating factor (PAF). Thus, sPLA2 activity eventually generates a wide array of bioactive lipid mediators.

Several mammalian and venom sPLA2s have been identified. The different venom sPLA2s have been classified into 4 groups based on their primary structures. The bee venom sPLA2 was the founding member of group III. The overall 3-dimensional structure of bee venom group III sPLA2 reveals the striking features of the PLA2 fold, including a well-defined Ca2+ loop, 3 large α helices, and a β wing–like structure. Group III sPLA2 from bee venom is made up of about 135 amino acids and has 5 disulfide bridges. Consistent with these catalytic and structural similarities between venom and mammalian sPLA2, evidence has been obtained that venom sPLA2 can indeed behave like mammalian sPLA2 and induce physiologic effects such as migration of endothelial cells and neurite outgrowth.8,9 Dendritic cells (DCs) affect both innate and adaptive immune responses.10 DCs elicit and tune antigen-specific T- and B-cell responses. According to a well-accepted concept that has emerged during recent years, the maturation state of DCs swings the decision between tolerance and immunity.11 While immature DCs maintain tolerance by silencing antigen-specific T lymphocytes or by actively converting them into regulatory T cells, mature DCs promote immunity. Maturation is induced by a multitude of signals frequently referred to as “danger” signals,12 and DCs have learned to respond to these signals using various receptors of the tumor necrosis factor (TNF) or toll-like receptor families.11 A hallmark of “danger” is inflammation. Infectious agents usually provoke inflammatory responses characterized by the production of many cytokines including TNF-α and interleukin 1 β (IL-1β), which are both known to trigger DC maturation.13 TNF-α and IL-1β are also among the most potent inducers of lipid mediator metabolism.4 TNF-α and IL-1β, for instance, augment the expression of PLA2 enzymes and cyclooxygenases (COXs), resulting in the production of prostaglandins (PGs) such as PGE2, which has also been described to contribute to DC maturation.14,15

In the present work we examined the role of sPLA2s in DC biology and found that sPLA2s can act on DC membranes to mobilize lipid mediators and induce DC maturation.

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Materials and methods

Secretory phospholipase A2

sPLA2 from bee venom was obtained from Cayman Chemical Company (Ann Arbor, MI). To test for potential endotoxin (lipopolysaccharide [LPS]) contamination, sPLA2 was subjected to the Limulus amebocyte lysate (LAL) assay from Biowhittaker (Walkersville, MD). At the sPLA2 concentrations used in this study (1 μg/mL and 10 μg/mL) endotoxin concentrations were 1.1 pg/mL and 11 pg/mL, respectively. At these concentrations, LPS fails to induce DC activation (data not shown). sPLA2 activity was monitored using the colorimetric sPLA2 assay kit (Cayman Chemical Company), which is based on the synthetic substrate diheptanoyl thio phosphorylcholine. Results are expressed as time-dependent absorbance increase (mOD414/min) using 5 points in time with 1-minute increments. Mean values of triplicate measurements plus or minus standard deviation (SD) are shown.

DC culture

Blood monocytes were isolated to high purity using the magnetic-activated cell sorting (MACS) technology from Miltenyi Biotec (Bergisch Gladbach, Germany). Monocytes (3 × 10^6 cells/3 mL) were subsequently cultured for 5 days in 6-well plates in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS; vol/vol) in the presence of granulocyte macrophage–colony-stimulating factor (GM-CSF; 800 U/mL) and IL-4 (500 U/mL). In some experiments, lipoprotein-deficient (LPD) FCS was used (Sigma-Aldrich, Vienna, Austria). From day 5 to day 7, maturation was induced with TNF-α (100 U/mL) and IL-1β (1 ng/mL) in the presence or absence of sPLA2 (1 μg/mL or 10 μg/mL).

Fatty acid release

To assess fatty acid release, day-5 DCs were labeled overnight with [14C]AA (1 μCi/mL) from Amersham Biosciences (Freiburg, Germany). The cells were washed to remove unincorporated radioactivity and reseeded in triplicate in RPMI 1640 medium supplemented with 1% FCS (vol/vol) and 1 mg/mL fatty acid–free bovine serum albumin (BSA; Sigma) for stimulation with TNF-α (100 U/mL) and IL-1β (1 ng/mL) in the presence or absence of sPLA2 (1 μg/mL or 10 μg/mL). After 90 minutes, supernatants and cells were harvested and radioactivity was determined by scintillation counting. The percentage release was calculated using the formula [S/(S+P)] × 100, where S and P represent the radioactivity measured in the supernatant and in the cell pellet, respectively. Values are mean values of quadruplicate measurements plus or minus SD.

Flow cytometry

To determine surface antigen expression, DCs were stained with anti-CD83–phycoerythrin (PE) or anti-CD86–fluorescein isothiocyanate (FITC) for 30 minutes on ice and then washed extensively. The endocytic activity of DCs was assessed using fluorescent tracer molecules. FITC-dextran was used to rate the fatty acid–releasing function of exogenous sPLA2, which cooperate to induce maturation of monocyte-derived DCs, have also been shown to efficiently promote AA mobilization in related cell types. To examine a potential role of endogenous sPLA2 in DC maturation we stimulated day-5 DCs with TNF-α alone (1000 U/mL) or in combination with IL-1β (5 ng/mL) with or without PGE2 (1 μM) for 48 hours and measured sPLA2 activity in culture supernatants (Figure 1). sPLA2 activity could not be detected under any condition. When increasing amounts of exogenous sPLA2 were added (0.1 μg/mL-10 μg/mL), sPLA2 activity could dose dependently be recovered after 48 hours (Figure 1) also indicating that the added sPLA2 was active throughout the 48-hour cell culture period.

Exogenous sPLA2 induces AA release from DC membranes

To assess the fatty acid–releasing function of exogenous sPLA2, day-5 DCs were pulsed overnight with [3H]-AA, which is incorporated into cellular membranes. Figure 2 shows that sPLA2 alone
was able to release substantial amounts of [3H]-AA from DC membranes in a dose-dependent manner. Low doses of TNF-α (100 U) plus IL-1β (1 ng/mL) also stimulated [3H]-AA release, which was further enhanced dose dependently in the presence of sPLA2. TNF-α plus IL-1β and sPLA2 at 10 μg/mL mobilized more than 15% of AA within 90 minutes. Also of note was the observation that even unstimulated, immature DCs released several percent of AA within this short time (Figure 2).

Exogenous sPLA2 has no immediate effects on the endocytic activity of DCs

To study the influence of sPLA2 on the endocytic activity of DCs, endocytosis assays were performed in the presence or absence of sPLA2. Mannose receptor–mediated endocytosis assessed as uptake of FITC-dextran was dose-dependently inhibited in the presence of sPLA2 (data not shown), an effect attributable to binding of sPLA2 to the mannose receptor. The inhibition must therefore be considered a competitive inhibition and not a consequence of sPLA2 enzymatic activity. sPLA2 had little influence on macropinocytosis measured as uptake of FITC-BSA (data not shown). Taken together, no convincing evidence could be obtained that sPLA2 has short-term effects on the endocytosis of DCs.

DCs respond to exogenous sPLA2 and undergo maturation

We of course wondered whether sPLA2 action on DC membranes is associated with the surface remodeling characteristic of DC maturation. We assessed expression of CD83, a very reliable marker of DC maturation, and expression of CD86, a well characterized costimulator of T-cell activation. Figure 3 demonstrates that sPLA2 alone (10 μg/mL) could increase the percentage of CD83+ cells (Figure 3A) and the mean fluorescence intensities (MFIs) of both CD83 and CD86 (Figure 3B). Since sPLA2 may also act on serum lipoproteins to release lipid mediators, identical experiments were performed in parallel using LPD-FCS. The percentages of CD83+ cells were similar or even modestly increased in LPD-FCS. In contrast, increases in CD83 and CD86 MFIs induced in FCS by sPLA2 alone were hardly detectable in LPD-FCS. However, increases in CD83 and CD86 MFIs induced by sPLA2 in combination with TNF-α plus IL-1β were still measurable in LPD-FCS, suggesting that the effects of sPLA2 alone on CD83 and CD86 MFIs occur mainly via sPLA2 digestion of serum lipoproteins, while sPLA2 effects in the presence of TNF-α plus IL-1β are a consequence of direct action of sPLA2 on DC membranes.

sPLA2 treatment enhances the migratory capacity of the DCs

Previous work has shown that sPLA2 can affect the migratory activity of endothelial cells. To test the effects of sPLA2 on DC migration we used a well-established assay of MIP-3β–directed migration of DCs. Figure 4 shows enhancement of DC migration after treatment with sPLA2 alone or in combination with TNF-α plus IL-1β. Migratory responses were modestly reduced when treatments were performed in LPD-FCS but migratory patterns induced by the various stimuli were almost identical (Figure 4).

DCs treated with sPLA2 have enhanced immunostimulatory capacity

The primary purpose of DC maturation is enhancement of the immunostimulatory capacity. As a next step we examined whether the phenotypic changes induced by sPLA2 were accompanied by increased stimulatory capacity of the DCs in the allogeneic MLR. Figure 5 depicts [3H] thymidine incorporation data demonstrating that sPLA2 treatment of DCs enhances proliferative alloresponses. Proliferation data shown in Figure 5 indeed reflect the phenotypic changes shown in Figure 3. In addition, cytokines accumulating in the allogeneic MLR supernatants were measured after 24 hours. Figure 6 confirms that...
Figure 5. sPLA2 treatment of DCs enhances their stimulatory capacity in the allogeneic MLR: proliferative responses. Day-5 DCs were treated in the presence of FCS or LPD-FCS for 48 hours with sPLA2 alone (10 μg/mL) or in combination with TNF-α (100 U/mL) plus IL-1β (1 ng/mL). Cells were washed and used as stimulators of 3 different preparations of allogeneic PBMCs depleted of CD14+ cells. Proliferation was determined as [3H] thymidine incorporation. Results from 1 of 2 almost identical experiments are shown. Mean values of triplicate measurements ± SD are shown. LPD indicates lipoprotein-deficient.

Figure 6. sPLA2 treatment of DCs enhances their stimulatory capacity in the allogeneic MLR: cytokine responses. Day-5 DCs were treated in the presence of FCS or LPD-FCS for 48 hours with sPLA2 alone (10 μg/mL) or in combination with TNF-α (100 U/mL) plus IL-1β (1 ng/mL). Cells were washed and used as stimulators of 3 different preparations of allogeneic PBMCs depleted of CD14+ cells. Triplicate supernatants were harvested and pooled at 24 and 96 hours. Cytokine profiles were determined using a commercial cytokine bead array and a flow cytometer (both from BD Biosciences). The 24-hour values are shown. LPD indicates lipoprotein-deficient.

Discussion

This is one of the first studies that investigates the effects of a secreted enzyme on DCs. DCs generated from monocytes in the presence of IL-4 lack endogenous sPLA2 activity (Figure 1) but respond to exogenous sPLA2 (Figures 2-6). Exogenous sPLA2 induced AA release from DC membranes (Figure 2), up-regulation of surface markers (Figure 3), and an increase in migratory (Figure 4) and immunostimulatory capacities (Figures 5 and 6). Confirming evidence for stimulatory effects of sPLA2 on DCs has recently also been obtained by Perrin-Cocon and colleagues.21

A principle of DC maturation that emerges is the cooperation of 2 groups of mediators. Ligands of TNF or toll-like receptors initiate the process of DC maturation.11 The concurrent activation of PLA2 enzymes initiates phospholipid metabolism and generates lipid mediators that cooperate with the ligands of TNF or toll-like receptors to enhance DC maturation.

The major phospholipid of the outer leaflet of the plasma membrane is phosphatidylcholine (PC). Lipolysis of PC by sPLA2 thus results in the release of a free fatty acid such as AA and lyso-PC (Figure 7). Lyso-PC has recently been shown to activate DCs.22 AA can be metabolized by COX and the respective synthase toward PGE2, which cooperates with TNF-α in DC maturation.14 Using a sensitive radioimmunoassay we were unable to detect PGE2 in culture supernatants of DCs treated with sPLA2 with or without TNF-α plus IL-1β (data not shown). We therefore conclude that the maturation-enhancing effects of sPLA2 are mediated by lyso-PC.

In addition to the direct action on cellular membranes, sPLA2 may act on serum lipoproteins to release lipid mediators, including lyso-PC.19 Side-by-side analysis in complete FCS or LPD-FCS revealed that part of the observed sPLA2 effects are indeed mediated by lipoprotein-derived mediators (Figures 3-6) indicating that sPLA2 may use either membrane phospholipids or serum lipoproteins as substrate. During inflammatory processes vascular leakage results in the influx of serum lipoproteins which increase substrate availability for sPLA2.

Taken together, sPLA2 activity may lead to the concomitant generation of 2 different lipid mediators that both enhance DC maturation, lyso-PC and PGE2. However, monocyte-derived DCs generated in the presence of IL-4 lack endogenous sPLA2 activity (Figure 1) and instead fail to produce PGE2.23 DCs obviously depend on exogenous sPLA2. In fact, one major function of mammalian secreted PLA2 may be to act on cells such as DCs which themselves lack endogenous enzyme activity. Such a mechanism of transcellular DC activation in which one cell, for instance a macrophage or stromal cell, secretes PLA2 which then induces transactivation of adjacent DCs has so far not been described. sPLA2 is considered an amplifier of inflammatory responses, which expands inflammation by activating neighboring cells. Our finding that sPLA2 activates DCs points to an important role of sPLA2 in the induction and amplification of immune responses. Transcellular DC activation may lead to more systemic DC maturation, which appears to be required for the induction of robust immunity. In this context the expression patterns of sPLA2...
classes may also be of interest. While sPLA2 IIA is found at very high levels in inflammatory lesions, the group V enzyme is abundant in macrophages and group X sPLA2 is selectively expressed in spleen, thymus, and peripheral blood leukocytes. Lambeau and Lazdunski M. Receptors for a growing family of secreted phospholipases A2. Trends Pharmacol Sci. 1999;20:162-170.


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