Lipids in dendritic cell biology: messengers, effectors, and antigens

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Abstract: Dendritic cells (DC) are the most professional APC, which induce and coordinate immune responses. The principal task of DC is T cell activation, although DC also interact with and regulate other cell types. The present review serves to illustrate the increasing evidence that lipids play an important role in DC biology. In addition to being fuel stores and structural components of cellular membranes such as in other cell types, lipids act as second messengers and as effectors throughout all steps of DC differentiation and regulate important DC functions. The recent finding that DC synthesize lipid antigens in response to bacterial stimulation and induce antibacterial, CD1-restricted T cells through antigenic mimicry further emphasizes the important role of lipids and DC at the blurring boundaries of innate and adaptive immunity. J. Leukoc. Biol. 81: 154–160; 2007.

Key Words: phospholipids · sphingolipids · phospholipase A2 · prostaglandins · CD1 · antigen-presentation

INTRODUCTION

Dendritic cells (DC) are professional APC, which induce and coordinate T cell responses [1, 2]. DC can be divided into at least two major subsets [1, 2]: the myeloid DC, which include the Langerhans cells of the skin as well as the interstitial DC of all other tissues, and the plasmacytoid DC, which secrete large amounts of Type I IFN after viral infection. In peripheral tissues, DC pick up protein antigens through various endocytic mechanisms and subsequently, subject them to endosomal processing. The resulting peptides are presented on the DC surface in the context of MHC molecules. In addition, lipid antigens may be presented by CD1 molecules on DC [3], with or without prior endocytic uptake and intracellular processing [4, 5]. Prompted by inflammatory signals at the sites of infection, DC run through a series of phenotypic and functional changes referred to as maturation and then chemotactically migrate to the T cell areas within adjacent secondary lymphoid organs to activate peptide-specific, MHC-restricted T cells or lipid-specific, CD1-restricted T cells [2, 3]. The last decade has also been characterized by multifaceted attempts to exploit DC as therapeutic vaccines against cancer [2, 6].

LIPIDS REGULATE DC DIFFERENTIATION

A tremendous effort in understanding human DC biology has been based on research performed with a two-step culture system that generates DC from monocytes in the presence of GM-CSF and IL-4 (Step 1), followed by a maturation phase (Step 2) induced by proinflammatory stimuli of microbial origin or by T cell-derived signals (Fig. 1) [2, 7–9]. One profound effect of IL-4 on peripheral blood monocytes in this system is the induction of PPAR-γ [10–12], a ligand-dependent nuclear receptor that has been implicated in cellular differentiation and function. A variety of synthetic and naturally occurring lipids can activate PPAR-γ. It is intriguing that IL-4 concomitantly induces 15-lipoxygenase (LOX) expression, a lipid-peroxidating enzyme that can oxygenate free polyunsaturated fatty acids and phospholipids in biomembranes [13]. In human DC, 15-LOX, for instance, converts arachidonic acid into 15-HETE (Figs. 1 and 2) [14]. Thus, IL-4 concomitantly induces the expression of PPAR-γ and the biosynthesis of PPAR-γ ligands, thereby generating an autocrine loop with anti-inflammatory effects. Continuous activation of PPAR-γ by endogenous ligands in the presence of IL-4 may be important to prevent macrophage deviation and to keep DC in the immature state, thus preventing spontaneous DC maturation and undesired T cell responses in the absence of proinflammatory signals.

The anti-inflammatory effect of IL-4 in this culture system also involves suppression of proinflammatory cytokines such as TNF-α and IL-1β [15] as well as the down-regulation of endogenous PGE2 biosynthesis [16]. Absence of proinflammatory factors appears to be a requirement for coordinate DC differentiation from monocytes, as the presence of such factors causes DC desensitization and failure to mature into cytokine-producing, CD83+ immunostimulatory DC [17]. This condition of desensitization also occurs during endotoxin tolerance and is considered responsible for the immunosuppressed state after fatal sepsis [18]. Likewise, chronic inflammation may result in DC desensitization and immunosuppression. Tissue damage in the absence of effective immunosurveillance may eventually pave the way for tumorogenesis.

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LIPIDS MODULATE DC MATURATION

A paradigmatic, proinflammatory factor that induces DC maturation is LPS, a glycolipid and common constituent of the outer membranes of gram-negative bacteria [19]. The biologically active structure of LPS is the lipid-A moiety, a phosphorylated, multiply acylated glucosamine disaccharide. LPS complexes with a serum protein known as LPS-binding protein (LBP). The LPS-LBP complex then binds to CD14, resulting in signal transduction via TLR-4 [20].

Mycobacteria such as bacille-Calmette-Guérin also induce DC maturation [21, 22], although they lack LPS. Mycobacteria instead produce another type of glycolipid, the LAM, which also binds to CD14, resulting in signal transduction via TLR-2 [23]. LAM, like LPS, induces or modulates DC maturation and IL-12 production [24, 25]. In the context of DC stimulation with LAM, Nigou and colleagues [26] demonstrated for the first time that engagement of the mannose receptor provides an inhibitory signal for TLR-induced IL-12 production.

LPS and LAM readily induce inflammation. Inflammatory lesions are characterized by the abundance of phospholipases such as the sPLA2, a 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 (Fig. 2A) [27]. If the phospholipid is phosphatidylycerol (PC, also known as lecithin), sPLA2 generates lyso-PC. Treatment of DC in vitro with sPLA2 or its product lyso-PC results in DC maturation, as evidenced by CD83 expression, cytokine production, and enhanced stimulatory activity in T cell assays [28–30]. If the free fatty acid, which is liberated from the phospholipid by sPLA2, is arachidonic acid, various eicosanoids can be formed (Fig. 2B). Arachidonic acid can be metabolized by cyclooxygenase (COX) and the respective terminal synthase (isomerase) toward prostaglandins (PG) E2. It is well established that PGE2 cooperates with TNF-α in inducing DC maturation [31, 32]. PGE2 acts via four seven-transmembrane domain, G protein-coupled receptors, termed EP1–EP4 [33]. One possible reason for the potent effect of exogenous PGE2 in DC maturation may be the limited ability of DC to provide substantial amounts of endogenous PGE2 as a result of IL-4-mediated suppression of the eicosanoid cascade in monocyte-derived DC generated in the presence of IL-4 [16]. PGE2 is an unstable lipid and subject to nonenzymatic hydrolysis, which results in formation of the cyclopentenone PGA2 (Fig. 2B). Although cyclopentenone PGs have been implicated mainly in anti-inflammatory processes, PGA2 also cooperated with TNF-α in inducing the maturation of immunostimulatory CD83+ DC [34], suggesting that the effects of PGE2 may in part be mediated by its degradation product PGA2.

Yet another PG that has been implicated in the regulation of DC maturation is PGD2, which is formed by the respective PGD2 synthase when DHA is the substrate. PGD2 is a weak proinflammatory PG and a weak agonist of TLR-4 on DC. PGD2 is generated by cyclooxygenase (COX) and the respective terminal synthase (isomerase) toward prostacyclins (PGI2), which is a potent vasodilator and antiplatelet agent.

The major lipid components of the outer leaflet of plasma membranes are phosphatidylcholine (PC) and sphingomyelin (SM). (A) sPLA2 uses PC as a substrate and generates lyso-PC as well as a free fatty acid. (B) If the free fatty acid is arachidonic acid, the eicosanoid cascade can be initiated, resulting in the formation of prostaglandins (PGs), leukotrienes (LTs), and lipoxins (LXs). (C) SM can be metabolized by SMase to generate ceramide, which is the precursor for S1P as well as for lipid antigens (sulfatides and gangliosides).

Fig. 2. The major lipid components of the outer leaflet of plasma membranes are phosphatidylcholine (PC) and sphingomyelin (SM). (A) sPLA2 uses PC as a substrate and generates lyso-PC as well as a free fatty acid. (B) If the free fatty acid is arachidonic acid, the eicosanoid cascade can be initiated, resulting in the formation of prostaglandins (PGs), leukotrienes (LTs), and lipoxins (LXs). (C) SM can be metabolized by SMase to generate ceramide, which is the precursor for S1P as well as for lipid antigens (sulfatides and gangliosides).
terminal synthase from PGH2 (Fig. 2B). PGD2 is produced in several tissues, most notably in mast cells and in brain [35]. IgE and other stimuli cause mast cells to release PGD2 together with histamine and other mediators. Similar to PGE2 metabolism, PGD2 can also be nonenzymatically dehydrated and isomerized to form 15d-PGJ2 (Fig. 2B). PGD2 exerts its effects via D prostanoid (DP1 and DP2) receptors and via a chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes [35]. No specific receptor for 15d-PGJ2 has been identified. 15d-PGJ2 instead acts via DP1 and DP2. In addition, PGD2 and 15d-PGJ2 can affect cellular differentiation and function via binding to PPAR-γ. PGD2 and 15d-PGJ2 were found to alter DC maturation by suppressing IL-12 production (PGD2) and by affecting the patterns of costimulatory molecule expression (PGD2 and 15d-PGJ2) [36]. In addition, 15d-PGJ2 can activate PPAR-γ in human monocyte-derived DC and thereby inhibit TLR-dependent DC activation via MAPK NF-kB pathways [12].

In contrast to monocyte-derived DC, little is known about the effects of PGs on plasmacytoid DC. In a recent report, PGE2 was found to be a negative regulator of plasmacytoid DC, as pretreatment of plasmacytoid DC with PGE2 caused cell death and inhibited IFN-α production in response to virus and CpG in a dose-dependent manner [37]. Although PGE2 impaired survival of plasmacytoid DC, PGE2 conferred survival-dependent apoptosis resistance in monocyte-derived DC [38].

Inflammatory responses are accompanied by the production of reactive oxygen species, which mediate lipid peroxidation. The resulting oxidized phospholipids, which can also bind to PPAR-γ, negatively regulate DC maturation [39]. The formation of oxidized phospholipids is considered a negative feedback loop that prevents excessive inflammatory and immune responses.

LIPIDS SIGNAL DC MIGRATION

Once knowing its prominent effect on DC maturation [31, 32], potential effects of PEG2 on DC migration were subsequently evaluated. Scandella et al. [40] tested various maturation stimuli and demonstrated that addition of PGE2 was absolutely required to induce productive migration of DC in response to CCL19 and CCL21, both ligands of the chemokine receptor CCR7. PGE2 did not alter CCR7 cell surface expression on DC but rather controlled migration of mature DC by facilitating CCR7 signal transduction [41]. Likewise, DC migration toward the chemokine CXCL12 and complement component C5a was also dependent on PGE2 pretreatment [42]. It is intriguing that PGE2 was stringently required during the early phase of DC maturation to enable chemotaxis, and delayed addition was ineffective. PGE2 exerted its effects on DC migration via EP2 and EP4 receptors [42]. These findings support the view that an early PGE2 stimulus is generally required for DC migration, irrespective of the chemotactic signal. The finding that the PGE2 signal is required early during DC maturation is intriguing, in light of the fact that inflammatory PGE2 production is biphasic [27]. Mobilization of arachidonic acid, the precursor of PGE2, by macrophages and mast cells usually occurs in two phases. The immediate phase, which is characterized by a burst of arachidonic acid liberation, takes minutes. The delayed phase, which spans several hours, depends on the continuous supply of arachidonic acid and involves cytosolic PLA2 activation [43]. Taken together, these findings suggest that the initial burst, which occurs immediately upon encounter with proinflammatory factors, generates the first wave of PGE2, which prompts DC to migrate from the site of antigen contact to adjacent lymph nodes. Such a mechanism may ensure that DC selectively internalize and present antigens from the microbial invader, which has elicited the early PGE2 signal.

Myself and others [31] have shown previously that PGE2 induces IL-12p40 production in DC. Very recently, Khader et al. [44] demonstrated that IL-12p40 is required for DC migration, as DC from mice deficient in IL-12p40 failed to migrate to draining lymph nodes for naïve T cell activation. Thus, PGE2 appears to prompt DC migration via induction of IL-12p40.

In contrast to PGE2, PGD2 has been shown to inhibit chemokine-induced migration of DC [36]. In a rat model of carrageenin-induced pleurisy, inducible COX-2, which is considered a predominantly proinflammatory enzyme, was responsible for PGE2 production at 2 h [45]. It is important that a second rise in COX-2 at 48 h resulted in the production of PGD2 and 15d-PGJ2, which contribute to the resolution of inflammation [45]. Thus, although early PGE2 prompts DC migration, delayed production of PGD2 and 15d-PGJ2 inhibits DC migration and thereby prevents excessive immune responses as a result of prolonged antigen presentation.

Another type of lipid that has also been shown to influence DC migration is LTC4 (Fig. 2B). The LTs received attention by the DC field after Randolph and colleagues [46] had shown that the ATP-binding cassette protein, multixidation resistance protein 1 (MRP-1), is important for DC migration. MRP-1 is an effective membrane pump that mediates the efflux of many drugs, including chemotherapeutic agents from the intracellular space, and thereby contributes to drug resistance. One physiological role of MRP-1 is the export of endogenously produced cysteinyl LTs [47], which fail to passively cross the cell membrane. Together, these data suggested that the cysteinyl-LTs are important for DC migration. In a subsequent work, it was demonstrated that DC migration from skin to draining lymph nodes was reduced greatly in mice lacking MRP-1 and that migration was restored by exogenous LTC4 [48]. The mechanism through which LTC4 exerts its effects on DC migration or other functions is currently unknown, although Thivierge and colleagues [49] have shown that cysteinyl-LT receptors are regulated during DC maturation. LTC4-mediated DC migration does not represent a general principle, as DC emigration from the lung to draining lymph nodes does not depend on MRP-1 [50].

A third lipid type that has been shown to regulate DC migration is S1P [51], which can be generated from ceramide through the action of ceramidase, which forms sphingosine, and sphingosine kinase finally phosphorylates sphingosine to form S1P (Fig. 2C). S1P acts via five related G protein-coupled receptors. S1P, which is abundant in human serum (up to 1 μM), has been shown previously to affect leukocyte migration,
ory T cells (TCM) are involved in secondary responses and can be subdivided based on their specific functions. Central memory (CM) T cells, which develop in secondary lymphoid organs, are involved in immediate protection. Obviously, signal strength is determined by the degree of DC maturation, as the expression of MHC (Signal 1) and costimulatory molecules (Signal 2) as well as the production of cytokines are up-regulated during DC maturation. PGE2 effectively costimulates the TNF-α-induced maturation of DC, which then become homogenously CD83+ and express high levels of various molecules related to antigen presentation. Thus, the strong signal provided by DC matured in the presence of PGE2 would likely drive the differentiation of TEM, which would be characterized by effector cytokine production and limited proliferative potential.

In particular, IL-5 production, which can be detected after in vitro T cell stimulation with PGE2-exposed DC and after vaccination with PGE2-exposed DC, has been shown to be restricted entirely to TEM. In addition, CFSE dye dilution analysis to determine antigen-induced proliferation after DC vaccination revealed only limited T cell cycling in response to the vaccine-control antigen keyhole limpet hemocyanin (KLH). In contrast, incubation of patient T cells with IL-7 plus IL-15 resulted in progressive dye dilution and formation of multiple daughter populations (M. Thurnher, unpublished observation). The fact that vaccination with PGE2-exposed DC promotes the production of large amounts of IFN-γ and IL-5 by responding T cells along with the observation that the proliferative potential of the responding T cells is limited suggest that fully mature DC generated in the presence of PGE2 provide an enhanced signal for T cells, which drives TEM differentiation. In contrast, less mature DC, for instance, generated with TNF-α alone, may favor the development of TCM (Fig. 3A). These considerations also raise the question of whether DC-based antitumor vaccination protocols should be redesigned to increasingly induce TCM to generate long-term memory, which may prolong patient survival.

A problem of many studies, which examined the role of PGE2 in immune regulation, is that PGE2 is often used as a single agent. However, such experimental conditions do not represent the physiological situation. PGE2 arises in inflammatory lesions as a result of the activity of proinflammatory as it regulates lymphocyte traffic within lymph nodes as well as lymphocyte egress from lymph nodes.

Idzko et al. demonstrated that in immature human DC, S1P stimulates chemotaxis, whereas these responses were lost upon DC maturation. In another study by Czeloth et al., S1P selectively induced the migration of mature murine DC. S1P-induced migration of murine DC could be inhibited effectively by the S1P agonist FTY720 in vitro and in vivo. FTY720 is tested in Phase III clinical trials for prevention of allograft rejection. In a study by Lan et al., FTY720 promotes the retention of murine DC in the circulation, most likely by inhibiting endothelial adhesion and transmigration. Although further experimental evidence is required, S1P appears to be important for the migration of mature DC toward secondary lymphoid organs.

LIPIDS DETERMINE THE T CELL-STIMULATORY CAPACITY OF DC

PGE2 is important already during early T cell development. Stromal cell-derived PGE2 in the thymus supports early thymocyte proliferation and differentiation as well as the maturation of the CD4+ Th cell lineage. At the level of the mature T cell, PGE2 can protect T cells from activation-induced cell death via PGE2-induced down-regulation of Fas ligand in activated T cells. The impact of PGs and in particular, of PG-exposed DC on Th cell differentiation is the subject of sometimes controversial discussions.

Almost a decade ago, myself and others found that PGE2 cooperates with TNF-α in inducing DC maturation. Based on a commercial ELISA, which detected IL-12 p40 and the bioactive IL-12p70 heterodimer consisting of p40 and p35, PGE2 appeared to induce IL-12 production in synergy with TNF-α. Kalinski et al. then demonstrated that PGE2 is a selective inducer of IL-12p40 and emphasized the Th2-driving function of PGE2. However, the original notion that DC generated in the presence of PGE2 exclusively promote Th2-type cytokine production in maturing human naïve Th cells could not be confirmed. Instead, such DC promote the efficient production of IFN-γ in vitro T cell assays and also after DC vaccination in ex vivo T cell assays. In addition, cytotoxic T lymphocytes have been induced by DC vaccination in these clinical studies. It is intriguing that IL-5 but not IL-4 production has sometimes been observed after DC vaccination (M. Thurnher, unpublished observation). Likewise, in the allogeneic MLR, DC, matured with TNF-α plus PGE2, induced substantially more IFN-γ and IL-5 compared with DC matured with TNF-α alone. Together, these findings argue against PGE2 as an exclusively Th2-driving force. PGE2 rather appears to drive concomitant differentiation of Th1 and Th2 cells.

According to a well-established model of memory T cell generation, the strength of the TCR and cytokine stimulation drives progressive T cell differentiation. In this model, signals of medium strength generate memory T cells, which can be subdivided based on their specific functions. Central memory T cells (TCM) are involved in secondary responses and long-term protection, whereas effector memory T cells (TEM) are involved in immediate protection. Obviously, signal strength is determined by the degree of DC maturation, as the expression of MHC (Signal 1) and costimulatory molecules (Signal 2) as well as the production of cytokines are up-regulated during DC maturation. PGE2 effectively costimulates the TNF-α-induced maturation of DC, which then become homogenously CD83+ and express high levels of various molecules related to antigen presentation. Thus, the strong signal provided by DC matured in the presence of PGE2 would likely drive the differentiation of TEM, which would be characterized by effector cytokine production and limited proliferative potential.

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cytokines, which stimulate the production of PGE2 and other mediators. Thus, PGE2 usually acts in concert with these cytokines and mediators. PGE2 appears to be essential for DC migration and thus for the induction of T cell responses, irrespective of a Th bias. PGE2 exerts its migration-inducing effects [42] via the induction of IL-12p40 [31], which is required for DC migration from peripheral tissues to draining lymph nodes [44]. PGE2 should therefore not be considered a force that exclusively drives Th2 responses. PGE2 rather contributes to the inflammatory process, stimulates DC migration, and regulates DC signal strength. PGE2 may thus shape the memory T cell repertoire by favoring the differentiation of Type 1 and Type 2 TEM. The final outcome of PGE2 action critically depends on the timing, kinetics, and the actual context of PGE2 action.

LIPID ANTIGENS ARE PRESENTED BY DC

In addition to their roles as fuel stores, structural components, second messengers, and effectors, lipid antigens may be recognized by the immune system. To be recognized by T cells, lipids have to be presented by CD1 proteins, which can bind lipids [3]. The human CD1 family consists of at least five members. Based on their sequence similarity, CD1a, CD1b, and CD1c are classified as Group 1 CD1 proteins. Group 2 consists of CD1d only, and CD1e again represents a separate group. Although T cells restricted by Group 1 CD1 antigens share many properties with peptide-specific, MHC-restricted T cells, the majority of CD1d-restricted T cells is represented by NKT cells, which express NK cell markers along with a semi-invariant TCR. Among the various lipid antigens presented on CD1 is sulfatide, which binds promiscuously to all CD1 isoforms and may induce sulfatide-specific, CD1-restricted T cells [3, 4, 70]. A recent study by Manolova et al. [71] has shown that sulfatide, which is abundant in serum, stabilizes surface expression of functional CD1a, which is considered a marker for DC in general and particularly, for Langerhans cells [1]. Homogenous CD1a expression has been considered a quality feature of in vitro DC generation, and as long as DC were cultured in the presence of serum (usually 10% FBS), the great majority of the DC was indeed CD1a+ [8, 9]. When DC research was translated into the clinic, serum-free media were used to generate clinical-grade patient DC. Under these conditions, CD1a expression was reduced strongly [72]. Doubts were raised about the genuineness of these DC, and the existence of various subsets was discussed [73]. The study by Manolova et al. [71] now unravels the mystery and demonstrates that serum lipids and particularly, sulfatide are required to stabilize surface CD1a.

Recent studies have demonstrated that T cell-based recognition of lipid antigens is important for antibacterial immunosurveillance and that lipid-specific responses occur at higher frequencies than suspected previously [3]. In this context, a recently identified mechanism deserves particular attention. De Libero et al. [70] have shown that bacterial infection promotes T cell recognition of self-glycolipids. Immediately after infection, APC such as DC respond to bacterial components such as LPS by up-regulating the synthesis of endogenous glycosphingolipids (GSLs). Endogenously produced GSL are subsequently presented on CD1, resulting in the stimulation of GSL-specific T cells in a CD1-restricted and TCR-dependent manner (Fig. 4). The novelty of this finding also relates to the fact that DC synthesize self-antigens in response to bacterial stimulation and activate autoreactive T cells that detect bacterial infection through molecular mimicry [74]. Usually, DC take up exogenous antigen through endocytosis or produce endogenous viral antigens during virus infection. This mechanism, which causes the blurring of the boundaries of the innate and the adaptive immune system, allows a rapid response to bacterial infection and in addition, may represent a helper function for MHC-restricted T cell responses, as cytokines produced by CD1-restricted T cells will activate local APC further and thus enhance CD1-restricted and MHC-restricted T cell responses (Fig. 4).

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