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The Influence of Lysophosphatidic Acid on the Functions of Human Dendritic Cells

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Lysophosphatidic acid (LPA) is a bioactive lipid mediator which is generated by secretory phospholipase A_2. In this study, we studied the biological activity of LPA on human dendritic cells (DCs), which are specialized APCs characterized by their ability to migrate into target sites and secondary lymphoid organs to process Ags and activate naive T cells. We show that immature and mature DCs express the mRNA for different LPA receptors such as endothelial differentiation gene (EDG)-2, EDG-4, and EDG-7. In immature DCs, LPA stimulated pertussis toxin-sensitive Ca^{2+} increase, actin polymerization, and chemotaxis. During the maturation process, DCs lost their ability to respond toward LPA with Ca^{2+} transients, actin polymerization, and chemotaxis. However, LPA inhibited in a pertussis toxin-insensitive manner the secretion of IL-12 and TNFα as well as enhanced secretion of IL-10 from mature DCs. Moreover, LPA did not affect the endocytic or phagocytic capacities and the surface phenotype of DCs, although it increased the allostimulatory function of mature DC and inhibited their capacity to induce Th1 differentiation. In summary, our study implicates that LPA might regulate the trafficking, cytokine production, and T cell-activating functions of DCs. *The Journal of Immunology,* 2002, 169: 4129–4135.

Lysophosphatidic acid (LPA)[3] is a natural occurring water-soluble phospholipid that was originally identified as a key molecule in de novo lipid biosynthesis. LPA is also regarded as an important extracellular mediator regulating a broad range of biological functions (1, 2). At present, three different stimulus-coupled cellular pathways for rapid synthesis and release of this mediator are known. The first pathway involves sphingomyelinase conditioning of cell-derived plasma membrane vesicles, phospholipase C- and/or phospholipase D-dependent liberation of phosphatidic acid and its conversion to LPA by secretory type II phospholipase A_2 (PLA_2) (3, 4). In the second pathway, activated diacylglycerol kinase generates phosphatidic acid, which again can be converted by secretory type II PLA_2 to LPA (5, 6). Finally, production of LPA in minimal oxidized low-density lipoproteins results from specific oxidative degradation (7). Independently of the generation process, LPA is stored within the cell at concentrations up to 60 μM and it may accumulate in extracellular fluids such as in the inflammatory exudate at concentrations as high as 10 μM (8). It has been shown that LPA stimulates cell proliferation, regulates keratinocyte differentiation, induces smooth muscle contraction, and triggers chemotaxis of epithelial cells and leukocytes (9–13). It is thought to be involved in progression of ovarian cancer, wound heeling, platelet aggregation, and inflammation (10, 14–18). Recently, several studies suggested a role for LPA as regulator of immunological functions. For instance, LPA has chemotactic activity, regulates secretion of IL-2, and prevents apoptosis in T cells (16–18). In addition, LPA is a growth factor for B cells, and it stimulates adhesion of monocytes and neutrophils to endothelial cells (19, 20).

Cellular recognition of LPA is mediated by G protein-coupled receptors of the endothelial differentiation gene (EDG) receptors. Among this family, EDG-2, EDG-4, and EDG-7 are specific receptors for LPA (21). The downstream biochemical events linking LPA to its pleomorphic activities are complex, since these receptors couple to different pertussis toxin (PTX)-sensitive and -insensitive G proteins. Depending on cell type, G_i as well as $G_{q/11/12}$ couple EDG-2, -4, and -7 to phospholipases, trigger tyrosine phosphorylation, and regulate rho-dependent actin reorganization (22–24).

Dendritic cells (DCs) are APCs specialized in the activation of naive T lymphocytes and the initiation of immune responses (25, 26). DCs originating from hemopoietic stem cells migrate to peripheral tissues such as the skin, where they are adapted to capture Ags and alert for danger signals such as microbial products, inflammatory cytokines, and cytoplasmic molecules released in the extracellular environment as a consequence of cell necrosis (27). Upon exposure to these factors, DC undergo maturation, a process that involves acquisition of high levels of membrane MHC and costimulatory molecules, and the production of a broad panel of cytokines, including TNF-α, IL-10, and IL-12 (25). Mature DCs migrate to secondary lymphoid organs to prime T cells to direct the development of immune responses (26, 28).

Recently, we showed that sphingosine-1 phosphate, the ligand of EDG-1, -3, -5, and -6, regulates the chemotaxis and cytokine

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[3] Abbreviations used in this paper: LPA, lysophosphatidic acid; DC, dendritic cell; PLA_2, phospholipase A_2; EDG, endothelial differentiation gene; PTX, pertussis toxin; C5a, complement fragment 5a; MIP-3β, macrophage inflammatory protein-3β; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; f-actin, filamentous actin.

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release of human DCs (29). Because generation of LPA in acute lung injury is mediated by soluble PLA_2 and huge amounts of soluble PLA_2 activity could be detected in nasal and bronchoalveolar lavage fluids in allergic patients after Ag challenge (30–33), we characterized the biological activity of LPA and the expression of EDG-2, -4, and -7 in DCs. We show that immature and mature DCs express the mRNA for the LPA receptors. LPA induces intracellular Ca^{2+} transients, actin polymerization, and chemotaxis in immature DCs. Maturation of DCs results in the loss of these responses, but LPA enhances IL-10 production and inhibits IL-12 and TNF-α secretion in maturing DCs. Furthermore, LPA increases the capacity of mature DCs to induce proliferation of allogeneic naive T cells while impairing the capacity of DCs to promote Th1 differentiation.

Materials and Methods

Reagents

LPA, recombinant human complement fragment 5a (C5a), PTX, and lysophosphatidylcholine were obtained from Sigma-Aldrich (Deisenhofen, Germany). Macrophage inflammatory protein-3β (MIP-3β) was obtained from PeproTech (London, U.K.).

Preparation of human DCs

PBMC were isolated from buffy coats by Ficoll centrifugation (29). The mononuclear cells were further separated with anti-CD14 mAb-coated MicroBeads using MACS single-use separation columns from Miltenyi Biotec (Bergisch Gladbach, Germany). The $CD14^+$ cells were resuspended in RPMI 1640 containing 10% FCS, 1% glutamine, 50 IU/ml penicillin, 50 μg/ml streptomycin, 1000 U/ml IL-4, and 200 ng/ml GM-CSF (Promocell, Heidelberg, Germany) at 37°C with 5% CO_2 for 5 days. DCs differentiated from monocytes were $<$5% $CD14^+$ and $>$95% $CD1a^+$, $CD80^{low}$, $CD86^{low}$, $CD83^{low}$, and $CD115^{high}$. Further differentiation into mature DCs was induced by treatment with 3 μg/ml LPS (Sigma-Aldrich) for 24–38 h. Mature cells were $<$5% $CD14^+$ and $>$95% $CD1a^+$, $CD80^{high}$, $CD86^{high}$, $CD83^{high}$ and $CD115^{low}$.

Semiquantitative RT-PCR analysis

mRNA was isolated with QIAshredder and RNeasy kits (Qiagen, Hilden, Germany). mRNA, Moloney murine leukemia virus reverse transcriptase, and $pd(N)_6$ primers (Life Technologies, Gaithersburg, MD) were used to obtain cDNA. All oligonucleotides used as primers in RT-PCR were designed to recognize sequences specific for each target cDNA. Primer sequences are as follows: EDG-2 receptor (464-bp product), sense, 5′-CGG CGG GTA GTG GTG GTC-3′, antisense, 5′-TTG TCG CGG TAG GAG TAA ATG ATG-3′; EDG-4 receptor (375-bp product), sense, 5′-TCC CCG CAC AGC CCG ACT-3′, antisense, 5′-CGC CGC CGC ACG TAG AAG A-3′; EDG-7 receptor (310-bp product), sense, 5′-TCG CGG CAG TGA TCA AAA ACA GA-3′, antisense, 5′-ATG GCC CAG ACA AGC AAA ATG AGC-3′; and β_2-microglobulin (259-bp product), sense, 5′-CCT TGA GGC TAT CCA GCG TA-3′, antisense, 5′-GTT CAC ACG GCA GGC ATA CT-3′. A total of 30 PCR cycles were run at 94°C (denaturation, 1 min), 62°C (annealing, 1 min), and 72°C (extension, 1 min). The generated products were subjected to electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining. The intensity of the bands in PCR gels was quantified by measuring the OD with a OneDscan computer software package (Biometra, Goettingen, Germany). To assure linear cDNA amplification in our experiments, different amplifying cycles (22–36 cycles) were checked. These experiments revealed linear amplification between 24 and 34 cycles. The identity of the PCR products was confirmed by sequencing after cloning using pCRII vectors. Controls run without reverse transcriptase yield no PCR products.

Intracellular Ca^{2+} measurement

Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were measured in DCs loaded with (1-[2-(5-carboxy-oxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2′-amino-5′-methyl-phenoxy)-ethane-*N*,*N*,*N*,*N*′-tetraaceticacid, pentaacetoxymethyl ester) (fura-2-AM) (Calbiochem, La Jolla, CA) using the digital fluorescence microscope unit Attofluor (Zeiss, Oberkochen, Germany) (34).

Actin polymerization

The content of filamentous actin (f-actin) was analyzed by flow cytometry with *N*-(7-nitrobenz-2-oxa-1, 3-diazol-4yl)-phallacidin (BD PharMingen, Heidelberg, Germany) staining according to Dichmann et al. (35).

Migration assay

Experiments were performed in 48-well plates (Nucleopore, Tübingen, Germany). Stimuli were placed into bottom wells, and cells (1×10^5/well) were added to the upper compartment and incubated at 37°C for 90 min in a humidified atmosphere. Upper and lower compartments were separated by a 5-μm pore polycarbonate membrane. The chemotactic index was calculated as the ratio between stimulated cells and cells in the control medium. Each experiment was performed in triplicate (29, 34).

Phagocytosis and endocytosis assays

Immature or mature DCs were washed, resuspended in complete medium, and pulsed with 1 mg/ml Texas red-conjugated BSA or FITC-labeled 2-μm latex beads (Molecular Probes, Eugene, OR). DCs were then incubated at 37°C or 4°C, and at selected time points, uptake was stopped by adding cold PBS containing 2% FCS and 0.01% NaN_3. Cells were then washed four times and analyzed by flow cytometry. Surface binding values obtained by incubating cells at 4°C were subtracted from values measured at 37°C. Results are expressed as Δ mean fluorescence channel numbers.

Cytokine assays

IL-10 was measured in DC supernatants by ELISA using matched pairs of mAbs from BD PharMingen. TNF-α and IL-12 were analyzed using ELISA kits from Amersham Pharmacia Biotech (Piscataway, NJ). Samples were assayed in triplicate for each conditions.

Primary MLR assay

$CD4^+$ T lymphocytes were purified from the heavy density fraction (50–60%) of Percoll gradients by two rounds of immunomagnetic depletion using a mixture of anti-MHC class II, anti-CD8, and anti-CD19 mAb-conjugated beads (Dynal Biotech, Oslo, Norway). Naive T cells were separated by incubation of $CD4^+$ cells with anti-CD45RO mAb followed by a goat anti-mouse Ig coupled to immunomagnetic beads. Purified ($>$95% $CD4^+CD45RA^+$) T cells were then cocultured (5×10^4 cells/well) with allogeneic DCs in 96 flat-bottom well plates in complete medium containing 5% human serum instead of 10% FCS. Cocultures were pulsed at day 5 with 1 μCi/well [^{3}H]thymidine (Amersham, Little Chalfont, U.K.) for $\sim$16 h at 37°C, and then harvested onto fiber-coated 96-well plates (Packard Instruments, Groningen, The Netherlands). Radioactivity was measured in a beta counter (Topcount; Packard Instruments). Results are given as mean cpm $\pm$ SD of triplicate cultures.

Cytokine detection

T cells from the MLR assay (day 6) were expanded by adding 20 U/ml human rIL-2 every 2 days. After $\sim$2 wk, T cells were restimulated with PMA (10^{-7} M; Sigma-Aldrich) plus ionomycin (1 μg/ml; Sigma-Aldrich) in RPMI 1640 containing 5% human serum and incubated in the presence of Golgi-Stop (BD PharMingen). After a 6-h stimulation, cells were fixed, permeabilized, stained with FITC-conjugated mouse anti-IFN-γ and PE-conjugated rat anti-IL-4 (BD PharMingen), and finally analyzed with a FACScan (BD Biosciences, Mountain View, CA). In control samples, staining was performed using isotype-matched control Ig (29).

Statistical analysis

Unless otherwise stated, data are expressed as the mean $\pm$ SEM. ANOVA was used to compare experimental groups to control values. When the global test of differences was significant at the 5% level, pairwise tests of differences between groups were applied (Tukey's multiple comparison test).

Results

Human DCs express the mRNA for EDG-2, EDG-4, and EDG-7 receptor subtypes

Expression of mRNA for the different LPA receptor subtypes was analyzed in immature and mature DC by RT-PCR. Fig. 1 shows that immature and LPS-matured DCs expressed similar amounts of the mRNA for EDG-2, -4, and -7 receptor subtypes. No products were obtained after omitting reverse transcription in the reaction (data not shown). Moreover, incubation of DCs up to 48 h with

LPA did not alter the expression of EDG-2, -4, and -7 (data not shown).

LPA induces Ca^{2+} mobilization, actin polymerization, and chemotaxis in immature DCs

Stimulation of immature DC with LPA induced a rapid and dose-dependent $[Ca^{2+}]_i$ increase with maximal and half-maximal responses at 10^{-5} M and 10^{-7} M LPA concentrations (Fig. 2*A*). To investigate the involvement of $G_{i/o}$ proteins in this response, DCs were incubated with 4 μM PTX for 2 h. As shown in Fig. 2*B*, PTX pretreatment strongly inhibited LPA-induced $[Ca^{2+}]_i$ increase in immature DCs. In addition, preincubation of DCs with EGTA (4 mM) did not influence the LPA-initiated Ca^{2+} rise, implicating that Ca^{2+} rise was due to mobilization of Ca^{2+} from the intracellular stores (data not shown). Strikingly, LPA did not elicit $[Ca^{2+}]_i$ transients in mature DCs (Fig. 2*C*). To check the responsiveness of LPS-differentiated DCs, experiments with 10^{-7} M MIP-3β were performed. Controls showed a ratio of 0.94 $\pm$ 0.05, while MIP-3β-stimulated cells displayed a ratio of 1.20 $\pm$ 0.02 ($p < 0.01$).

Actin reorganization is a prerequisite for migration of leukocytes. The effect of LPA on the actin network of immature and mature DCs was analyzed by flow cytometry. A dose-dependent effect of LPA on actin polymerization is shown in Fig. 3. LPA caused in immature DCs a rapid polymerization of the actin molecules, with a 100% increase in the f-actin content within 25 s after stimulation. Maximal and half-maximal effects were observed at 10^{-6} and 10^{-8} M doses, respectively. In contrast, no actin reorganization was seen in mature DCs upon stimulation with LPA. Pretreatment of immature DCs with PTX inhibited the LPA-induced actin response (data not shown). Lack of response of mature DCs to LPA was not due to refractoriness of the cells, because MIP-3β was still able to induce actin polymerization in these cells (3.05 $\pm$ 0.26).

We then analyzed the influence of LPA on DC chemotaxis. LPA dose-dependently chemoattracted immature DCs; accordingly with the fact that LPA did not induce actin polymerization in mature DC, this stimulus did not induce any migration in mature DCs (Fig. 4*A*). C5a (10^{-8} M) and MIP-3β (100 ng/ml) were used as positive controls for immature and mature DCs, respectively. The chemotactic index for C5a in immature DCs was ~2.13 $\pm$ 0.17, and the index for MIP-3β in LPS-differentiated cells was ~2.05 $\pm$ 0.11. Moreover, the chemotactic activity of LPA in immature DCs did not depend on the supplement of the media during the cell culture, since DCs supplemented with 1% human serum or plasma showed similar chemotactic index as DCs supplemented with 10% FCS (data not shown). In addition, down-regulation of the chemotactic activity during maturation was not linked to LPS[8] since comparable effects were observed in TNF-α- and CD40 ligand-differentiated DCs. However, preincubation of immature DCs with PTX inhibited the chemotactic effect of LPA (Fig. 4*B*).

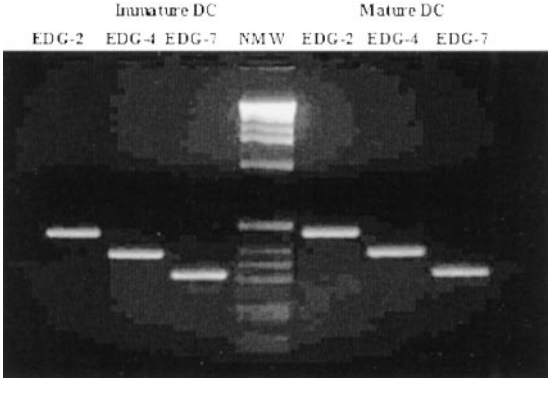

FIGURE 1. Immature and mature DCs express the mRNA for EDG-2, EDG-4, and EDG-7 receptors. RT-PCR analysis was performed on mRNA isolated from purified monocyte-derived DCs either immature or treated for 48 h with LPS. Experiments were repeated four times with identical results.

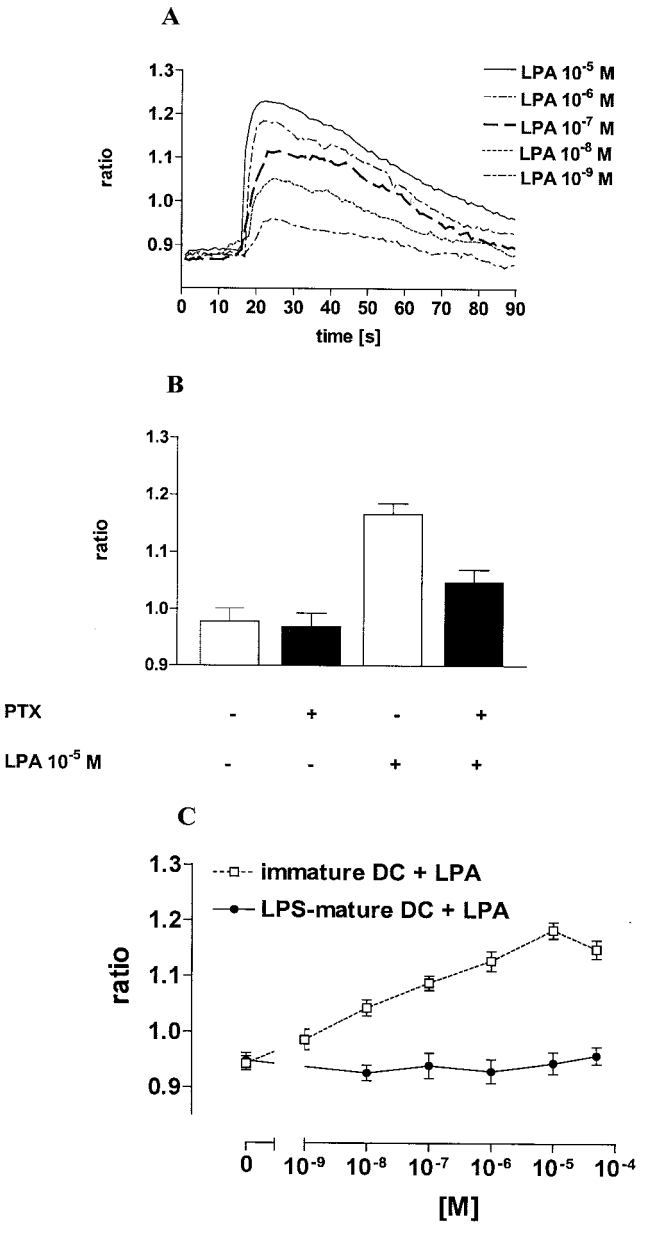

FIGURE 2. LPA triggers Ca^{2+} transients in immature but not mature DCs via a PTX-sensitive mechanism. *A*, Immature DCs were labeled with fura 2 and stimulated with the indicated concentrations of LPA ($n = 5$). *B*, Immature DCs were incubated with or without 10 μg/ml PTX for 2 h, and then labeled with fura 2 and stimulated with 10^{-5} M LPA. Data are means $\pm$ SEM ($n = 3$). *C*, DC were incubated for 48 h with and without LPS. Thereafter, DC were washed, labeled with fura 2, and stimulated with indicated concentrations of LPA. Global differences between groups: $p < 0.001$, immature vs mature DCs.

LPA reduces IL-12 and TNF-α production and increases IL-10 release from maturing DCs

In the following experiments, we studied whether LPA could affect cytokine secretion and Ag-presenting capacity of human DCs.

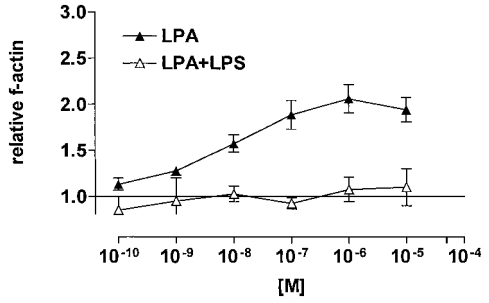

FIGURE 3. LPA induces actin polymerization in immature, but not mature DCs. DCs were incubated with and without LPS for 48 h. Thereafter, DCs were washed and stimulated with the indicated LPA concentrations. The relative f-actin content was analyzed after 25 s. Data are means ± SEM ($n = 5$). Global differences between groups: $p < 0.001$, immature vs mature DCs.

LPA did not induce changes in the expression of CD54, CD80, CD86, CD83, and MHC class I and II molecules in immature or LPS-differentiated DCs (data not shown). Moreover, LPA did not alter the capacity of DCs to take up albumin (Fig. 5) or 2-μm latex beads (data not shown). However, LPA added together with LPS dose-dependently inhibited the production of IL-12 (Fig. 6*A*) and TNF-α (Fig. 6*B*), while it increased the release of IL-10 in maturating DCs (Fig. 6*C*). Basal cytokine production from immature DCs was not affected by LPA (Fig. 6, legend). PTX did not abrogate the effect of LPA on cytokine production in LPS-differentiated DCs (Table I).

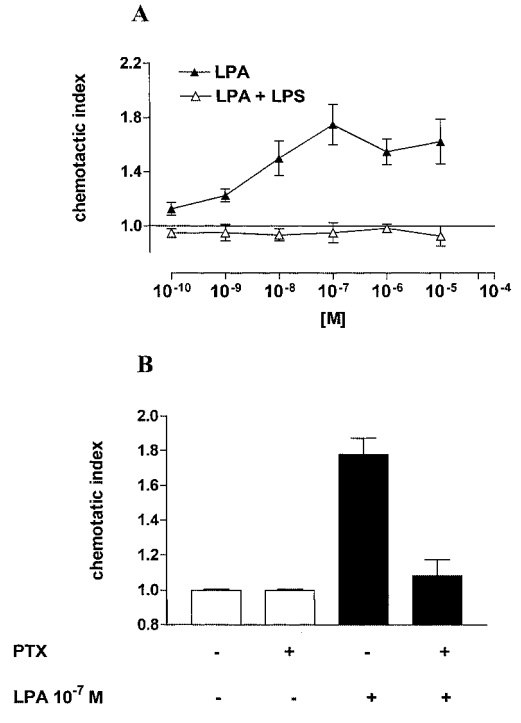

FIGURE 4. LPA elicits chemotaxis of immature, but not mature DCs. DCs were incubated with and without LPS for 48 h. Thereafter, DCs were washed and exposed to the indicated concentrations of LPA for 90 min at 37°C in a Boyden chamber. Data are expressed as mean ± SEM ($n = 5$). *B*, After pretreatment with and without 10 μg/ml PTX for 2 h, immature DCs were exposed to 10^{-7} M LPA for 90 min at 37°C in a Boyden chamber. Data are means ± SEM ($n = 4$).

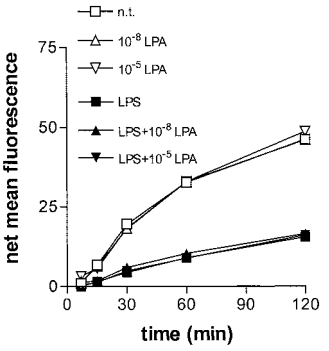

FIGURE 5. LPA does not affect the endocytic capacity of DCs. DCs were treated (filled symbols) or not (open symbols) with LPS in the presence or not of two different concentrations of LPA for 18 h at 37°C. Thereafter, DCs were washed and incubated with 1 mg/ml Texas red BSA at 37°C. At the indicated time points, cells were stopped with cold PBS and the BSA accumulation was measured by flow cytometry. Fluorescence of cells incubated at 4°C was subtracted from the fluorescence of cells incubated at 37°C. The results are representative of three different experiments.

DCs matured in the presence of LPA display enhanced T cell allostimulatory capacity but reduced Th1-inducing activity

The Ag-presenting functions of DCs were investigated in the primary allogeneic MLR assay using purified naive $CD4^+CD45RA^+$ T cells as responders. DCs that were induced to mature in the presence of LPA exhibited a higher T cell-activating capacity in the allogeneic MLR assay (Fig. 7*A*). In contrast, no significant effects were observed in immature DCs treated with LPA (Fig. 7, *A* and *B*). On a per cell basis, LPA-treated mature DCs induced a 2-fold increase in the T cell response (Fig. 7*C*). Next, we studied the ability of DCs maturated in the presence of LPA to direct the differentiation of naive T cells. To this end, T cells from the MLR assay were expanded for 2 wk with IL-2, and then analyzed for intracellular cytokine expression. T cells stimulated with mature DCs were predominantly of the Th1 type (Fig. 8*A*). In contrast, DCs maturated in the presence of LPA induced the differentiation of a higher percentage of Th0 and Th2 cells, and in parallel, of a lower percentage of IFN-γ single positive cells (Fig. 8, *B* and *C*).

Discussion

Secretory type II PLA_2 is thought to be involved in the pathogenesis of inflammatory reactions via generation of LPA (3, 4). This simple phospholipid binds to at least three different G protein-coupled receptors and has biological activities toward T cells, B cells, monocytes, and neutrophils (16–21). Recently, involvement of inflammation-associated secretory type II PLA_2 and LPA in acute lung injury and pathogenesis of asthma as well as rhinitis allergica has been suggested (30–33). Under pathological conditions, LPA can be generated through cleavage of phosphatidic acid, which is produced either through sphinomyelinase conditioning of plasma membrane vesicles and phospholipase C, and by phospholipase D or diacylglycerol kinase activation (3–6). Independently of the synthesis pathway, up to 30–60 μM LPA can be stored within the cells. In extracellular fluids including serum and in the inflammatory exudate, accumulation of LPA at concentrations as high as 10 μM can be expected (8); therefore, the effects reported in the present study are likely to be of physiological relevance.

We analyzed the biological activity of LPA on DCs, which are the major immunological cell residing in the epidermis, nasal epithel, and lung (25). In immature DCs, LPA triggered intracellular Ca^{2+} transients, actin reorganization, and chemotactic migration, but it does not affect the capacity of DCs to internalize proteins or

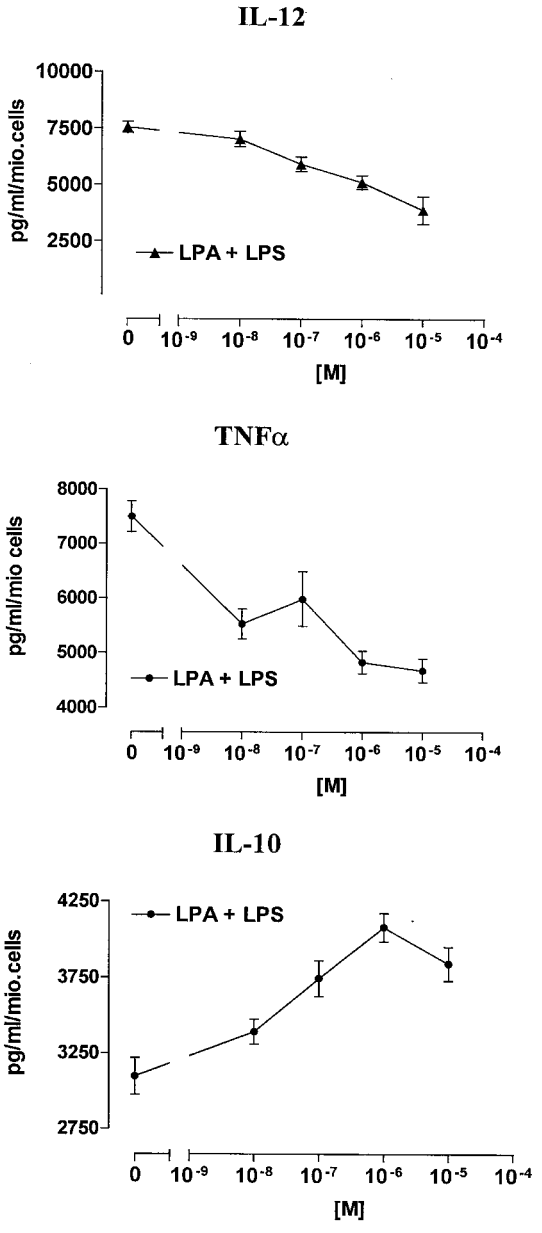

FIGURE 6. LPA inhibits IL-12 and TNF-α release, whereas LPA increases IL-10 production in maturing DCs. DCs were treated with 3 μg/ml LPS in the presence or the absence of the indicated concentrations of LPA. Supernatants were harvested 24 h after stimulation with LPS along with and without LPA, and the different cytokines were evaluated by ELISA. The results are expressed as means ± SEM ($n = 4$). Global differences between groups: $p < 0.001$, immature vs mature DCs. Baseline cytokine production in immature DCs in the absence or presence of 10^{-5} M LPA was the following: 245 (± 47) and 331 (± 65) pg IL-12/ml/10^6 cells, 590 (± 53) and 475 (± 125) pg TNF-α/ml/10^6 cells, and 454 (± 118) and 560 (± 160) pg IL-10/ml/10^6 cells. Data are means ± SEM ($n = 4$).

particles. Half-maximal and maximal chemotaxis responses are observed upon stimulation with 10^{-8} and 10^{-6} M LPA, respectively. The increase in the $[Ca^{2+}]_i$ in response to LPA is due to mobilization of stored Ca^{2+} via activation of $G_{i/o}$ proteins. The mechanism underlying actin reorganization is presumably regulated by interaction of phosphoinositides with the actin-binding proteins and requires activation of G_i proteins, and presumably of small GTP-binding proteins of the rho family (21, 22). In LPS-differentiated DC, LPA-induced $G_{i/o}$ protein-dependent responses and signaling events are no longer present, although mature DCs

Table I. *PTX does not affect LPA-induced cytokine modulation*[a]

	IL-12	IL-10	TNF-α
Control	7.42 ± 0.25	2.98 ± 0.18	7.86 ± 0.34
LPA	4.76 ± 0.33	4.01 ± 0.21	4.98 ± 0.28
PTX	7.45 ± 0.29	2.78 ± 0.23	7.65 ± 0.43
LPA + PTX	4.37 ± 0.34	4.16 ± 0.27	5.03 ± 0.24

[a] DCs were treated with 3 μg/ml LPS in the presence and the absence of 10 μg/ml PTX and along with and without 10^{-5} M LPA for 24 h. Supernatants were harvested and the cytokines were evaluated by ELISA. Data are expressed as mean (± SEM) nanograms per milliliter per 10^6 cells ($n = 3$).

are still responsive to other stimuli. The trafficking of DCs, which originate from hemopoietic stem cells in the bone marrow and migrate to peripheral target sites to take up Ags, is thought to be controlled by chemokines and the sequential expression of their receptors during the different maturation stages (28). Similar to platelet-activating factor, monocyte chemotactic proteins 1–4, sphingosine-1-phosphate, and adenosine, LPA could be involved in the accumulation of immature DCs at peripheral target sites,

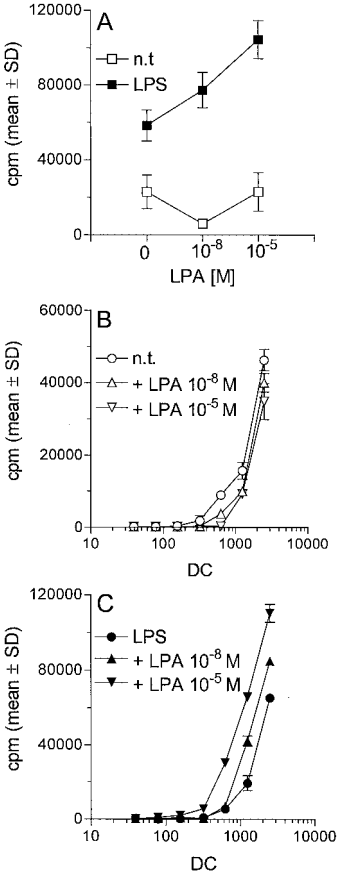

FIGURE 7. Treatment with LPA enhances the capacity of mature DCs to activate T cells. *A*, DCs were treated (■) or not (□) with LPS in the presence or not of two different concentrations of LPA for 18 h at 37°C. Thereafter, DCs were washed and cocultured (2.5×10^3 DC/well) with purified allogeneic $CD4^+CD45RA^+$ T cells (5×10^4 cells/well) in 96-well plates. Grading numbers of immature (*B*) or mature (*C*) DCs, treated or not with LPA, were cocultured with purified allogeneic $CD4^+CD45RA^+$ T cells (5×10^4 cells/well). [^{3}H]thymidine incorporation was measured after 5 days. Background T cell proliferation was <1000 cpm. In *A*, differences in T cell proliferation were significant ($p < 0.02$) at both LPA doses. In *C*, differences in T cell proliferation were significant ($p < 0.01$) at DC number >620/well (LPA 10^{-5} M) and 1250/well (LPA 10^{-5} M). Results are representative of four different experiments.

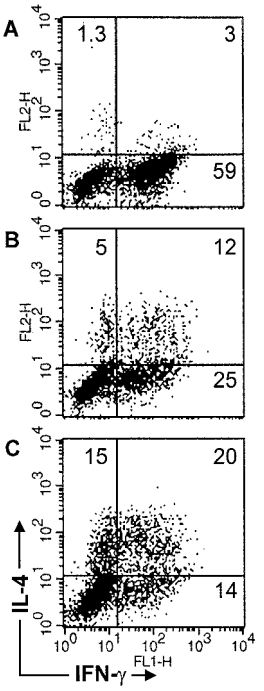

FIGURE 8. LPA decreases the capacity of DCs to initiate Th1 responses. DCs were treated with LPS alone (*A*) or in the presence of 10^{-8} M (*B*) or 10^{-5} M (*C*) LPA for 18 h. Then, DCs were washed and used to activate purified naive (>95% $CD45RA^+$) allogeneic T cells. After expansion with IL-2 for 14 days, T cells were restimulated with PMA plus ionomycin for 6 h, and finally examined for intracellular IFN-γ and IL-4 by double-color flow cytometry. The numbers indicate the percentage of positive cells in each quadrant. Three experiments gave comparable results.

then loss of $G_{i/o}$ protein-dependent sensitivity to LPA during DC maturation could clear the way for MIP-3β-driven migration of DCs to secondary lymphoid organs (28).

Monocyte chemotactic proteins 1–4, platelet-activating factor, and adenosine lose their chemotactic activity during maturation due to transcriptionally regulated down-regulation of their receptors (28, 34). In contrast, we showed that the mRNA levels of EDG-2, -4, and -7, the known LPA receptors, are comparable in immature and LPS-differentiated DCs. However, in this context, it is worthy to notice that this is true also for the sphingosine receptors EDG-1, -3, -5, and -6 (29). Due to the lack of selective agonists, antagonists, or Abs to stimulate or block specific EDG receptor types, at present we cannot attribute different responses to activation of single receptors. To explain the lack of $G_{i/o}$ protein-dependent sensitivity to LPA in LPS-differentiated DCs, one can speculate that LPA receptors could be differentially coupled to intracellular signaling pathways due to changes in the expression pattern of different G protein subunits in immature and mature DCs. This seems to be the case according to the findings that EDG receptor-dependent responses (e.g., inhibition of IL-12 and TNF-α, and stimulation of IL-10 secretion) are PTX-insensitive in mature DCs. In contrast, posttranslational modifications of different G protein subunits as a consequence, for example, of palmitoylation, cannot be excluded (36–38). These findings suggest that modulation of cytokine production in maturing DCs is regulated through PTX-insensitive G proteins such as $G_{q/11/12}$. Moreover, we found that LPA enhanced the Ag-presenting capacities of mature DCs toward allogeneic naive T cells, an effect that was not observed in immature DCs. The increased T cell stimulatory functions of DCs matured in the presence of LPA cannot be attributed to a higher expression of membrane-presenting and costimulatory molecules. Consistent with the capacity of LPA to inhibit IL-12 release, DCs maturated in the presence of LPA induce a higher percentage of Th0/Th2 cells compared with the predominant Th1 differentiation promoted by mature DCs. This later finding is interesting since involvement of inflammation-associated secretory type II PLA_2 and LPA in acute lung injury and pathogenesis of asthma as well as rhinitis allergica has been implicated (30–33). Based on these reports and our data, it is certainly allowed to speculate that LPA might be involved in induction and/or sustaining of Th2-dominated immunity in patients with asthma and rhinitis allergica.

In summary, we showed that LPA at concentrations similar to those present in the tissues during inflammation can exert multiple effects on immature and mature DCs and modulate their biological functions.

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