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Costimulation of Dectin-1 and DC-SIGN Triggers the Arachidonic Acid Cascade in Human Monocyte-Derived Dendritic Cells

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Inflammatory mediators derived from arachidonic acid (AA) alter the function of dendritic cells (DC), but data regarding their biosynthesis resulting from stimulation of opsonic and nonopsonic receptors are scarce. To address this issue, the production of eicosanoids by human monocyte-derived DC stimulated via receptors involved in Ag recognition was assessed. Activation of FcγR induced AA release, short-term, low-grade PG biosynthesis, and IL-10 production, whereas zymosan, which contains ligands of both the mannose receptor and the human β-glucan receptor dectin-1, induced a wider set of responses including cyclooxygenase 2 induction and biosynthesis of leukotriene C4 and IL-12p70. The cytosolic phospholipase A2 inhibitor pyrrolidine 1 completely inhibited AA release stimulated via all receptors, whereas the spleen tyrosine kinase (Syk) inhibitors piceatannol and R406 fully blocked AA release in response to immune complexes, but only partially blocked the effect of zymosan. Furthermore, anti-dectin-1 mAb partially inhibited the response to zymosan, and this inhibition was enhanced by mAb against DC-specific ICAM-3-grabbing nonintegrin (SIGN). Immunoprecipitation of DC lysates showed coimmunoprecipitation of DC-SIGN and dectin-1, which was blocked AA release in response to immune complexes, but only partially blocked the effect of zymosan. Furthermore, anti-dectin-1 mAb partially inhibited the response to zymosan, and this inhibition was enhanced by mAb against DC-specific ICAM-3-grabbing nonintegrin (SIGN). Immunoprecipitation of DC lysates showed coimmunoprecipitation of DC-SIGN and dectin-1, which was confirmed using Myc-dectin-1 and DC-SIGN constructs in HEK293 cells. These data reveal a robust metabolism of AA in human DC stimulated through both opsonic and nonopsonic receptors. The FcγR route depends on the ITAM/Syk/cytosolic phospholipase A2 axis, whereas the response to zymosan involves the interaction with the C-type lectin receptors dectin-1 and DC-SIGN. These findings help explain the distinct functional properties of DC matured by immune complexes vs those matured by β-glucans.


Dendritic cells (DC) detect the presence of pathogens and can be in two functional states defined by their ability to uptake Ags and by phenotypical markers linked to cell surface receptor expression. Whereas detailed attention has been paid to the cytokines derived from DC, there are few data regarding their production of arachidonic acid (AA) metabolites, despite the evidence that eicosanoids play a relevant role in DC function and the finding that there are prominent changes in the profile of lipid metabolism along the process of monocyte differentiation.

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§Abbreviations used in this paper: DC, dendritic cell; AA, arachidonic acid; ALLN, N-acetyl-leucyl-leucyl-norleucinal; COX, cyclooxygenase; cPLA2, cytosolic phospholipase A2; DC-SIGN, DC-specific ICAM-3-grabbing nonintegrin; IC, immune complex; LT, leukotriene; MR, mannose receptor; PGN, peptidoglycan; PMN, polymorphonuclear leukocytes; HA, hemagglutinin.

Copyright © 2008 by The American Association of Immunologists, Inc. 0022-1767/08/$2.00 received by M-CSF and IL-4 (1). PGE2 is required for human DC migration in response to chemokines (2, 3) and, consistent with this pivotal function, failure of DC to produce PGE2 has been considered a major obstacle for the successful application of DC in therapy (4, 5).

PG biosynthesis involves several steps catalyzed by different enzymes, but the limiting step for the biosynthesis of eicosanoids is hydrolysis of AA from phospholipids by cytosolic phospholipase A2 (cPLA2). Cyclooxygenases (COX) 1 and 2 (COX-1 and -2) convert the AA released by cPLA2 to PG endoperoxide H2, which is the precursor of series 2 prostanoids such as PGD2 and PGE2. Unlike COX-1, COX-2 is an inducible enzyme involved in the sustained production of prostanoids by many cell types. Notably, COX-2 activity is necessary for strong Ab response following vaccination, especially when vaccines are poorly immunogenic or the target population is poorly responsive to immunization (6). In addition to the COX-2 route for AA metabolism, there are pathways dependent on constitutively expressed 5-lipoxygenase and COX-1, which are triggered shortly after cell activation. With regard to 5-lipoxygenase products, deficient extracellular export of leukotriene (LT) C4 is associated to a decreased migratory response of DC (7) and cysteinyl-LT increase IL-10 production by myeloid DC (8). Recent studies have disclosed lipoxins as a unique class of lipoxygenase interaction metabolites with a strong ability to suppress the production of IL-12 and the function of DC, a phenomenon termed DC paralysis (9).

In previous studies, we have observed a robust release of AA in human monocytes (10) and polymorphonuclear leukocytes (PMN) (11) by two pathogen-associated molecular signatures, namely, peptidoglycan (PGN) and mannose-based polymers, thus defining pathogen-associated molecular patterns as chief stimuli for AA.

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metabolism. In the present study, we addressed the effect of a set of stimuli acting through receptors involved in recognition of microbial components by DC. We found that ligands of opsonic and nonopsonic receptors such as the human β-glucan receptor dectin-1 (12), the mannose receptor (MR), and FcγRs elicited a robust release of AA. Binding of zymosan and mannose-based stimuli (13, 14) was followed by the induction of COX-2 expression and the production of IL-12p70, whereas FcγR cross-linking was associated with IL-10 generation. The Syk kinase inhibitors piceatannol and R406 completely inhibited AA release in response to FcγR cross-linking, but they only partially blocked the response to zymosan. Anti-dectin-1 mAb inhibited the effect of zymosan and this inhibition was further enhanced by mAb against DC-specific ICAM-3-grabbing nonintegrin (SIGN) (15). Immunoprecipitation of DC lysates with anti-DC-SIGN mAb showed coprecipitation of dectin-1. These data show for the first time the occurrence of a robust metabolism of AA in human DC in response to immune complexes (IC), β-glucan particles, and mannose-based molecular patterns.

Materials and Methods

Reagents

Zymosan, the soluble β-glucan laminarin, mannarin from Saccharomyces cerevisiae, OptiPrep, porcin mucin-3, and piceatannol were purchased from Sigma-Aldrich. Anti-human CD206/MSR, anti-human CD209/DC-SIGN, and anti-CD45 mAb were obtained from BD Pharmingen. Anti-human dectin-1 (GE2 mAb) was generated as previously described (16). Goat anti-dectin-1 Ab was purchased from R&D Systems. The cPLA2 inhibitor pyrrolidine 1 was purchased from Calbiochem. The Syk kinase inhibitor R406 (17) was a generous gift from Dr. E. Masuda (RIGEL Inc., South San Francisco, CA). Preparation of IgG/OVA equivalence IC and opsonization inhibitor R406 (17) was a generous gift from Dr. E. Masuda (RIGEL Inc., South San Francisco, CA). Preparation of IgG/OVA equivalence IC and opsonization

Immunoblots and immunoprecipitations

Proteins were separated by electrophoresis in SDS-PAGE and transferred to nitrocellulose membranes. The membranes were used for immunodetection of COX-2 with a goat Ab (SC-1745) from Santa Cruz Biotechnology. The phosphorylation of cPLA2 was assayed using anti-cPLA2 phospho-specific Ab (Ser195), catalog no. 2851 from Cell Signaling Technology. For the assay of phosphotyrosine, phosphotyrosine mAb 4G10 from Upstate Biotechnology was used. Syk phosphorylation was addressed using phospho-specific anti-human Syk Ab reactive to the activation loop site (Ser505; catalog no. 2711; Cell Signaling Technology). Quantitation of the blots was conducted using Bio-Rad Quantity One gel imaging software. Immunoprecipitations were conducted as previously described (20). Briefly, cells were lysed in a medium containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, 1% Nonidet P-40, 1 mM Na3VO4, 10 μg/ml aprotinin and leupeptin, 100 μg/ml soybean trypsin inhibitor, and 1 mM PMSF and clarified by centrifugation at 15,000 rpm for 20 min. The clarified lysates were preabsorbed on protein G-Sepharose and then incubated with precipitating mAb for 4 h, followed by overnight incubation with protein G-Sepharose beads. IC were extensively washed, suspended in Laemmli sample buffer, and subjected to SDS-PAGE. Blots were stained to assess the input protein and the coimmunoprecipitation of C-type lectins. Experiments to address the effect of the ectopic expression of the C-type lectin receptors and the potential interactions with Syk were conducted in HEK293 cells. Transfections were conducted by the calcium phosphate method with 5 μg of plasmid DNA completed up to 15 μg with pE4F empty vector in plates containing 3 × 106 cells.

Real-time RT-PCR of COX-2

Purified RNA was depleted of genomic DNA by treatment with DNase (Turbo-DNA free; Ambion) and used for reverse transcriptase reactions. The resulting cDNA was amplified in a PTC-200 apparatus equipped with a Chromo4 detector (Bio-Rad) using SYBR Green I mix containing Hot-Star polymerase (ABgene). The sets of primers for PCR were selected in exons 1 and 2 and were: forward 5′-CAATTTGTCATACGCTTGC-3′ and reverse 5′-GTGGAAACGAAAGGTGTG-3′. Cycling conditions were as follows: 94°C for 5 min, followed by 40 cycles of 94°C for 30 s, 60°C for 30 s, and extension at 72°C for 30 s. GAPDH was used as a housekeeping gene to assess the relative abundance of the different mRNA, using the comparative cycle threshold method.

Confocal microscopy

DC were seeded in glass coverslips for 1 h and then stimulated with Alexa Fluor 488-labeled zymosan particles at the concentration of five particles per cell. Cells were fixed with 10% formaldehyde in PBS and stained with goat anti-mouse IgG Ab labeled with Alexa Fluor 594. The coverslips were observed by confocal microscopy using a Bio-Rad Laser Scanning System Radiance 2100 with LaserSharp2000 software coupled to a Nikon inverted microscope, with a ×60 oil immersion objective. Green fluorescence (zymosan) was monitored at 488 nm of argon excitation using HQ500LP and HQ540SP blocking filters (Chroma Technology). Red fluorescence was monitored at 590 nm using a HQ570LP blocking filter.

Statistics

For statistical analysis of data, paired and unpaired Student’s t tests were performed (PRISM version 4.0; GraphPad) as appropriate. Values of p < 0.05 were considered significant.

Results

Monocyte-derived DC show a robust metabolism of AA via the cPLA2 route

Since the functional properties of DC change along the process of maturation, experiments were conducted in both immature and mature DC. TNF-α-induced maturation of DC was characterized by an increased expression of CD83 and a parallel decrease of the surface display of CD14. The expression of DC-SIGN, MR, and dectin-1 increased above the levels detected in monocytes upon the addition of GM-CSF and IL-4, but decreased after treatment with

5728 AA METABOLISM IN DC
TNF-α. Of the different types of FcγR, FcγRII showed the highest level of expression, whereas the expression of FcγRI and FcγRIII was low (Fig. 1A).

Preliminary experiments using zymosan and IC as stimuli showed the release of similar amounts of [3H]AA from both 5-day immature and 7-day TNF-α mature DC. On this basis and unless otherwise indicated, experiments were conducted with 5-day immature DC. [3H]AA release induced by zymosan and IC steadily increased up to ~90 min (Fig. 1B) and was observed with concentrations as low as 0.1 mg/ml zymosan and 10 μg/ml IC (Fig. 1C). Mucin-3, a glycoprotein from the gastrointestinal tract, which is an endogenous ligand of the MR, also showed a robust [3H]AA-

**FIGURE 1.** Expression of cell surface markers and [3H]AA release. A, DC were cultured for 5 days (5-d DC) in the presence of GM-CSF and IL-4, and after this time the culture medium was supplemented with 100 U/ml TNF-α for 2 days (7-d DC) and 5 days more (10-d DC). At the days indicated, cells were collected and used for the detection of a set of surface markers by flow cytometry. The dotted line marked IAb on the dectin-1 panel shows the binding of an isotype control Ab. B, DC labeled with [3H]AA were stimulated with zymosan and IC, and the supernatants were assayed for the release of [3H]AA. Results are expressed as percentage of total [3H]AA incorporated into cell phospholipids. C and D, Fixed-time experiments with different concentrations of stimuli were conducted at 60 min. Data represent mean ± SEM of three time-course and six fixed-time experiments in duplicate. Zymosan-C3bi indicates zymosan particles treated with serum to allow complement opsonization. IC-C3bi indicates IC opsonized with serum. Zymosan-IgG indicates zymosan opsonized with purified rabbit IgG.

**FIGURE 2.** Effect of different inhibitors on [3H]AA release. A–C, DC cells were preincubated at 37°C with the indicated inhibitors for 30 min before the addition of the stimuli. All of the Ab used in the experiments were used at the concentration of 10 μg/ml. After 1 h, the supernatants were collected and used for the assay of [3H]AA release. D, Binding and phagocytosis of Alexa Fluor zymosan by DC. DC were preincubated with the indicated additions for 30 min at 4°C as described in Materials and Methods and then five particles of Alexa Fluor zymosan per cell were added. DC were either maintained at 4°C or transferred into a cell incubator at 37°C for 60 min. At the end of this period, cells were collected for the detection of bound and phagocytosed particles by flow cytometry. Mean fluorescence intensity was 325 U for cells maintained at 37°C and 21 U for cells kept at 4°C. Data represent mean ± SEM of six independent experiments of [3H]AA release and four experiments of binding. *p < 0.05 as compared with DC incubated with medium or irrelevant Ab.
releasing activity (Fig. 1C), whereas mannan induced \(^{3}\text{H}\)AA release to a lower extent (Fig. 1D). Given that complement coating of both zymosan and IC modifies the ability of these stimuli to release AA in human monocytes (18, 20), zymosan and IC were treated with normal human serum to allow the covalent coupling of C3bi moieties. Unlike the results observed in monocytes, neither C3bi coating nor opsonization with rabbit IgG modified the ability of these stimuli to release \(^{3}\text{H}\)AA (Fig. 1D). These data indicate that the ability of zymosan to induce AA release in DC is independent of its ability to interact with complement factors and IgG.

Initial attempts to delineate the pathway involved in AA release were conducted with the cPLA2 inhibitor pyrrolidine 1. As shown in Fig. 2A, pyrrolidine 1 fully inhibited \(^{3}\text{H}\)AA release, which

![FIGURE 3.](image)

**FIGURE 3.** DC were incubated for 24 h with several stimuli in the presence and absence of different inhibitors and the production of IL-12p70 (A) and IL-10 (B) was determined. Data represent mean ± SEM of four independent experiments for IL-12p70 and six experiments for IL-10. *, p < 0.05 as compared with cells processed in the absence of drugs.

![FIGURE 4.](image)

**FIGURE 4.** Induction of COX-2 protein and mRNA expression. A, Expression of COX-2 in lysates from immature (iDC) and mature DC (mDC) incubated with the various stimuli for 8 h. B and C, COX-2 protein expression in DC stimulated for 8 h with different concentrations of stimuli. D, Time course of COX-2 mRNA expression in DC stimulated with zymosan. The amount of protein loaded in the different lanes was assessed by using anti-\(\beta\)-actin mAb. Blots are representative of at least three independent experiments.

![FIGURE 5.](image)

**FIGURE 5.** PGE\(_2\) production by DC. A and B, DC were stimulated for the times indicated and the production of PGE\(_2\) was assayed in the supernatants. Data represent mean ± SEM of three independent experiments (A) or a representative experiment of three with identical trend (B). C, The effect of a preincubation with 1 \(\mu\)M ionomycin for 30 min before the addition of zymosan and IC on the production of PGE\(_2\) is shown in the left columns. The effect of a preincubation with the stimuli before ionomycin is shown in the right columns. PGE\(_2\) was assayed 24 h after the addition of the stimuli. D, Inhibition of PGE\(_2\) production by pyrrolidine 1. DC were treated for 30 min with pyrrolidine 1 or vehicle and then incubated in the presence of zymosan or LPS. PGE\(_2\) was assayed in supernatants 48 h after addition of stimuli. Data represent mean ± SEM of three independent experiments. E, Production of LTC\(_4\) and PGD\(_2\) in response to zymosan and IC. LTC\(_4\) was assayed 1 h after addition of the stimuli. PGD\(_2\) was assayed at 24 h in medium containing ITS liquid medium supplement (insulin, transferring, sodium selenite) to avoid the immunoreactivity of FCS in the assay. Data represent mean ± SEM of three independent experiments.
suggests a complete dependence of $[^{3}H]AA$ release from cPLA$_2$-catalyzed reactions (Fig. 2A). Zymosan-induced $[^{3}H]AA$ release was inhibited by laminarin, mannan, and anti-dectin-1 and anti-DC-SIGN mAb, which was most evident when the inhibitors were used in combination (Fig. 2, B and C). However, when zymosan was used at nonsaturating levels, inhibition with laminarin was almost complete (Fig. 2B). These data indicate receptor cooperation in zymosan-induced AA release. To obtain further insight into the type of receptors involved in the recognition of zymosan by DC, the binding of Alexa Fluor 488 zymosan was studied in the presence of different inhibitors. These experiments were conducted at both 4 and 37°C to differentiate binding from the combination of agonist:antagonist ratios were used and the experiments were performed under identical conditions to those used for AA release. As shown in Fig. 2D, laminarin, mannan, anti-DC-SIGN, and anti-dectin-1 mAb could block zymosan binding. Combination of these inhibitors enhanced the blockade of zymosan binding at both 4 and 37°C, although the amount of particles associated with the cells was higher at 37°C than at 4°C (325 vs 21 mean fluorescence intensity units, respectively), consistent with enhanced uptake at 37°C. Taken together, this data show the existence of a cPLA$_2$-dependent route for AA release in DC, which can be triggered by engaging FcγR and by binding to dectin-1 and DC-SIGN in the case of zymosan particles.

The production of IL-12p70 and IL-10 was assayed to correlate with the presence of different inhibitors. These experiments were conducted with HEK293 cells transfected with pEF-HA-Syk and pEF4-Myc-dectin-1 vectors and then stimulated with 1 mg/ml zymosan for 10 min. At the end of this period, cell lysates were immunoprecipitated with 2.5 μg of anti-HA mAb and immunoblotted with both anti-HA and anti-Myc mAb. P-Y, Phosphotyrosine; P-Syk, phospho-Syk; P-cPLA$_2$, phosphorylated cPLA$_2$; IP, immunoprecipitation.

AA release can be coupled to COX-2 induction and eicosanoid production

To delineate the routes of AA metabolism downstream of cPLA$_2$ that can be operative in DC, the induction of COX-2 and the production of PGE$_2$, PGD$_2$, and LTC$_4$ were addressed. Zymosan and mucin-3 induced COX-2 protein expression in immature DC (Fig. 3A), whereas only SC-236 partially inhibited the effect of LPS. Pyrrolidine 1 also inhibited the production of IL-10 elicited by zymosan, but was ineffective in the case of IC (Fig. 3B). These data would suggest a partial dependence of cytokine production on the biosynthesis of eicosanoids by DC.

To assess whether AA release was coupled to eicosanoid production, we next assayed the production of PGE$_2$, PGD$_2$, and LTC$_4$. As shown in Fig. 5A, PGE$_2$ mirrored the time course of AA release induced by both IC and zymosan, whereas LPS did not
elicit a release of AA at early time points (data not shown). When PGE$_2$ was assayed after longer periods of stimulation, the results were consistent with those observed for COX-2 protein induction, because zymosan and mannan stimulated PGE$_2$ production at an extent similar to that observed for LPS, whereas IC and laminarin lacked any significant effect (Fig. 5B).

The effect of zymosan and IC was compared with that produced by ionomycin, a nonspecific activator which mobilizes calcium ions and can induce AA release. As shown in Fig. 5C, ionomycin induced low levels of PGE$_2$ and enhanced the production elicited by IC and zymosan. Because LPS was a potent inducer of PGE$_2$ production, but it did not produce an acute release of AA, the possible involvement of the cPLA$_2$ route in the delayed production of PGE$_2$ was studied by using pyrrolidine 1. As shown in Fig. 5D, pyrrolidine 1 inhibited PGE$_2$ production in resting cells and in response to stimuli. Unlike IC and LPS, zymosan induced the production of LTC$_4$ with a time course paralleling AA release, since maximal levels were assayed at 1 h (Fig. 5E). PGD$_2$ production was observed in response to zymosan and, at a lower extent, to IC (Fig. 5E). Altogether, these data indicate a metabolism of AA in DC dominated by COX products.

**Syk activity is involved in AA release**

The protein tyrosine kinase Syk plays a central role in cell signaling through both Fc receptor-mediated Ag presentation and DC maturation (22). The involvement of Syk in [H]$^1$AA release and COX-2 induction was then assessed by examining tyrosine phosphorylation of this kinase (a measure of Syk activation) and the effect of Syk inhibitors. As shown in Fig. 6A, both IC and zymosan induced activation of Syk in DC. Treatment of DC with piceatannol significantly inhibited IC-induced, but only marginally affected zymosan-induced cPLA$_2$ phosphorylation (Fig. 6B). In agreement with this result, piceatannol inhibited the release of [H]$^1$AA by 96 and 54% in response to IC and zymosan, respectively, R406, a very specific Syk inhibitor, also inhibited completely the response to IC and reduced zymosan-induced [H]$^1$AA release by 30% (Fig. 2A). Zymosan-induced Syk phosphorylation was also inhibited with the addition of laminarin, but not by anti-DC-SIGN mAb (Fig. 6C). We also examined the effect of Syk inhibition on the induction of COX-2 and observed that piceatannol could inhibit the induction of this protein partially (Fig. 6D). Piceatannol also blocked the induction of COX-2 elicited by PGN, which is in agreement with the recent observation that piceatannol could inhibit LTC$_4$ production in response to PGN (23); however, this inhibitor did not affect COX-2 induction by LPS. HEK293 cells transfected with expression vectors encoding dectin-1 and Syk showed a clear association of dectin-1 and Syk following zymosan addition (Fig. 6E), further supporting the involvement of Syk in dectin-1 signaling. Taken collectively, these results are consistent with the notion that Syk activity is completely necessary for IC-induced AA release, but it is only partially involved in the signaling mechanism whereby zymosan elicits AA release and COX-2 induction in DC.

Zymosan induces the activation of NF-$\kappa$B and we examined the involvement of this pathway in the induction of COX-2 in DC. By analyzing IxB$\alpha$ degradation (Fig. 7A), we observed a robust activation of the NF-$\kappa$B route by zymosan, which occurred between 30 and 60 min and was followed by resynthesis of IxB$\alpha$ by 2 h (Fig. 7A). Moreover, blockade of this pathway with N-acetyl-leucyl-leucyl-norleucinal (ALLN) inhibited the induction of COX-2 protein in response to all stimuli, suggesting an involvement of NF-$\kappa$B-driven transcriptional regulation (Fig. 7B).

**DC-SIGN coimmunoprecipitates with dectin-1**

The ability of combinations of laminarin/anti-dectin-1 and anti-DC-SIGN mAb to inhibit [H]$^1$AA suggested a cooperation between DC-SIGN and dectin-1 for the generation of this response. Indeed, we could demonstrate that dectin-1 coimmunoprecipitated
with DC-SIGN, particularly after the stimulation of DC with zymosan (Fig. 8A). A control anti-GST mAb did not immunoprecipitate DC-SIGN or dectin-1, demonstrating the specificity of this assay. Additional experiments in HEK293 cells transfected with vectors encoding DC-SIGN and Myc-dectin-1 showed a robust coimmunoprecipitation of both C-lectin receptors when immunoprecipitation was conducted with either anti-DC-SIGN mAb or anti-Myc mAb (Fig. 8B). These results are consistent with a system for zymosan recognition in DC involving the interaction of dectin-1 and DC-SIGN.

This possible interaction was further addressed by confocal microscopy. As shown in Fig. 9A, DC-SIGN was found to cluster in areas of contact with zymosan particles (Fig. 9A), but not around engulfed particles as judged from the analysis of images taken after 10 min, where ingested particles were not surrounded by DC-SIGN staining. This finding agrees with recent reports indicating that DC-SIGN is a mannann-inhibitable zymosan receptor, but does not mediate phagocytosis (15, 24). In contrast, engulfed zymosan particles were clearly surrounded by dectin-1 (Fig. 9B). Clustering of DC-SIGN and dectin-1 staining was not observed in resting cells (Fig. 9, C and D) or in cells stained for the transmembrane protein CD45 following zymosan challenge (Fig. 9E). These findings also suggest the cooperative involvement of dectin-1 and DC-SIGN in the induction of AA metabolism by zymosan in DC.

**Discussion**

The present data demonstrate that there is a robust metabolism of AA in DC challenged with stimuli of both the innate and the adaptive immune response. The profile of metabolites is dominated by COX products and the amounts of prostanoids produced are comparable to those assayed in macrophages. AA release via the cPLA2 route was similar in immature and mature DC. In contrast, PG production linked to COX-2 induction occurred in immature DC stimulated with zymosan, whereas TNF-α-matured DC showed a diminished induction of COX-2 upon zymosan challenge. Because PGE2 is important for DC migration from peripheral tissues toward secondary lymph organs, these results suggest an autocrine mechanism of PG production which helps us to understand the functional responses of DC. These are novel findings, since most studies on the role of AA in DC biology have focused on the responses to eicosanoids and the process of DC migration mediated by E prostanoid (25, 26), cysteinyl-LT (7, 8), LTB4 (27), and lipoxin receptors (9) rather than on eicosanoid production. It has, however, been proposed that the differentiation of monocytes into DC is associated with a decreased expression of cPLA2, due to the action of IL-4, an essential component of the cytokine mixture used to promote DC differentiation (4, 5). A possible reason for this discrepancy could be the use of different experimental approaches. Zelle-Rieser et al. (5) cultured monocytes for 5 days in the presence of either GM-CSF or GM-CSF plus IL-4 and the differences in PGE2 production in response to LPS were attributed to the effect of IL-4 on cPLA2. However, although LPS is a well-established inducer of COX-2 expression and delayed prostanoid production, it has only a priming effect (28, 29) or no effect at all on early AA release and LT biosynthesis in PMN, macrophages (30, 31), and DC. Our data show stimulus-specific patterns for AA metabolism in DC similar to those recently reported in a murine macrophage cell line, where there were depicted a pattern associated with TLR agonist pathways and another one linked to stimuli able to generate their response via a sustained intracellular Ca2+ elevation (32). Our results can be summarized in three different programs of DC activation: 1) a response triggered by FcγR crosslinking, which leads to Syk-dependent cPLA2 activation, early PG biosynthesis, and IL-10 production; 2) a program involving cPLA2 activation, early biosynthesis of LTC4 and PG, delayed PG production linked to COX-2 induction, and IL-12 production, which is triggered by ligands of C-type lectin receptors; and 3) a route activated by LPS which involves IL-12 and COX-2 induction in the absence of acute AA release. To explain the distinct pattern of AA metabolism induced by LPS, it should be taken into account that cooperation of secreted phospholipases A2 and cPLA2 is involved in the delayed phase of prostanoid production in different cell types (33–38). This depends on COX-2 induction and occurs without accompanying LT synthesis. The existence of a similar mechanism in DC might explain our findings.

The time course of AA release observed in the present study is similar to that reported in monocytes stimulated with IC (18), PMN treated with mannann and PGN (11), and RAW264.7 macrophages activated with TLR agonists. In contrast, stimulation of
RAW264.7 cells via purinergic receptors shows optimal AA release at ~10 min (32) and activation of mast cells via FceRI releases AA in 2–3 min (39). Whether the different time courses observed upon stimulation of FceRI and FcγRI are due to differences in the structure of the ITAMs or to other elements involved in the signaling cascades is not known.

The association between COX-2 induction and IL-12 production may be linked to posttranscriptional mechanisms or to a mechanism of transcriptional regulation common to both proteins. The first hypothesis is supported by the synergistic effect of PGE2 on TNF-α-induced production of IL-12 (40) and by the diminution of IL-12p70 protein we observed upon COX-2 inhibition (Fig. 3A). In contrast, the regulation of COX-2 and IL-12 by NF-κB transcription factors, which may show stimulus-specific activation patterns, would be consistent with the second hypothesis. For example, TNF-α activates RelA but not c-Rel and is a weak inducer of IL-12p35 mRNA (41) and COX-2. In contrast, LPS activates both RelA and c-Rel (42) and is a strong inducer of both IL-12 p35 and COX-2 expression. In the absence of chromatin remodeling, tightly packaged nucleosomes are also an obstacle for accessibility of transcription factors to regulatory sequences in COX-2 (43), IL-12p35 (44), IL-12p40 (45), and IL-10 (46), which may explain transcriptional repression in the presence of active transcription factors. Taken together, these data indicate that a complex combination of cell responses involving activation of transcription factors and covalent modification of histones could explain the distinct transcriptional responses observed in DC.

The inhibition of IL-10 production by pyrrolidine 1 suggests a connection between IL-10 production and AA metabolism. This might be explained by two different mechanisms: 1) an indirect one, where IL-10 is up-regulated by IL-12 as a feedback mechanism to inhibit the proinflammatory response (47) and/or 2) a direct induction of IL-12p35 mRNA (48). These findings are relevant to understand DC function, because DC matured in the presence of PGE2, although phenotypically identical in many aspects to DC matured with other stimuli, are more sensitive to chemokine-mediated migration and are more efficient in eliciting T cell responses (25). In addition, DC matured in medium supplemented with PGE2 generate optimal yields of DC producing IL-12p70 (49). Autocrine production of PGE2 is also a central factor for DC to switch from a strongly adhesive to a highly migratory phenotype through a mechanism involving podosome disassembly and loss of αβ2 integrin-mediated adhesion to the substrate (50). Taken together, these data suggest that part of the specific responses elicited via innate immune system receptors on DC might be mediated by endogenous PGE2.

Our data underscore the ability of zymosan to induce AA release from DC, as compared with monocytes and PMN, where C3bi coating is required for this response (18, 51). This could be explained by the high levels of expression of dectin-1 on DC; however, the identification of the receptor(s) involved in zymosan recognition by these cells has not yet been resolved. In fact, dectin-1-deficient mice mount a normal cytokine response to Saccharomyces cerevisiae zymosan (52), although the response to Sparassis crispa glucan is abolished (53). Another explanation for our findings could be cooperation of dectin-1 with other receptors and/or regulatory molecules. Indeed, dectin-1 can interact with other proteins such as the leukocyte tetraspanin CD37, which has been shown to inhibit dectin-1-mediated IL-6 production (54). In addition, dectin-1, TLR2, and the MR have been shown cooperate in the recognition of Candida albicans mannans and glucans (55, 56). Given that DC show high levels of DC-SIGN expression, cooperation of dectin-1 and DC-SIGN in triggering AA release seems likely. This hypothesis is supported by the inhibition of [3H]AA release by combinations of anti-DC-SIGN and anti-dectin-1 mAb and by the communoprecipitation of dectin-1 and DC-SIGN in DC and in cells overexpressing these receptors. These findings suggest a system for zymosan recognition in DC involving the synergistic interaction of several receptors.

Unlike monocytes (18), coupling of C3bi to IgG does not result in loss of AA-releasing activity in DC. The C3bi moiety incorporated into IC binds to CR3 (Mac-1, CD11b/CD18, αβ2 integrin) and drives the immune load from FcγRII to CR3, thus promoting phagocytosis by monocytes in the absence of inflammatory response. Notably, productive binding of C3bi to leucocyte-expressed CR3 needs an interaction with FcγRII to form a CR3/FcγRIII complex, the formation of which is impeded in DC (57). DC show a high expression of CD11c, i.e., the α-chain of CR4, another integrin with C3bi-binding properties. In addition, DC β2 integrins are in an inactive state which hampers binding to cognate ligands (58). In the light of these data, plausible explanations for the distinct responses of monocytes and DC to C3bi-coupled IC could be an impaired binding to CR3, a preferential binding to CR4 or a constitutively inactive state of DC integrins.

Stimuli leading to AA release also induce Syk phosphorylation, although their ability to phosphorylate Syk does not correlate with their ability to induce the release of AA. Inhibition of Syk blocks AA release in response to IC, but only partially in response to zymosan. Since blockade of DC-SIGN shows an additive effect with laminarin and since DC-SIGN cannot activate Syk, signals triggered through DC-SIGN binding might account for the Syk-independent component of this response (59). This concept is supported by a recent report demonstrating that DC-SIGN activates the phospholipase Cγ2 route by a mechanism involving Src family kinases, but not Syk (60). Syk activation itself is not sufficient to induce COX-2, as judged from the absence of COX-2 induction by IC. Because monocyte-derived DC lose the expression of FcγRI and FcγRIII (42, 61, 62), it seems likely that the isolated cross-linking of FcγRIIA and triggering of Syk is sufficient for the activation of the cPLA2, whereas COX-2 induction requires additional signals to initiate β2-driven transcriptional activation, as it has been reported in other systems (63).

Recent studies on dectin-1-mediated signaling suggest the occurrence of two different pathways: a dectin-1/Syk pathway, which depends on CARD9, and a dectin-1/TLR2/MyD88 pathway that might be at least partially independent from Syk and CARD9 (21, 64). The first pathway is involved in the production of IL-2, IL-23, and IL-10, whereas the MyD88-dependent module is involved in the production of IL-6, IL-12, and TNF-α. Our data allow the integration of AA metabolism within this dual signaling scheme (Fig. 10): AA release showing a partial dependence on Syk activation through DC-SIGN binding might account for the Syk-dependent component of this response. A route of dectin-1/Syk signaling is sufficient to induce COX-2, as judged from the absence of COX-2 induction by IC. Because monocyte-derived DC lose the expression of FcγRI and FcγRIII (42, 61, 62), it seems likely that the isolated cross-linking of FcγRIIA and triggering of Syk is sufficient for the activation of the cPLA2, whereas COX-2 induction requires additional signals to initiate β2-driven transcriptional activation, as it has been reported in other systems (63).

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DISCLOSURES

The authors have no financial conflict of interest.

REFERENCES


