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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 picetinam 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 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.
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 picelan-annol and R406 completely inhibited AA release in response to ligands of opsonic and nonopsonic receptors. The Syk kinase inhibitors piceatin, mannan, and mAb against dectin-1 and DC-SIGN, DC were incubated for 30 min before the addition of zymosan particles. One hour after addition of zymosan particles, cells were taken from the plates and transferred into FACS tubes (BD Pharmingen) for analysis by flow cytometry in a Beckman Coulter Epics XL cytofluorometer.

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 p47phox was assayed using an anti-p47phox phospho-specific Ab (Ser424), catalog no. 2831 from Cell Signaling Technology. For the assay of phospho-Src, antiphosphotyrosine mAb 4G10 from Upstate Biotechnology was used. Syk phosphorylation was addressed using phospho-specific anti-human Syk Ab reactive to the activation loop site pY525/p526 (ref. 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).

Materials and Methods

Reagents

Zymosan, the soluble β-glucan laminarin, mannan from Saccharomyces cerevisiae, OptiPrep, porcine mucin-3, and picelanannol were purchased from Sigma-Aldrich. Anti-human CD180/MR, 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). Production of IgG/GOV2 equivalent IC and coprecipitation of zymosan particles were conducted as described elsewhere (18). IL-12p70, IL-10, and PGE2 were assayed with Biotrack ELISA systems from Amersham Biosciences according to the manufacturer’s instructions. The detection limit of these assays is 3 pg/ml for IL-12p70 and IL-10 and 2.5 pg/ml for PGE2. ELISA for LTC4 and PGD2 were obtained from Cayman Chemical. A pE8-hemagglutinin (HA) expression vector encoding porcine spleen tyrosine kinase (Syk) was provided by Dr. T. Mustelin (Burnham Institute, La Jolla, CA). The cDNA of human β-glucan receptor was inserted into the EcoRI/XbaI sites of a pE8-F4-Myc vector. A pcDNA3 vector encoding human DC-SIGN cDNA and anti-DC-SIGN (MR1 mAb) for immunoprecipitation assays were provided by Dr. A. Corbi (Centro de Investigaciones Biológicas, Madrid, Spain). Endotoxin levels in the reagents were below 1 ng/ml as determined by the Limulus amebocyte lysate assay (Cambrex). Moreover, addition of 200 µg/ml polymyxin B to these reagents did not modify the effect of the different stimuli, excluding a possible involvement of LPS in the responses studied.

Harvesting of monocyte-derived DC

Mononuclear cells were collected from buffy coats of healthy donors by centrifugation on Ficoll-Hypaque cushions. The mononuclear cell ring was recovered in 3 ml of OptiPrep and then layered below a solution of 7 ml of Ficoll (density 1.072 g/ml). A discontinuous gradient was formed by adding OptiPrep (density, 1.0686 g/ml) containing 0.5% BSA and 1 mM EDTA, followed by a HEPES-buffered saline solution. The mixture was centrifuged for 25 min at 725 × g at 19°C and the lymphocyte-depleted cell solution was collected and treated again to enhance the purity of the cell preparation. Cells were maintained for 2 h at 37°C to allow the adherence of monocytes. Nonadhered cells were removed and the remaining cells were cultured in the presence of GM-CSF (800 U/ml) and IL-4 (500 U/ml) for 5 days. Maturation was achieved by incubation in the presence of recombinant human TNF-α (100 U/ml) and assessed by flow cytometry of CD40, CD80, CD83, and CD86. Labeling of monocyte-derived DC with [3H]TAAs was conducted as previously described (18).

Phagocytosis of Alexa Fluor 488-labeled zymosan particles by DC

DC (3 × 10³ in 0.5 ml) were incubated with zymosan particles conjugated with Alexa Fluor 488 at the concentration of five particles per 100 µg with Alexa Fluor 488 to address particle binding or at 37°C to address both binding and phagocytosis as previously reported (18, 19). For inhibition studies with laminarin, mannan, and mAb against dectin-1 and DC-SIGN, DC were incubated for 30 min before the addition of zymosan particles. One hour after addition of zymosan particles, cells were taken from the plates and transferred into FACS tubes (BD Pharmingen) for analysis by flow cytometry in a Beckman Coulter Epics XL cytofluorometer.

Confluent microarray

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 different mAbs and 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

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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-release.
releasing activity (Fig. 1C), whereas mannan induced [3H]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 [3H]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 [3H]AA release, which

![FIGURE 3.](image)

**FIGURE 3.** DC were incubated for 24 h with several stimuli in the presence 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-β-actin mAb. Blots are representative of at least three independent experiments.

![FIGURE 5.](image)

**FIGURE 5.** PGE2 production by DC. A and B, DC were stimulated for the times indicated and the production of PGE2 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 μM ionomycin for 30 min before the addition of zymosan and IC on the production of PGE2 is shown in the left columns. The effect of a preincubation with the stimuli before ionomycin is shown in the right columns. PGE2 was assayed 24 h after the addition of the stimuli. D, Inhibition of PGE2 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. PGE2 was assayed in supernatants 48 h after addition of stimuli. Data represent mean ± SEM of three independent experiments. E, Production of LTC4 and PGD2 in response to zymosan and IC. LTC4 was assayed 1 h after addition of the stimuli. PGD2 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 [3H]AA release from cPLA2-catalyzed reactions (Fig. 2A). Zymosan-induced [3H]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. 2B, 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 receptors involved in the recognition of zymosan by DC, which can be operative in DC, the induction of COX-2 and the production of PGE2, PGD2, and LTC4.

**FIGURE 6.** Effect of zymosan and IC on Syk phosphorylation in DC. A, Cells were stimulated for 10 min with the indicated stimuli and cell lysates were used for the immunodetection of phosphorylated Syk using 4G10 mAb and phospho-specific mAb reactive to the two adjacent phosphorylated tyrosines in the Syk activation loop. Anti-β-actin mAb was used to assess the amount of protein loaded into the different lanes. B, DC were preincubated with piceatannol or vehicle for 30 min before the addition of the stimuli, and 60 min thereafter cell lysates were collected to address cPLA2 phosphorylation with phospho-specific anti-cPLA2 Ab. C, Effect of anti-DC-SIGN mAb and laminarin on Syk phosphorylation. DC were incubated for 30 min at 37°C in the presence of anti-DC-SIGN mAb, laminarin, and a combination thereof and then incubated for 10 min in the presence of zymosan and IC. Cell lysates were collected for the assay of Syk phosphorylation. The amount of Syk in the blots was assessed with anti-Syk Ab. D, DC were incubated with piceatannol at the concentrations indicated for 30 min before the addition of the stimuli and, 8 h thereafter, cell lysates were collected for the immunodetection of both COX-2 and β-actin. The amount of COX-2 protein was quantitated by densitometric scanning and normalized for the β-actin control. E, HEK293 cells were 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-cPLA2, phosphorylated cPLA2; IP, immunoprecipitation.

The production of IL-12p70 and IL-10 was induced by all of the stimuli (Fig. 3B). Treatment with pyrrolidine 1, piceatannol, and the COX-2 inhibitor SC-236 inhibited the production of IL-12p70 in response to zymosan, 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.

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

To delineate the routes of AA metabolism downstream of cPLA2 that can be operative in DC, the induction of COX-2 and the production of PGE2, PGD2, and LTC4 were addressed. Zymosan and mucin-3 induced COX-2 protein expression in immature DC (Fig. 4A), whereas both IC and TNF-α at the concentration used to induce DC maturation failed to do so (Fig. 4B, right panels). COX-2 protein induction was less prominent in mature DC than in immature DC (Fig. 4A) and showed a dose dependence similar to that observed for [3H]AA release (Fig. 4, B and C). Although COX-2 mRNA was detected as soon as 30 min after the addition of zymosan (Fig. 4D), COX-2 protein was only detectable after 4 h and persisted up to 48 h (Fig. 4E).

To assess whether AA release was coupled to eicosanoid production, we next assayed the production of PGE2, PGD2, and LTC4. As shown in Fig. 5A, PGE2 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 FcγR and dectin-1 in murine DC (21), and is a key element in Fc receptor-mediated Ag presentation and DC maturation (22). The involvement of Syk in [^{3}H]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 [^{3}H]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 [^{3}H]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-κB and we examined the involvement of this pathway in the induction of COX-2 in DC.** By analyzing IkBα degradation (Fig. 7A), we observed a robust activation of the NF-κB route by zymosan, which occurred between 30 and 60 min and was followed by resynthesis of IkBα by 2 h (Fig. 7A). Moreover, blockade of this pathway with N-acetyl-leucinyl-leucinyl-norleucinal (ALLN) inhibited the induction of COX-2 protein in response to all stimuli, suggesting an involvement of NF-κB-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 [^{3}H]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 cross-linking, 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 mannan 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 FcγR 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 pyrroolidine 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-10 by PGE2 (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 α5β1 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 tetraspan 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 FcγRI 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 Cγ 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 εB-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 activity and COX-2 induction showing a full dependence on NF-κB activation triggered by TLR2 engagement (65). A route of dectin-1 signaling involving TLR2-independent NFAT activation has also been recently reported. Although this mechanism might explain a portion of COX-2 induction by zymosan in murine macrophages, it is significantly less potent than the canonical TLR2 route (66). Taken collectively, our data reveal an active metabolism of AA in human DC, which is triggered by physiological stimuli of DC. Stimulation of FcγRs is associated with the activation of the cPLA2 enzyme and early production of COX-1 metabolites, whereas fungal stimuli are also inducers of COX-2 expression and endow DC with the ability to produce high-level, long-lasting PG biosynthesis.
FIGURE 10. Diagram of AA metabolism in DC stimulated with zymosan particles. The mannan and β-glucan components of zymosan are recognized by DC-SIGN, TLR2, and dectin-1. This gives rise to a series of signaling events implicating activation of Syk and Src families of tyrosine kinases. Both routes converge to activate phospholipase Cγ and the generation of diacylglycerol activate protein kinase C and phospholipase Cβ. The mannan and β-glucan components of zymosan are recognized by at least DC-SIGN, TLR2, and dectin-1. This gives rise to a novel pattern recognition pathway for C type lectins.

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Disclosures

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