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A Critical Role for Prostaglandin E2 in Podosome Dissolution and Induction of High-Speed Migration during Dendritic Cell Maturation

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Dendritic cells (DCs) are professional APCs of the immune system that play a key role in regulating T cell-based immunity. The capacity of DCs to activate T cells depends on their maturation state as well as their ability to migrate to the T cell areas of draining lymph nodes. In this study, we investigated the effects of DC maturation stimuli on the actin cytoskeleton and β1 integrin-dependent adhesion and migration. Podosomes, specialized adhesion structures found in immature monocyte-derived DCs as well as myeloid DCs, rapidly dissolve in response to maturation stimuli such as TNF-α and PGE2, whereas the TLR agonist LPS induces podosome disassembly only after a long lag time. We demonstrate that LPS-mediated podosome disassembly as well as the onset of high-speed DC migration are dependent on the production of PGs by the DCs. Moreover, both of these processes are inhibited by Ab-induced activation of β1 integrins. Together, these results show that maturation-induced podosome dissolution and loss of α5β1 integrin activity allow human DCs to undergo the transition from an adhesive to a highly migratory phenotype. The Journal of Immunology, 2006, 177: 1567–1574.

Dendritic cells (DCs) are the most potent APCs of the immune system (1). Upon Ag uptake and exposure to inflammatory stimuli, these cells undergo a remarkable transition from a tissue-resident, endocytic cell type to a highly migratory APC type, a process known as DC maturation (1, 2). This phenotypical conversion not only involves up-regulation of MHC and costimulatory molecules, but is also accompanied by extensive changes in cytoskeletal organization and cell adhesion (3–6).

The ability of DCs to migrate is essential for the induction of immune responses. Upon Ag recognition and exposure to inflammatory signals, DCs migrate out of the tissues, via the blood or lymph vessels into the lymph nodes, where they activate T cells. We recently demonstrated both in preclinical mouse models (7) and in human cancer patients (3) that DC vaccine efficacy directly correlates with DC maturation and the capacity of these cells to migrate into the lymph nodes. The migration of mature DCs (mDCs) can be defined as high-speed migration (8), comparable to the migration of T cells (9). This type of migration is characterized by short-lived and low-affinity interactions with the substrate. Immature DCs (iDCs), in contrast, show an adhesive and low-speed migratory behavior that is dependent on strong interactions with the extracellular matrix (ECM), similar to fibroblasts (8). β1 integrins are the main adhesion molecules responsible for these DC-ECM interactions (10, 11). Although the fibronectin-binding integrins α5β1 and αvβ3 are both expressed by DCs, we and others have shown that adhesion of DCs to fibronectin is predominantly α5β1 mediated (3, 12, 13). In their high-affinity state, integrins form multimeric complexes in the plasma membrane that enhance binding to the ECM. The integrin cytoplasmic domains in turn recruit a large number of structural and signaling proteins into higher order complexes that connect integrins to the actomyosin cytoskeleton. In most cells, such adhesive contacts are known as focal adhesions (14). However, cells of myeloid origin, such as macrophages, osteoclasts, and DCs, form adhesion complexes distinct from focal adhesions, known as podosomes (15). Unlike focal adhesions, podosomes are not linked to contractile actin stress fibers, but rather consist of an actin-dense core surrounded by a ring of cytoskeletal proteins (16, 17).

Although a large variety of stimuli, including cytokines, CD40 ligation, or TLR ligands, induce DC maturation (18–20), these stimuli not always bring out all the characteristics that mDCs need to fulfill their function (21). Particularly, migration appears to be insufficiently developed in response to some stimuli. To effectively manipulate DC migration in clinical applications, a more detailed knowledge of how maturation factors mobilize DCs is needed. In this study, we investigated the effects of different maturation stimuli on cell adhesion and migration in DCs. We find that high-speed DC migration, induced by maturation signals, requires dissolution of podosomes as well as loss of β1 integrin activity. In the presence of a β1-activating Ab, both podosome dissolution and cell migration are effectively inhibited. Moreover, we demonstrate that PGs

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5 Abbreviations used in this paper: DC, dendritic cell; ECM, extracellular matrix; iDC, immature DC; MCM, monocyte-conditioned medium; mDC, mature DC; DC-SIGN, DC-specific ICAM-3-grabbing nonintegrin.
such as PGE$_2$, produced by DCs during maturation, mediate podosome disassembly as well as the onset of high-speed migration.

**Materials and Methods**

**Chemicals and Abs**

The following Abs were used: anti-HLA class I (W6/32), anti-HLA-DR/DP/DQ (Q5/13), anti-CD80 (all from BD Biosciences), anti-CD14 and anti-CD83 (both from Beckman Coulter), anti-CD16 (BD Pharmingen), anti-CD209/DC-SIGN (anti-DC-SIGN) (22), anti-$\beta$-integrin-activating epitope (TS2/16), anti-$\beta$-integrin-blocking epitope (AIIB2) (Developmental Studies Hybridoma Bank), anti-$\beta$-integrin recognizing an active conformation (1G10) (24) (a gift from M. Humphries, Faculty of Life Sciences, University of Manchester, Manchester, U.K.), anti-$\alpha$-integrin (SAM-1), -$\alpha$-vinculin (Sigma-Aldrich), mlgG1 (BD Biosciences), mlgG2a, mlgG2b, and rIgG1 isotype (BD Pharmingen). Alexa Fluor 488-labeled secondary Abs were from Molecular Probes (Molecular Probes), and FITC-labeled secondary Abs were from Zymed Laboratories and MP Biomedicals. Texas Red-conjugated phalloidin (Molecular Probes) was used to stain F-actin. Indomethacin was obtained from Sigma-Aldrich.

**Preparation of DCs**

DCs were generated from PBMCs, as described previously (25, 26). Monocytes were derived fromuffy coats or from a leukapheresis product. Plastic-adherent monocytes were cultured in X-VIVO 15 medium (BioWhitaker) supplemented with 2% pooled human serum (PAA Laboratories), IL-4 (500 U/ml), and GM-CSF (800 U/ml) (both obtained from Schering-Plough International) (27), or in RPMI 1640 medium (Invitrogen Life Technologies) supplemented with 10% (v/v) FCS (Greiner Bioscience), IL-4 (500 U/ml), and GM-CSF (800 U/ml) (18). iDCs were harvested on day 7. DCs were matured with LPS (2 mg/ml) (Sigma-Aldrich), TNF-$\alpha$ (75 ng/ml) (a gift from G. Adolf, Bender, Vienna, Austria), or a combination of monocyte-conditioned medium (MCM) (50% v/v), TNF-$\alpha$ (10 ng/ml), and PGE$_2$ (10 mg/ml) (Pharmacia & Upjohn) for 2 days. mDCs were harvested on day 9. Expression of MHC class I/II, costimulatory molecules, and DC-specific markers on DCs was measured by flow cytometry (data not shown), and the expression of MHC molecules, costimulatory molecules, and DC markers was similar to what was described before (3). iDCs expressed MHC class I and II, the costimulatory molecule CD86, the DC-specific marker CD209/DC-SIGN, and low levels of the DC-specific marker CD83. Maturation resulted in the induction of CD83; up-regulation of MHC class I and II, CD86, and CD80; and a down-modulation of CD80/DC-SIGN expression.

**Isolation of myeloid DCs**

Myeloid DCs (CD19$^+$ CD1c$^+$) were isolated from PBMCs derived from buffy coats. PBMCs were labeled with a FITC-conjugated mAb to CD3 (DakoCytomation), and T and B cells were depleted using anti-FITC and anti-CD19 beads (both Miltenyi Biotec). Myeloid DCs were then isolated from the CD3$^+$ CD19$^-$ population using a biotinylated anti-CD1c (BDCA-1) Ab and anti-biotin beads (both Miltenyi Biotec), following the manufacturer’s instructions. To assess purity, isolated cells were labeled with streptavidin Cy5 (Jackson ImmunoResearch Laboratories) and a PE-conjugated anti-CD19 Ab (Miltenyi Biotec), PE-conjugated anti-CD14 (Beckman Coulter), or anti-CD11c (BD Biosciences) Abs. Myeloid DCs were identified as cells that expressed CD1c and CD11c, but were negative for CD14. The myeloid DC population thus obtained was 99% pure and represented 0.3% of the total number of PBMCs.

**Flow cytometry**

Cells (1 x 10$^6$) were incubated with 2% (v/v) human serum in PBA (PBS containing 1% (w/v) BSA and 0.05% (w/v) NaN$_3$) for 15 min at 4°C. After washing with cold PBA, the cells were incubated with primary Ab (5 mg/ml) for 30 min at 4°C. Subsequently, the cells were incubated with FITC-labeled goat anti-mouse. Cells were washed and resuspended in 100 $\mu$l of PBA. Fluorescence was measured using a FACS Calibur with CellQuest software (BD Biosciences).

**Fluorescence microscopy**

Coverslips were coated with fibronectin (20 mg/ml) (Roche) in PBS for 1 h at 37°C. Cells were seeded on fibronectin-coated coverslips, left to adhere for 1–12 h, and fixed in 3.7% (w/v) formaldehyde in PBS for 10 min. Cells were permeabilized in 0.1% (v/v) Triton X-100 in PBS for 5 min and blocked with 2% (w/v) BSA in PBS. The cells were incubated with primary Ab for 1 h. Subsequently, the cells were incubated with Alexa Fluor 488-labeled secondary Abs for 45 min. Subsequently, cells were incubated with Texas Red-conjugated phallolidin for 30 min. Images were collected on a Leica DMRa fluorescence microscope with a ×63 PL APO 1.3 NA oil immersion lens (or a ×40 PL FLUOTAR 1.0 NA oil immersion lens for overview images) and a Cohu high performance integrating charge-coupled device camera (Cohu). Pictures were analyzed with Leica Qhturo version 1.2.0 (Leica Microsystems) and Adobe Photoshop 7.0 (Adobe Systems) software.

**Migration assay**

Ninety-six-well flat-bottom plates (Costar) were coated with fibronectin and blocked with gelatin. Cells (7 x 10$^5$/ml) were labeled with calcine-AM (25 mg/ml) in PBS (Molecular Probes) for 30 min at 37°C, either untreated or preincubated for 10 min at room temperature with blocking Ab (100 mg/ml), and seeded on the fibronectin-coated plates (3 x 10$^5$/well) for 45 min at 37°C in the presence or absence of activating Ab (1 mg/ml). Non-adherent cells were removed by washing with 0.5% (w/v) BSA in TBSA (150 mM NaCl, 10 mM Tris-HCl, 2 mM MgCl$_2$, 1 mM CaCl$_2$ (pH 8.0)) at 37°C. Adherent cells were lysed in 100 $\mu$l of lysis buffer (50 mM Tris, 0.1% (w/v) SDS), and fluorescence was quantified using a cytometer (Applied Biosystems). Results are expressed as the mean percentage of adhesion of triplicate wells.

**Transfection and live imaging of DCs**

DCs (3 x 10$^5$) were electroporated with pCMV-β-actinGFP on day 5 using Amaza nucleofection (Amaza Biosystems). Transfected DCs were seeded on fibronectin-coated Wellco glass-bottom dishes (Wellco Wells). Cells were imaged at 37°C in RPMI 1640 without phenol red (Invitrogen Life Technologies) supplemented with 10% (v/v) FCS using a Zeiss LSM 510-meta microscope equipped with a type S heated stage CO2 controller and PlanAchromatic ×63 1.4 NA oil immersion DIC lens (Zeiss). Podosomes were imaged for 20 min with 20-s intervals without PGE$_2$ and for 17 min with 10-s intervals with PGE$_2$ (10 mg/ml). In addition, podosomes were imaged for 60 min without TNF-$\alpha$, and for 20 min in the presence of TNF-$\alpha$ (75 mg/ml) at 30-s intervals. Cells were imaged using Zeiss LSM Image Browser version 32 (Zeiss), and images were processed with Image J (version 1.32) software (National Institutes of Health, http://rsb.info.nih.gov/ij/).

**Statistical analysis**

An ANOVA test or two-tailed Student’s t test was used for statistical analysis.

**Results**

**Induction of high-speed migration in response to DC maturation stimuli**

The ability of DCs to migrate is essential for the efficient induction of T cell responses. In earlier work, we have shown that the capacity of DCs to migrate on fibronectin in vitro correlates well with their migratory behavior in vivo (3). Consistent with these previous findings, the majority of the iDCs were slowly migrating with a mean speed of 0.5 ± 0.1 $\mu$m/min (Fig. 1A). In contrast, DCs matured with a maturation mixture (MCM, PGE$_2$, TNF-$\alpha$), routinely used in clinical applications (3, 26), were highly migratory, showing random migration at a mean speed of 5 ± 0.5 $\mu$m/min (Fig. 1B). The effects of maturation on cell migration were noticeable after 16 h of stimulation, whereas a full migratory capacity was obtained after 24 h of stimulation (Fig. 1C).
The activity of $\alpha_5\beta_1$ integrin is down-regulated during DC maturation

DC maturation is associated with a significant loss in adhesion to fibronectin (3). The expression of active $\beta_1$, as determined by an Ab (12G10) that exclusively recognizes the active conformation of this integrin (24), was almost completely absent on mDCs. At the same time, expression levels of $\beta_1$ integrins were unaffected during maturation (Fig. 2A). These data suggest that the regulation of $\beta_1$ integrin activity, rather than expression, controls DC adhesion. More importantly, adhesion of mDCs to fibronectin could be completely restored using a $\beta_1$-activating Ab (TS2/16), while the adhesion of iDCs could not be further enhanced (Fig. 2B). Ab-induced adhesion was still $\alpha_5\beta_1$ mediated, because an $\alpha_5$-blocking Ab (SAM-1) effectively interfered with this interaction (Fig. 2B). These data demonstrate that, while the $\alpha_5\beta_1$ integrin expressed on mDCs is still capable of mediating adhesion to fibronectin, its activity is specifically down-regulated during DC maturation.

iDCs display podosomes enriched in active $\beta_1$ integrin

The dramatic changes in cell adhesion and migration, as they occur during DC maturation, are also reflected in the morphology and cytoskeletal organization of these cells. When plated on fibronectin, iDCs exhibited extensive cell spreading accompanied by the formation of numerous podosomes (Fig. 3A, upper panel). In sharp contrast, mDCs were much less spread and no longer contained podosomes (Fig. 3A, lower panel). Importantly, both the $\beta_1$ and $\alpha_5$ chain of the fibronectin-binding $\alpha_5\beta_1$ integrin were present in podosomes, and the active form of the $\beta_1$ integrin, as detected with the 12G10 Ab, was highly enriched in podosomes (Fig. 3B). These results indicate that adhesion of iDCs to fibronectin is mediated by active $\alpha_5\beta_1$ integrin molecules present in these podosomes.

DC maturation signals induce rapid disassembly of podosomes

Because DC maturation leads to dramatic changes in adhesive behavior and migration, we explored the effect of maturation-inducing factors on the actin cytoskeleton. In the immature state, most DCs displayed podosomes. However, in most cells, podosomes were lost within 5–10 min in response to either the maturation mixture or PGE$_2$ alone, while stimulation with TNF-α induced podosome dissolution in a subset of the DCs (Fig. 4, A and B). Prolonged stimulation did not further affect the number of cells displaying podosomes (Fig. 4B). A moderate effect of MCM on podosome dissolution was observed after prolonged stimulation (16 h). These results identify PGE$_2$ as the most active component in the maturation mixture. A concentration of 10 μg/ml PGE$_2$, which is routinely used in the maturation mixture, was the lowest concentration to induce efficient podosome dissolution (Fig. 5). Surprisingly, LPS, a TLR ligand and potent DC maturation factor, failed to induce podosome dissolution after 5–10 min, while prolonged stimulation resulted in a nearly complete loss of podosomes (Fig. 4B). These findings demonstrate that, while most maturation stimuli tested induce dissolution of podosomes, the extent to which

![Figure 1](http://example.com/figure1.png)

**FIGURE 1.** mDCs migrate on fibronectin, and migration is initiated after 16–24 h. A and B, iDCs and mDCs were added to fibronectin-coated plates and monitored for 60 min. Plots of 50 individual tracks, aligned at their starting positions, are shown. The mean speed ± SEM (μm/min) is depicted in the upper right corner as well as the percentage of migrating cells ± SD. C, DCs were incubated for different time periods (0, 5, 16, 24, 41, and 48 h) with a combination of MCM, PGE$_2$, and TNF-α, added to fibronectin-coated plates, and migration was monitored for 60 min. The percentage of migrating cells ± SD is depicted. Significant differences ($p < 0.05$) compared with the percentage of migrating DCs after 48 h of maturation are indicated by ∗.

![Figure 2](http://example.com/figure2.png)

**FIGURE 2.** Active $\beta_1$ integrin is down-regulated upon maturation. A, Active $\beta_1$ integrin expression on DCs decreases upon maturation. iDCs and mDCs were stained using mAbs recognizing $\beta_1$ integrin (AIIB2) or a specific activation epitope of the $\beta_1$ integrin (12G10). Expression was determined by flow cytometry. Open graphs represent isotype controls. B, Adhesion of mDCs to fibronectin can be enhanced by Ab-induced activation of $\beta_1$ integrins. Calcein-AM-labeled iDCs and mDCs were added with or without preincubation with a blocking Ab against $\alpha_5$ integrin (SAM-1) to fibronectin-coated plates, and incubated in the presence or absence of an anti-$\beta_1$ integrin-activating Ab (TS2/16). Cells were lysed and the fluorescence was quantified using a cytofluorimeter. Results are depicted as the mean percentage of adhesion of triplicate wells ± SD. Adhesion of iDCs and mDCs incubated with SAM-1 before and after activation with TS2/16 is significantly decreased in comparison with nontreated control cells. Adhesion of mDCs using TS2/16 is significantly increased in comparison with adhesion of DCs without a $\beta_1$ integrin-activating Ab ($p < 0.05$).
this occurs as well as timing differ between agonists. To examine the kinetics of podosome dissolution in response to maturation stimuli, we expressed an β1-actinGFP construct in iDCs and performed live cell imaging. We observed a rapid turnover of individual podosomes in iDCs expressing β1-actinGFP (lifetime of individual podosomes was 10 min), while the total number of podosomes remained relatively constant (Fig. 6 and data not shown). Addition of PGE2 or TNF-α to iDCs caused a rapid and complete dissolution of podosomes within minutes after stimulation (Fig. 4C and data not shown). Together our results show that podosomes in DCs are highly dynamic and that podosome dissolution is the earliest effect observed in response to DC maturation factors such as PGE2 and TNF-α.

Activation of β1 integrins interferes with podosome dissolution and high-speed migration

We have shown that β1 integrins are prominently present in podosomes and DC maturation is initiated by a loss of these adhesion sites, as well as a global decrease in β1 integrin activity. Therefore, we examined whether and how stimulation of β1 integrin activity using a β1 integrin-activating Ab (TS2/16) affects cytoskeletal changes and the onset of high-speed migration in response to DC maturation. Stimulation with TS2/16 effectively interfered with PGE2-induced podosome dissolution (Fig. 7, A and B) as well as the induction of rapid migration after prolonged incubation (Fig. 7C). These results demonstrate that loss of α5β1 integrin activity during DC maturation is key to the induction of high-speed migration.

LPS-induced podosome dissolution and high-speed migration are dependent on the production of PGs

The disassembly of podosomes in response to LPS occurs with a lag phase of 16 h (Fig. 4B), and therefore cannot be a direct consequence of TLR activation. We investigated whether podosome dissolution induced by LPS involves production of PGE2 by the DCs. Therefore, iDCs were seeded on fibronectin and stimulated with LPS for 16 h, either in the presence or absence of indomethacin. This drug inhibits the production of PGE2 and other PGs by...
inhibiting cyclooxygenases, enzymes involved in the production of PGs (29). Indeed, an effective inhibition of LPS-induced podosome dissolution was observed in the presence of indomethacin (Fig. 8, A and B). In the absence of LPS, no effect of indomethacin on podosomes was seen. Similarly, we observed that indomethacin affects the migratory capacity of LPS-matured DCs (Fig. 8C). These results identify PGs, such as PGE2, as important mediators of maturation-induced DC migration.

PGE2 and LPS induce podosome dissolution in myeloid DCs

To examine whether our findings also apply to DCs generated ex vivo, human myeloid DCs were isolated from peripheral blood by magnetic sorting of CD1c-positive/CD19-negative cells (Fig. 9A). These myeloid DCs, although much smaller than the monocyte-derived DCs, similarly formed podosomes on fibronectin, which rapidly dissolved in response to PGE2 stimulation (Fig. 9B and C). Furthermore, PGE2-induced podosome dissolution was efficiently blocked by stimulation with the β1 integrin-activating Ab TS2/16 (Fig. 9C), while stimulation with TS2/16 without PGE2 had no effect on podosomes.

We also investigated the effect of LPS on podosome dissolution in these myeloid DCs. Similar to monocyte-derived DCs, no immediate effects of LPS on podosome dissolution were observed, whereas a complete loss of podosomes was seen after 16-h stimulation with LPS (Fig. 9D). Again, LPS-induced podosome dissolution was effectively inhibited in the presence of indomethacin (Fig. 9D). These results indicate that both in monocyte-derived DCs as well as myeloid DCs, the effects of LPS on podosome dissolution are dependent on the production of PGs by the DC.

Discussion

In this study, we report how maturation-induced podosome dissolution and loss of αβ1 integrin activity allow DCs to switch from a strongly adhesive to a highly migratory phenotype. Although a substantial amount of reports describe the biogenesis of podosomes in different cell types (17, 30), mechanisms responsible for podosome disassembly have remained elusive. Consistent with earlier findings in osteoclasts (31), we show that these structures are very dynamic. Although podosomes are generally considered to be features of migratory cells (30, 32), we find a strong inverse correlation between the presence of podosomes and DC migratory capacity. This apparent discrepancy can be easily explained by the fundamental differences in the way iDCs and mDCs interact with the substrate to produce migration. Although iDCs display very strong membrane protrusive activity, as indicated by the presence of lamellipodia and membrane ruffles, strong interactions with the substrate (i.e., podosomes) only allow low-speed migration (0.5 μm/min). mDCs display short-lived, weak interactions with the substrate, resulting in high-speed migration, similar to the migration of T cells (9). Consistent with the notion that podosomes restrict high-speed migration, we observed that the active form of the αβ1 integrin is enriched in these structures. Moreover, DC maturation is accompanied by a nearly complete loss of αβ1 integrin-mediated adhesion as well as dissolution of podosomes. As a consequence, mDCs show migration speeds that are 10-fold higher (5 μm/min) than those observed in iDCs. We also show that loss of fibronectin binding, as it occurs during DC maturation, is due to loss of αβ1 integrin activity rather than expression. Furthermore, activation of the αβ1 integrin, using an activating Ab, prevents podosome disassembly and restricts high-speed migration in mDCs. Although preincubation with this integrin-activating Ab effectively inhibits podosome disassembly, the Ab failed to induce new podosomes in fully mDCs (data not shown). This suggests that, although down-regulation of integrin activity during DC maturation is important for the induction of high-speed migration, αβ1 integrin activation cannot fully reverse the effects of DC maturation on the cytoskeleton. Although there are many examples in which integrin activation of leukocytes triggers the induction of cell migration (33–35), we show in this study that down-regulation of integrin activity may be equally important. Similarly, the importance of integrin deactivation in the induction of cell migration was recently demonstrated for αβ1 integrin (LFA-1). Mice expressing a constitutively active variant of LFA-1 showed defective cell migration and compromised immune function due to impaired deadhesion from its ligand ICAM-1 (36).

A maturation mixture, consisting of MCM, PGE2, and TNF-α, was used to induce DC maturation. This combination effectively induces all aspects of DC maturation and is used in clinical DC-vaccination studies (26, 37). In this study, we show that PGE2 is an essential component in the induction of full migratory capacity by this cytokine mixture. mDCs matured in the presence of PGE2 not only migrate more efficiently than without, we also observed that PGE2 alone is sufficient to induce DC migration. The importance of PGE2-mediated signaling in DC migration and consequently the induction of a proper immune response is also supported by mouse models. Mice deficient in EP4, a PGE2 receptor, show impaired skin immune responses due to impaired migration and maturation of Langerhans cells (38). Moreover, it was shown in human DCs that PGE2 enhances both the expression and the sensitivity of

![Figure 5](http://www.jimmunol.org/) PGE2 is able to mediate complete podosome dissolution at a concentration of 10 μg/ml. iDCs were seeded on fibronectin-covered coverslips and left untreated (control) or stimulated for 5 min or 16 h with different concentrations of PGE2. Subsequently, the cells were stained with an anti-vinculin Ab and phalloidin-Texas Red to stain F-actin. The number of cells containing podosomes was counted in seven images per condition. The percentage of cells expressing podosomes ± SEM is depicted. One representative experiment is shown. Conditions that are different from the control situation (p < 0.05) are indicated with *.

![Figure 6](http://www.jimmunol.org/) The lifetime of individual podosomes is <10 min. iDCs were transfected with β-actin-GFP, seeded on fibronectin, and imaged for 60 min with a 30-s interval. The lifetime of individual podosomes in unstimulated cells was determined by counting in how many frames individual podosomes are observed. The lifetime of 50 podosomes was determined, and the distribution of podosome lifetimes is depicted.
Ex vivo generated myeloid DCs form podosomes on fibronectin and late endosomal/lysosomal proteins (p61Hck, CD63, and LYZAAT-1) to other processes in the cell that require high actin turnover.

The observation that podosomes are sites of active matrix degradation (46, 47) and that matrix metalloproteinases are needed for transendothelial migration of leukocytes (48) supports the idea that podosomes are required for crossing tissue barriers. In osteoclasts, podosomes are involved in bone resorption and remodeling (49), while in macrophages podosomes are considered regulators of cell adhesion and migration (50). In addition to a role during transmigration, podosomes in DCs may contribute to the movement of early DCs within peripheral tissues, while patrolling for Ags.

Disassembly of podosomes as it occurs in response to maturation-inducing agents may be an integral part of the DC maturation process, and may serve to redeploy actin and actin-regulatory proteins to other processes in the cell that require high actin turnover. Podosome biogenesis has been linked to lysosome exocytosis, and late endosomal/lysosomal proteins (p61Hck, CD63, and LYZAAT-1) localize to podosomes (51–53). Therefore, it has been put forward that late endosomal vesicles fusing with the plasma membrane contribute to podosome formation (53). During podosome dissolution, the opposite process could be taking place to fuel increased vesicle transport as it occurs during DC maturation.
assembly/disassembly of focal adhesions and podosomes (30, 55). Changes in gene expression. Spatial and temporal regulation. PGE2 on podosomes occur within minutes, which implies that.

Whereas the effects of LPS stimulation on podosome disassembly in human DCs appear to be indirect and require 16- to 24-h incubation, in mouse DCs a rapid, but transient dissolution of podosomes is seen minutes after LPS stimulation (6). We speculate that these are a direct consequence of PGE2 receptor activation rather than changes in LPS-mediated signal transduction between species (54). It will be interesting to determine whether prolonged exposure of mouse DCs to LPS also leads to a sustained podosome loss and whether these late responses are also dependent on the production of PGs such as PGE2.

How does PGE2 induce podosome disassembly? The effects of PGE2 on podosomes occur within minutes, which implies that these are a direct consequence of PGE2 receptor activation rather than changes in gene expression. Spatial and temporal regulation of actomyosin contractility was shown to be important for the assembly/disassembly of focal adhesions and podosomes (30, 55).

Two receptors for PGE2 (EP2/4) are expressed on DCs affecting DC migration (38, 39, 56). Both of these receptors couple to Gs, to increase cAMP levels, leading to activation of protein kinase A (57). Changes in activity regulate actomyosin contractility, either by affecting the activity of the small GTPase RhoA, a key regulator of myosin II function, or by inactivation of myosin L chain kinase (reviewed by Burridge and colleagues (58) and references therein). Future experiments will therefore be aimed at determining how these signal transduction pathways contribute to DC adhesion and migration.

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Disclosures

The authors have no financial conflict of interest.

References


FIGURE 9. Podosome dissolution in myeloid DCs. A, Myeloid DCs (CD1c/BDCA-1-positive cells) were isolated from PBMCs and analyzed by flow cytometry. These myeloid DCs were 99% pure (CD19− CD1c+) and expressed high levels of CD11c, while a subset expressed CD14. B, Myeloid DCs were seeded on fibronectin-coated coverslips and stimulated with PGE2 for 5 min. Subsequently, cells were stained with an anti-vinculin Ab (green) and phallolidin-Texas Red to detect F-actin (red). Representative images are shown. C, Myeloid DCs were seeded on fibronectin-coated coverslips and stimulated with PGE2 for 5 min in the presence or absence of TS2/16 Ab. Subsequently, the cells were stained with phalloydin-Texas Red to detect F-actin (red). The number of cells containing podosomes was counted in 15 images per condition. The percentage of cells expressing podosomes ± SEM is depicted. Conditions that are significantly different (p < 0.05) are indicated with *, D, Myeloid DCs were seeded on fibronectin-coated coverslips and stimulated with LPS in the presence or absence of indomethacin. Subsequently, cells were stained with an anti-vinculin Ab (green) and phallolidin-Texas Red to detect F-actin (red). The number of cells containing podosomes was counted in seven images per condition. The percentage of cells expressing podosomes ± SEM is depicted. Conditions that are significantly different (p < 0.05) are indicated with *. One representative experiment is shown.


