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Podosomes, But Not the Maturation Status, Determine the Protease-Dependent 3D Migration in Human Dendritic Cells

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Dendritic cells (DC) are professional Antigen-Presenting Cells scattered throughout antigen-exposed tissues and draining lymph nodes, and survey the body for pathogens. Their ability to migrate through tissues, a 3D environment, is essential for an effective immune response. Upon infection, recognition of Pathogen-Associated Molecular Patterns (PAMP) by Toll-like receptors (TLR) triggers DC maturation. Mature DC (mDC) essentially use the protease-independent, ROCK-dependent amoeboid mode in vivo, or in collagen matrices in vitro. However, the mechanisms of 3D migration used by human immature DC (iDC) are still poorly characterized. Here, we reveal that human monocyte-derived DC are able to use two migration modes in 3D. In porous matrices of fibrillar collagen I, iDC adopted the amoeboid migration mode. In dense matrices of gelled collagen I or Matrigel, iDC used the protease-dependent, ROCK-independent mesenchymal migration mode. Upon TLR4 activation by LPS, mDC-LPS lose the capacity to form podosomes and degrade the matrix along with impaired mesenchymal migration. TLR2 activation by Pam3CSK4 resulted in DC maturation, podosome maintenance, and efficient mesenchymal migration. Under all these conditions, when DC used the mesenchymal mode in dense matrices, they formed 3D podosomes at the tip of cell protrusions. Using PGE2, known to disrupt podosomes in DC, we observed that the cells remained in an immature status and the mesenchymal migration mode was abolished. We also observed that, while CCL5 (attractant of iDC) enhanced both amoeboid and mesenchymal migration of iDC, CCL19 and CCL21 (attractants of mDC) only enhanced mDC-LPS amoeboid migration without triggering mesenchymal migration. Finally, we examined the migration of iDC in tumor cell spheroids, a tissue-like 3D environment. We observed that iDC infiltrated spheroids of tumor cells using both migration modes. Altogether, these results demonstrate that human DC adopt the mesenchymal mode to migrate in 3D dense environments, which relies on their capacity to form podosomes independent of their maturation status, paving the way of further investigations on in vivo DC migration in dense tissues and its regulation during infections.

Keywords: dendritic cells, podosomes, 3D migration, toll-like receptor, maturation
INTRODUCTION

Dendritic cells (DC) are professional phagocytic and antigen-presenting cells, which populate the skin, mucosal surfaces, and most organs of the body (1, 2). They scan their environment in search for antigens, collect antigenic materials, and transport them via lymphatic vessels to draining lymph nodes where they trigger T lymphocyte activation and the onset of the adaptive immune response (2). Yet, while DC functions rely on their ability to migrate in tissues, the mechanisms underlying DC migration in three-dimensional (3D) environments are not completely understood.

Immature DC (iDC) have been shown in vivo to patrol randomly in tissues, such as the gut and the skin, constantly sampling the interstitium for potential pathogen entry (3, 4). Using micro-channels as an in vitro model of constrained migration, iDC alternate phases of rapid migration with phases of arrest, corresponding to random scanning of the environment and antigen capture (5–7).

During infection, Toll-like receptors (TLRs) mediate cellular responses to a large variety of pathogens (viruses, bacteria, and parasites) by inducing DC activation and maturation. DC maturation is characterized by changes in the surface expression pattern of CC-chemokine receptors. A decrease in the expression of CCR5, which is highly abundant in iDC and involved in their recruitment to the site of inflammation, is accompanied by an increase in the expression of CCR7 that is required for mature DC (mDC) migration toward its ligands CCL19 and CCL21 expressed by lymphatic vessels (2, 8–13). mDC also upregulate protein surface expression of antigen-presenting and co-stimulatory molecules for a proper activation of the T cell responses. Regarding the mechanisms of mDC migration, data from in vivo approaches and in vitro 3D collagen models showed that the so-called “amoeboid” migration mode, which refers to crawling amoeba, are used in porous environments. The amoeboid mode is integrin and protease independent, it involves cell contractility induced by activation of RhoA, the Rho-associated protein kinase ROCK and myosin II, and it is characterized by a round cell shape (1, 14–21).

Podosomes are adhesion cell structures, which are formed constitutively by macrophages, DC, and osteoclasts (22). The known podosome functions are cell adhesion, substrate rigidity sensing, and matrix degradation (22–28). In addition, podosomes and their cancer cell counterpart, invadopodia, are involved in the protease-dependent cell migration that takes place in dense 3D-environments. This mode is integrin-dependent and ROCK-independent. It is characterized by an elongated and protrusive cell shape, and it involves proteolytic degradation of the extracellular matrix (ECM) mediated by podosomes in macrophages and osteoclasts precursors (29–31). This migration mode is called mesenchymal migration. Interestingly, while TLR4-mediated human DC maturation by LPS induces the loss of podosomes (32–34), the TLR2-mediated maturation by Pam3CSK4 maintains podosome formation and stability (34), suggesting that DC migration capacity may be differentially regulated by TLR activation.

Therefore, in the present study, we hypothesized that the migration capacity of DC in 3D environments could be influenced by the architecture of the matrix, the cell maturation status, and the presence/absence of podosomes. We report that human monocyte-derived DC display amoeboid 3D migration in porous matrices of fibrillar collagen I, independent of their maturation status. We demonstrate that both iDC and mDC can adopt the mesenchymal migration mode to infiltrate 3D dense environments, a process that relies on their capacity to form podosomes.

MATERIALS AND METHODS

Dendritic Cell Differentiation and Activation

Human monocytes were obtained from blood donors (Etablissement Français de Sang, EFS, Toulouse). For this report, written informed consents were obtained from all the donors under EFS contract n°21/PLER/TOU/IPBS01/2013-0042. According to articles L1243-4 and R1243-61 of the French Public Health Code, the contract was approved by the French Ministry of Science and Technology (agreement number AC 2009-921). All subjects gave written informed consent in accordance with the Declaration of Helsinki. Monocyte-derived macrophages and DC were differentiated as previously described (29, 35).

Briefly, purified CD14+ monocytes were seeded in 24-well plates (5 × 10⁶ cells/well) with RPMI 1640 supplemented with 10% FCS, human IL-4 (29, 35), human GM-CSF (Miltenyi Biotec) at 20 ng/mL, and human GM-CSF (Miltenyi Biotec) at 10 ng/mL. Cells were allowed to differentiate for 5–7 days. Fresh culture medium was added at day 3 of differentiation. For DC activation, cells were stimulated overnight with either LPS (from Escherichia coli O111:B4, Sigma-Aldrich) at 10 ng/mL, Pam3CSK4 (Synthetic triacylated lipoprotein, Invivogen) at 100 ng/mL, or PGE2 (Prostaglandin E2, kindly provided by Agnès Coste (PharmaDev, Toulouse)) at 5 µM, then harvested and used for the following assays. We also used ultra-pure LPS (from Escherichia coli O111:B4, Invivogen) and obtained similar results as those obtained with LPS from Sigma.

Flow Cytometry

Immature DC, mDC-LPS, mDC-Pam3CSK4, and iDC treated with PGE2 were harvested by gentle flushing with 1 mL of culture medium, centrifuged for 5 min at 340 g, incubated in staining buffer (PBS, 2 mM EDTA, 0.5% FBS) with a 1:100 dilution of Human TruStain FcX (Biolegend) for 5 min at room temperature. Cells were then stained in cold staining buffer for 25 min with fluorochrome-conjugated antibodies (APC-Cy7 labeled anti-HLA-DR (clone: L243, 1/400), PE labeled anti-CD80 (clone: 2D10, 1/200), PerCP-Cy5.5 labeled anti-CD86 (clone: IT2.2, 1/400), Pacific Blue labeled anti-CD11c (clone: 29E.2A3, 1/400), PerCP-Cy5.5 labeled anti-CCR5 (clone: J418J1, 1/400), and PE labeled anti-CD86 (clone: 29E.2A3, 1/400), PerCP-Cy5.5 labeled anti-CCR5 (clone: J418J1, 1/400), and PE labeled anti-CD86 (clone: G043H7, 1/400) from BD Biosciences) and the associated BD FACSDiva software. Data were then analyzed using the FlowJo 7.6.5 software (TreeStar).

3D Migration Assay

Fibrillar (2.15 or 4 mg/mL) and gelled (5.15 mg/mL) collagen I, and Matrigel were prepared as previously described (29). Matrices...
control in all experiments. DMSO at the concentration of PI mix was used as a
leupeptin (6 µM), and pepstatin (2 µM) (29). Y27632 was used
E64c (100 µM), GM6001 (5 µM), aprotinin (0.04 TIU/mL),
20 ng/mL. The mixture of protease inhibitors (PI mix) comprises
CCL21 (Immunotools) were added in the bottom chamber at
60
phalloidin as previously described (38) and imaged using the
with anti-Vinculin (Sigma-Aldrich) and Texas-Red-coupled
1–3 h and stimulated as indicated. DC were then fixed and stained
for analysis. DCs were classified as being "out of spheroids" when located in the first
line of nuclei, and "inside" when entering the first line of nuclei.

Fluorescence Microscopy
Glass coverslips were coated with fibronectin (10 µg/mL, Sigma
Aldrich) in PBS for 1 h at 37°C. Cells were seeded on fibronectin-
coated coverslips (3 × 106/well in 24-well plate), left to adhere for
1–3 h and stimulated as indicated. DC were then fixed and stained with anti-Vinculin (Sigma-Aldrich) and Texas-Red-coupled
phalloidin as previously described (38) and imaged using the
60 × 1.4 objective on an FV1000 confocal microscope (Olympus).
The number of cells displaying podosomes was counted using a
Leica DM-IRB fluorescence microscope on least 100 cells per
experimental conditions.

3D Podosome Staining and Imaging
At the end of migration experiments, matrices were fixed with
3.7% (w/v) paraformaldehyde (PFA) and 15 mM sucrose for
45 min at room temperature. PFA was quenched with 50 mM
NH4Cl for 5 min. Cells embedded in matrices were permeabi-
liized with PBS–Triton X-100 0.1% supplemented with 3% (w/v)
BSA to perform saturation at the same time for 1 h. Afterward,
cells were stained anti-vinculin (Sigma-Aldrich) and secondary
AlexaFluor 488–coupled secondary antibody, phalloidin-Texas
Red (Invitrogen) and DAPI (0.5 mg/mL; Sigma-Aldrich). Cells
were imaged using an Olympus/Andor CSU-X1 spinning disk
with a 60× objective.

Scanning Electron Microscopy
Scanning electron microscopy (SEM) observations were per-
formed as previously described (39, 40). Briefly, at the end of the
3D migration assay, cells and matrices were fixed in 2.5%
glutaraldehyde/3.7% PFA/0.1M sodium cacodylate (pH 7.4) and
dehydration in a series of increasing ethanol. Critical point was
dried using carbon dioxide in a Leica EMCPD300. After coating
with gold, cells were examined with an FEI Quanta FEG250 scan-
nning electron microscope.

Tumor Cells and Spheroid Culture
Spheroids were generated as previously described (36). Briefly,
24-well tissue culture plates were coated with 500 µL of 2% agar
per well. The human breast cancer cell line SUM159PT (106
cells/20 µL) was plated in the lid of tissue culture plates. After
7 days, each spheroid was transferred into wells with 500 µL
culture medium. Preliminary studies have established that after
20–24 days of culture, spheroids reached a diameter of ~400 µm.
DC staining was performed using the cell-live permeant probe
CellTracker Red CMPTX (Molecular Probes, Invitrogen) at
0.5 µM in PBS, as described by the manufacturer. DC at day 7
of differentiation were distributed (104 cells) into agar-coated
wells containing a single spheroid and co-incubated for 3 days.
Formalin-fixed spheroids stained with DAPI were imaged in
chambers (CoverWell PCI-1.0; Grace Bio-Labs, Bend, OR) using a
Zeiss LSM710 microscope (10× objective, NA 0.3, voxel size
1.3 µm × 1.3 µm × 5.5 µm) with a multiphoton source at 715 nm
(coherent Chameleon) for z-stack acquisition of DAPI and
CellTracker fluorescence. With the cell counter plugin of ImageJ
software (National Institutes of Health, Bethesda, MD, USA),
CellTracker-stained DC associated to spheroids were counted.
DC were classified “out of spheroids” when located in the first
line of nuclei, and “inside” when entering the first line of nuclei.
At least three spheroids per condition were used.

Statistics
A Wilcoxon matched-paired signed rank test was used for statisti-
cal analyses performed using GraphPad Prism 6.0 (GraphPad
Software Inc.). The P < 0.05 was considered significant.

RESULTS
Immature DC Adopt Either the Amoeboid
or the Mesenchymal Migration Mode, and
Form Podosomes Depending on the
Matrix Architecture
To study the 3D migration ability of human monocyte-derived
DC, we used different matrices presenting distinct architectures
that were polymerized in transwells as thick layers (>1 mm) (29).
Fibrillar collagen I is a porous matrix used to mimick classical stro-
mal/interstitial ECM, and Matrigel is a dense matrix composed
of a mixture of ECM proteins particularly rich in laminin and
collagen IV (Figure 1A) (41). Since Matrigel and fibrillar collagen
I have distinct biochemical compositions, we also used collagen
I polymerized as a dense gel, called gelled collagen I, which dis-
plays a dense architecture and viscoelasticity parameters similar
to Matrigel (Figure 1A). As shown in Figure 1B, iDC migrated in
fibrillar collagen I, Matrigel and, to a lower extent, in gelled colla-
gen I. iDC, imaged inside gelled collagen I and Matrigel, displayed
an elongated cell shape with long protrusions (Videos S1 and S2
in Supplementary Material; Figure 1C). In fibrillar collagen I,
iDC displayed a round cell shape (Video S1 in Supplementary
Figure 1

A. Fibrillar Collagen I, Gelled Collagen I, Matrigel

B. % of migrating IDC and Mean Migration Distance (µm) over time (24h, 48h, 72h).

C. Images of cells on Fibrillar Collagen I, Gelled Collagen I, Matrigel, showing number of protrusions per cell.

D. F-actin, Vinculin, DAPI staining of cells on Collagen I.

E. % of IDC migrating in Fibrillar Collagen I, Gelled Collagen I, Matrigel.

F. % of IDC migrating in DMSO, Pnimix, Y27632.

G. % of IDC migrating in Gelled Collagen I.

H. % of IDC migrating in Matrigel.

**FIGURE 1** | Continued
we decided to compare the 3D migration capacity of these two cell types that form podosomes [Figure S2A in Supplementary Material (23, 27, 29)]. Macrophages and iDC were differentiated from monocytes isolated from the same donors. The percentage of macrophages and iDC migrating in fibrillar collagen I was similar, but macrophages covered a longer distance than DC at 24 h (Figure S2B in Supplementary Material). In Matrigel, iDC displayed a higher capacity to infiltrate the matrix compared to macrophages (percentage of migrating cells and migration distance) during the first 24 h; however, both cell types migrated equally after 3 days (Figure S2C in Supplementary Material). Therefore, macrophages and iDC display similar ability to migrate in both types of matrix.

**Podosomes, Rather Than the Maturation Status, Determine the Ability of DC to Perform Mesenchymal 3D Migration**

Next, we investigated the influence of DC maturation on the 3D migration capacity. iDC were stimulated by LPS (TLR4 agonist) or Pam3CSK4 (TLR2 lipopeptide agonist) to generate mDC-LPS or mDC-Pam3CSK4. LPS- and Pam3CSK4-induced DC maturation was confirmed by the up-regulation of maturation markers at the cell surface, such as HLA-DR, CD80, CD86 and PD-L1 (Figures S3A,B in Supplementary Material). On top of porous fibrillar collagen I, both iDC, mDC–LPS and mDC–Pam3CSK4 exhibited a round cell shape with large membrane ruffles and blebs, as shown by SEM (Figure 2A). They efficiently infiltrated the matrix and covered a similar migration distance (Figure 2C). SEM pictures of Matrigel revealed that iDC and mDC–Pam3CSK4 remodeled the matrix by forming infiltrating holes, while mDC–LPS failed to do so (Figure 2B). In line with these observations, unlike mDC–LPS that did not infiltrate Matrigel or gelled collagen I, iDC and mDC–Pam3CSK4 infiltrated dense matrices and covered a similar migration distance (Figure 2C). Similar to iDC, mDC–Pam3CSK4 displayed a round cell shape in fibrillar collagen I and an elongated cell shape in dense matrices (Video S3 in Supplementary Material).

Of note, we observed that LPS treatment triggered DC maturation along with podosome dissolution (32–34). By contrast, podosomes were maintained when DC maturation was induced by Pam3CSK4 (34). These previous observations were confirmed in Figures 2D,E. iDC and mDC–Pam3CSK4 formed 3D podosomes in gelled collagen I (Figure 2F) and membrane protrusions in dense matrices (Figure 2G). Altogether, these results suggest that the ability of DC to migrate in dense matrices is independent of their maturation status, but it relies on the capacity to form podosomes.
FIGURE 2 | Continued
To further characterize the role of podosomes in DC mesenchymal migration, we looked for a way to trigger podosome dissolution without inducing DC maturation. To address this question, PGE$_2$ was used as a potent inducer of podosome dissolution without inducing DC maturation. We report the following novel findings:

1. Both iDC and mDC use the amoeboid mode to migrate in 3D environments, independent of their maturation status. PGE$_2$ did not induce DC maturation, as the cell surface expression of HLA-DR, CD80, and CD86 remained unchanged compared to iDC (Figure S3B in Supplementary Material). We confirmed its capacity to disrupt podosomes and migrate in Matrigel were impaired without affecting amoeboid migration in fibrillar collagen I (Figures 2J, K).

Collectively, these results indicate that the presence of podosomes, rather than the maturation status of DC, determines the ability to migrate in dense 3D environments.

**CC-Chemokines Regulate 3D Migration of DC**

Since mDC-LPS migration in Matrigel is impaired, we next investigated whether it could be restored by chemokines. Upon TLR4 activation by LPS, the expression pattern of CC-chemokine receptors was modified with decreased expression of CCR5 and enhanced expression of CCR7 (Figure S4A in Supplementary Material), as previously described (8). Consequently, we used CCL5 or a combination of CCL19 and CCL21, as ligands for CCR5 and CCR7, respectively. In fibrillar collagen I, the percentage of migrating cells and the distance covered by iDC and mDC-LPS were enhanced by CCL5 and the combination of CCL19 and CCL21, respectively (Figure 3A). Under the influence of chemokines, both iDC and mDC-LPS exhibited the characteristic amoeboid round cell shape in fibrillar collagen I (Figure 3B), and their migration capacity was inhibited by Y27632 (Figures S4E, F in Supplementary Material). In Matrigel, however, CCL5 strongly increased the 3D migration and the distance covered by iDC, while CCL19 and CCL21 had no effect on these cells (Figure 3C). CCL5 also enhanced iDC migration in gelled collagen I (Figure S4B in Supplementary Material), and the percentage of migrating iDC under the influence of CCL5 was inhibited by Plmix in Matrigel and gelled collagen I (Figures S4G–H in Supplementary Material). Importantly, none of these cytokines triggered mDC-LPS migration in Matrigel. Under the influence of CCL5, iDC displayed an elongated cell shape in Matrigel while mDC-LPS remained on top of the matrix (Figure 3D). Finally, we did not observe any influence of these chemokines on the capacity of iDC and mDC-LPS to degrade the matrix (Figures S4C, D in Supplementary Material).

Due to the switch in CC-chemokine receptor expression pattern, we observed that the amoeboid migration of iDC and mDC-LPS is, as expected, influenced by CCR5 and CCR7 ligands, respectively. While the mesenchymal migration of iDC is influenced by CCL5, CCL19 and CCL21 failed to trigger mDC-LPS infiltration in Matrigel. We infer that this is likely due to the dissolution of podosomes induced by LPS resulting in mDC-LPS inability to degrade the matrix.

**Immature DC Use Both Migration Modes in Tumor Cell Spheroids**

To examine the migration of iDC in a tissue-like 3D environment, we used spheroids of the breast carcinoma cell line SUM159PT, which secrete several ECM proteins including fibronectin, laminin, and collagen IV (36). DC were stained with CellTracker and co-cultured with spheroids in the presence of DMSO (vehicle), the pan-matrix metalloprotease inhibitor GM6001 (a component of Plmix), or Y27632. After 3 days of co-culture, iDC infiltrated in spheroids were visualized using multiphoton microscopy (Figure 4A) and quantified. As shown in Figure 4B, iDC efficiently infiltrated tumor spheroids and both GM6001 and Y27632 significantly decreased the percentage of iDC inside spheroids (Figure 4B). Therefore, iDC are able to use both the mesenchymal and amoeboid modes in a complex tissue-like environment.

**DISCUSSION**

This study extends our knowledge on the migration ability of human DC in 3D environments and its modulation during TLR-induced DC maturation. We report the following novel findings: (1) both iDC and mDC use the amoeboid mode to migrate in porous 3D collagen I; (2) only DC forming podosomes migrate in dense environments, independent of their maturation status or the presence of chemokines; and (3) iDC use both the mesenchymal and amoeboid migration modes to infiltrate tumor cell spheroids.
Our first attempt was to investigate the migration capacity of iDC and the influence of TLR-induced maturation in 3D environments using different matrices with distinct architectures (29). We observed that, independent of their maturation status, DC adopt the amoeboid mode to migrate in porous collagen I as characterized by ROCK dependency and round cell shape. Hence, both iDC and mDC behave like other leukocytes, namely monocytes, T lymphocytes, macrophages, and neutrophils (14, 18, 29, 38, 45) with the 3D amoeboid mode as a common feature to migrate in porous 3D environments.

We demonstrate that, independent of their maturation status, DC migrate in dense matrices if they form podosomes as examined in 2D and 3D settings. Both the TLR4 and TLR2 agonists, LPS and Pam3CSK4, respectively, induce DC maturation. However, although LPS triggers podosome dissolution and loss of the mesenchymal migration capacity, Pam3CSK4 maintains podosomes and mesenchymal migration in dense matrices. These results suggest that in the context of infectious diseases, activation of distinct TLR triggers DC maturation with distinct impact on podosome formation and matrix degradation capacity. Using PGE2, which is synthesized downstream of
LPS stimulation and mediates podosome dissolution (44, 46), we showed that it maintained DC in an immature state and abolished migration in Matrigel concomitantly to podosome disruption. In contrast to the TLR4 signaling pathway, PGE2 is not produced upon TLR2 stimulation (47), and thus podosomes are maintained. Due to the unique ability of DC to dissolve their podosomes, this study further supports the critical role of these cell structures in 3D migration in dense environments. We also report that when DC form podosomes in 2D, they form 3D podosomes during migration in dense 3D environments, as previously described in macrophages (29, 30, 36). Altogether, these data provide evidence that when DC form podosomes, they have the dual 3D migration ability, using the amoeboid mode in a porous matrix and the mesenchymal mode in a dense environment.

Among leukocytes, only macrophages and osteoclast precursors perform mesenchymal migration, which is functionally linked to their capacity to form podosomes (26, 29, 31, 37, 38, 42, 48). Macrophages that form podosomes (in 2D and 3D environments) are able to degrade, ingest, and compact the matrix to form tunnels and create paths allowing migration into dense matrix (26, 30). Interestingly, gene deletion or knockdown of podosome effectors such as Hck, WASp, or Filamin A translate in reduced podosome stability, ECM proteolysis and mesenchymal migration without any effect on amoeboid migration (39, 49, 50). Conversely, the HIV-1 protein Nef, which stabilizes podosomes and increases their proteolytic activity, enhances the mesenchymal migration of human macrophages (37). Thus, a pathogen able to modify podosome formation and function alters the 3D migration of its host cell in dense matrices (37). A few other studies described the influence of pathogens on podosomes and the consequences on DC migration mainly studied in 2D. In 2D, the influence of podosomes on cell migration is likely related to their adhesion property rather than to their proteolytic activity. Gram-positive versus Gram-negative bacteria have distinct effects on podosomes. Gram-negative bacteria, such as Neisseria meningitidis or Salmonella enteritidis, induce podosome dissolution in DC associated with enhanced migration on 2D surface in a LPS- and TLR4-dependent manner (34). Gram-positive bacteria, such as Staphylococcus aureus or Streptococcus pneumoniae, maintain podosomes and do not influence 2D migration of DC (34). Infection of human DC with the parasite Toxoplasma gondii induces a rapid dissolution of podosomes (51, 52) and enhanced amoeboid 3D migration in fibrillar collagen I (53). Finally, Helicobacter pylori is able to induce podosome formation in cells devoid of podosomes. Hepatocytes infected with H. pylori form podosomes, degrade the matrix, and exhibit diminished 2D cell migration (54). Altogether, these data show that several pathogens target podosomes with potential consequences on cell adhesion, matrix degradation, and cell migration, likely influencing tissue immunopathology and activation of the adaptive immune response.

Although podosomes in macrophages and DC are involved in mesenchymal migration, they exhibit distinct behaviors in response to protease inhibitors. The presence of protease inhibitors in the culture medium disrupted podosomes in DC, as previously observed with a cathepsin B inhibitor (43), but they exhibited no effect on podosomes in macrophages (29). Thus, proteases in macrophages and DC are involved in the proteolytic activity of podosomes toward the ECM and regulate podosome dynamics only in DC. Additional comparative experiments are required to further characterize specific properties of podosomes between macrophages and DC.

Chemokines, together with the switch in CC–chemokine receptor expression operating during DC maturation, tightly regulate DC migration (2). Here, we observed that chemokines enhanced the 3D migration capacities of DC and the migration distance in both dense and porous matrices, but none of them modulated their matrix degradation capacities. In addition, when DC are devoid of podosomes, chemokines are unable to trigger mesenchymal migration. Thus, chemokine receptors do not directly govern the 3D migration mode used by DC, but they facilitate their intrinsic migratory capacities. In response to CCL19, the small Rho-GTPase Cdc42 is essential for an efficient unpolar and directional migration of mDC-LPS both in vivo and in vitro in a 3D collagen matrix (16), likely explaining the increased distance covered by DC in matrices. Moreover, PGE2 induced during TLR4 mediated maturation of DC plays a critical role in CCR7 expression and signaling for an efficient migration of mDC-LPS toward CCL19 and CCL21 (55–57). Our results are in line with these previous studies since CCR7 expression is up-regulated and the amoeboid migration of mDC-LPS is enhanced in response to CCL19 and CCL21, probably facilitating the amoeboid migration to lymph nodes (1). The dual capacity of CCL5 to enhance both amoeboid and mesenchymal migration in iDC suggests that activation of CCR5 might support cell migration in all types of matrix architecture. Whether common or distinct molecular mechanisms are involved downstream of CCR5 activation to stimulate both migration modes remains to be determined.

The role of proteases in DC migration has been already reported showing that the Matrix Metallo-Proteases MT1-MMP and MMP9 regulate CCL5-induced iDC migration (58, 59). Interestingly, MT1-MMP localizes on podosome protrusions in iDC where it mediates ECM degradation (60–63). Moreover, MMP9 and MMP2 activities are also involved in Langerhans and dermal DC emigration from ex vivo murine and human epidermis (64). However, in these studies, it was not investigated whether cells form podosomes and exhibit the characteristics of mesenchymal motility. In tumor cell spheroids, we found that the pan-MMP inhibitor reduced cell infiltration, indicating that MMPs are involved in mesenchymal migration used by DC to infiltrate a tissue-like environment.

In vivo, existence of podosomes in myeloid cells and their role in 3D migration have not been formally demonstrated, but correlations have been provided between the capacity of cells to form podosomes in 2D and their migration capacity in vivo. In the past, we showed that deficiency of the tyrosine kinase Hck in macrophages reduces podosome stability and mesenchymal migration in vitro, which correlated with impaired migration of macrophages in the peritoneal cavity during inflammation (39, 49). Conversely, the HIV-1 protein Nef, which increases the podosome stability and mesenchymal migration in vitro,
enhances the recruitment of macrophages in tumors in Nfat transgenic mice (37). Here, we showed that iDC infiltration in tumor cell spheroids was in part dependent on MMP activity, suggesting that DC might use proteases, probably through podosome formation, to migrate in tumors. Interestingly, abolition of tumor cell-secreted PGE₂ enhanced conventional DC1 infiltration in tumors, and this was associated with tumor rejection (65). Although the impact of PGE₂ on the actin cytoskeleton was not addressed, we hypothesize that tumor-derived PGE₂ by partly disrupting podosomes, may prevent protease-dependent DC accumulation and immune-dependent tumor rejection. In vivo, conventional DC subsets (cDC1 and cDC2) and monocyte-derived DC have a different ontogeny (66) and functions (2, 67, 68). Whether these DC subsets share the capacity to form podosomes and migrate in 3D in a protease-dependent manner remains to be explored.

In conclusion, DC adapt their migration mode to the matrix architecture: in a matrix with large pores, they use the amoeboid mode; in a matrix with a low porosity, they use the mesenchymal mode. Interestingly, we demonstrate that mesenchymal migration relies on the capacity to form podosomes and not on the DC maturation status. It is likely that differential regulation of podosome maintenance or dissolution during DC activation has consequences on DC migration in tissues and immune responses during infections and cancer.

ETHICS STATEMENT

For this report, written informed consents were obtained from all the donors under EFS contract n&176:21/PLER/TOU/IPBS01/2013-0042. According to articles L1243-4 and R1243-61 of the French Public Health Code, the contract was approved by the French Ministry of Science and Technology (agreement number AC 2009-921). All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

CC and IMP designed the study; EM, GLV, RP, and ON provided reagents and expertise; CC, CL, RG, RM, GLV, and RP performed experiments; CC and IMP wrote the manuscript, all authors provided a final approval of the version to be published.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fimmu.2018.00846/full#supplementary-material.

FIGURE S1 | (A) iDC were left untreated or treated with PImix or Y27632 for 16 h, and stained with phallicdin Texas-Red to detect F-actin (red) revealing podosomes, and DAPI to stain nuclei (blue). The percentage of cells forming podosomes was quantified. *p < 0.05. (C) iDC seeded on gelatin-FITC-coated glass coverslips were left untreated or treated with PImix or Y27632 for 16 h. Cells were stained with phallicdin Texas-Red to detect F-actin (red) and DAPI to stain nuclei (blue). Dark areas correspond to gelatin-FITC degradation. (D) The percentage gelatin-FITC matrix degradation was quantified. Results are expressed as mean ± SEM of at least three independent experiments. **p < 0.01 (related to Figure 1).

FIGURE S2 | (A) Macrophages and iDC were stained with an anti-vinculin Ab (green), phallicdin Texas-Red to detect F-actin (red) revealing podosomes, and DAPI to stain nuclei (blue). (B) The percentage of migrating cells and the mean migration distance of macrophages and iDC migrating in fibrillar collagen I were measured. Results are expressed as mean ± SEM of three independent experiments. (C) The percentage of migrating cells and the mean migration distance of macrophages and iDC in Matrigel were measured. Results are expressed as mean ± SEM of three independent experiments. *p < 0.01; **p < 0.001 compared to iDC condition (related to Figure 1).

FIGURE S3 | (A) Histograms showing the MFI of cell-surface maturation markers in iDC and mDC-Pam3CSK4. Results are expressed as mean ± SEM of 10 independent experiments. *p < 0.05; **p < 0.01; ***p < 0.001 compared to iDC condition. (B) Histograms showing the MFI of cell-surface maturation markers in iDC, mDC-LPS, and iDC+PGE₂. Results are expressed as mean ± SEM of 10 independent experiments. *p < 0.01; **p < 0.001 compared to iDC condition (related to Figure 2).

FIGURE S4 | (A) Histograms showing the MFI of cell-surface CCR5 or CCR7 in iDC and mDC-LPS. Results are expressed as mean ± SEM of 10 independent experiments. *p < 0.01; **p < 0.001 compared to iDC condition. (B) The percentage of iDC migrating in gelled collagen I was monitored after 24 h when none (white) or CCL5 (grey) was added in the lower chamber as chemoattractant. Results are expressed as mean ± SEM of three independent experiments. *p < 0.01 compared to none condition. (C) Quantification of gelatin-FITC degradation by iDC and mDC-LPS left untreated or treated with either CCL5 or CCL19 and CCL21 for 16 h. (D) iDC and mDC-LPS seeded on gelatin-FITC-coated glass coverslips were left untreated or stimulated with either CCL5 or CCL19 and CCL21 for 16 h. Cells were stained with phallicdin Texas-Red to detect F-actin (red) and DAPI to stain nuclei (blue). Dark areas correspond to gelatin-FITC degradation. (E) The percentage of iDC migrating in fibrillar collagen I was measured when none (white) or CCL5 (grey) was added in the lower chamber as chemoattractant, in control or drug-treated cells (PImix or Y27632). Results are expressed as mean ± SEM of at least three independent experiments. *p < 0.05 compared to DMSO condition. (F) The percentage of mDC-LPS migrating in fibrillar collagen I was measured when none (white) or CCL5 (grey) was added in the lower chamber as chemoattractant, in control or drug-treated cells (PImix or Y27632). Results are expressed as mean ± SEM of at least three independent experiments. *p < 0.05 compared to DMSO condition. (G) The percentage of iDC migrating in Matrigel was measured when none (white) or CCL5 (grey) was added in the lower chamber as chemoattractant, in control or drug-treated cells (PImix or Y27632). Results are expressed as mean ± SEM of at least three independent experiments. *p < 0.05; **p < 0.01 compared to DMSO condition. (H) The percentage of iDC migrating in gelled collagen I was measured when none (white) or CCL5 (grey) was added in the lower chamber as chemoattractant, in control or drug-treated cells (PImix or Y27632). Results are expressed as mean ± SEM of at least three independent experiments (related to Figure 3).

VIDEO S1 | Immature dendritic cell migrating in 3D matrices. iDC were seeded in transwells filled with fibrillar collagen I, gelled collagen I or Matrigel. z-series of
images were acquired after 24 h of migration at the surface of the matrices and at several depth (until 660 μm, 30 μm intervals) into the matrix.

**VIDEO S2** | Immature dendritic cells migrating in Matrigel. Time-lapse every 10 min during 13.5 h, using the 10× objective of an inverted video microscope.

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