FORMINS: THE MULTITASKING ACTIN NUCLEATORS IN DENDRITIC CELL BIOLOGY, A LITERATURE REVIEW

FORMINAS: AS NUCLEADORAS DE ACTINA MULTITAREFA NA BIOLOGIA DAS CÉLULAS DENDRÍTICAS, UMA REVISÃO DA LITERATURA

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ABSTRACT

Dendritic cells (DCs) are at the frontline of the immune defense. They are professional antigen-presenting cells capable of linking adaptive and innate Immune responses by interacting with T cells and by secreting cytokines. By performing such activities, they play an ambiguous role, either engaging the adequate immune response or initiating diseases, and thus, they pose as important therapeutic targets for immunotherapies. Such potential is even more significant if we consider the dynamics of the actin cytoskeleton remodeling to perform such roles. The Formin family of actin nucleators performs one of the three actin-regulatory networks. Formins play essential roles in virtually all eukaryotic cells, including DCs. In this way, we reviewed the most prominent functions of formins in DC biology. The most prominent formins playing roles were members of the Diaphanous and FHOS/FHOD subtypes, acting from pathogen recognition and uptaking, T-cell activation, formation of viral synapses/filopodia, migration stability to adhesion and could be considered as biomarkers of inactive and activated states of DCs. These important roles in DCs leave important open questions yet to be answered.

KEYWORD: Dendritic cell, Formins, Cellular Biology, Immunotherapy, Actin cytoskeleton.

RESUMO

As células dendríticas (DCs) estão na linha de frente da defesa imunológica. São células apresentadoras de antígenos profissionais, capazes de conectar respostas imunes adaptativas e inatas, interagindo com células T e secretando citocinas. Ao realizar tais atividades, elas desempenham um papel ambíguo, seja engajando a resposta imune adequada ou iniciando doenças, e portanto, representam alvos terapêuticos importantes para imunoterapias. Esse potencial é ainda mais significativo se considerarmos a dinâmica da remodelação do citoesqueleto de actina para desempenhar tais papéis. A família de Forminas de nucleadores de actina desempenha uma das três redes reguladoras de actina. As forminas possuem papéis essenciais em praticamente todas as células eucarióticas, incluindo as DCs. Dessa forma, revisamos as funções mais proeminentes das forminas na biologia das

DCs. As forminas mais proeminentes que desempenharam papéis são membros dos subtipos Diáfano e FHOS/FHOD, atuando desde o reconhecimento e captação de patógenos, ativação de células T, formação de sinapses/filopódios virais, estabilidade da migração até adesão, podendo ser consideradas como biomarcadores dos estados inativo e ativado das DCs. Esses papéis importantes nas DCs deixam importantes questões em aberto ainda sem resposta.
PALAVRAS-CHAVE: Célula dendrítica, Forminas, Biologia celular, Imunoterapia, Citoesqueleto de actina.

INTRODUCTION

Dendritic cells (DCs) are highly specialized antigen-presenting cells that connect adaptive and innate immune responses. They activate T cells and induce adequate immune responses to a wide variety of diseases, including cancer. These functions could be considered the “bright side” of DCs, however, in the past few years, it has been shown that DCs can contribute to the initiation and progression of diseases, revealing its “dark side"[1]. This complex Dendritic cell biology has an enormous therapeutic potential, especially in the field of immunotherapy and cancer vaccines. If we take into account that such a variety of functions involve changes in cell shape, motility and phagocytic properties, the cytoskeleton becomes a trending topic to be exploited, as those functions are fundamental in the process of migration, maturation and differentiation of this cell type, which moves through tissues to reach lymph nodes and increase their capacity for antigen presentation and activation of lymphocytes.

Understanding the contribution of the dynamically organized actin cytoskeleton in dendritic cell (DC) biology and its potential therapeutic applications highlights the roles of actin nucleator networks in DCs, thus, formins are candidates to be worth studying, in the context of Dendritic cell functions. Formins are a wide family of proteins and one of the three actin nucleator complexes. In the next sections we brought the general biology of dendritic cells and formins and also, by a literature review, we gathered information about which formins are known to play a role in Dendritic cells.

BIOLOGY OF DENDRITIC CELLS

Dendritic cells are immune cells that capture, process, and present antigens to T lymphocytes, crucial for activating adaptive immunity[2]. Dendritic cells are classified into two types: conventional (cDC) and plasmacytoid (pDC)[3]. Plasmacytoid dendritic cells (pDC) assist the immune system by secreting cytokines, especially type I interferon, which helps modulate the immune response, particularly against viral infections[4]. Conventional dendritic cells (cDC), on the other hand, are known as professional antigen-presenting cells. cDCs can capture an antigen and degrade it into several peptides. After this process, one of these processed peptides is exposed in the MHC groove. The set of antigenic peptide plus the MHC molecule on the DC membrane is essential for interaction with the TCR located on the T lymphocyte membrane in lymphoid organs, thereby activating the acquired immune response.[5]. cDCs then bridge the innate and adaptive immune responses[6]. cDCs can expose degraded peptides in MHC grooves, MHC I and MHC II[7]. It is known that the activation of T lymphocytes begins in lymphoid organs when a cDC presents the antigen via MHC-TCR interaction. This interaction triggers a second signal inducing the production of IL-2, a cytokine that induces cell proliferation. After activation of the T lymphocyte, a third signal occurs, which is based on the interaction of the so-called costimulatory molecules of the cDC and T lymphocytes[8]. The main cDC costimulatory molecules are B7.1 (CD80), B7.2 (CD86), CTLA4, and CD40; in T lymphocytes, CD28 and CD40L are the most prominent ones. The clustering between these molecules occurs as follows: CD80/CD86 binds to CD28, stimulating cytokine production or increasing T lymphocyte cytotoxicity[9]. CTLA-4 binds to CD28, inducing immunosuppression. CD40 binds to CD40L, inducing higher expression of costimulatory molecules, thereby increasing the production of cytokines and the cytotoxic activity of T lymphocytes[8].

Another evidence of cDCs' professional antigen-presenting function is their ability to perform cross-presentation. Cross-presentation consists of the same antigen-presenting cell presenting antigens to helper T lymphocytes (CD4+) and

cytotoxic T lymphocytes (CD8+) simultaneously, which occurs in lymph nodes, and is fundamental in antitumor immunity[5]. Due to their unique characteristics, dendritic cells have been extensively utilized in diverse immunotherapies to stimulate the innate immune response against antigenic threats[8].

FORMINS: FAR BEYOND CYTOSKELETON REMODELING

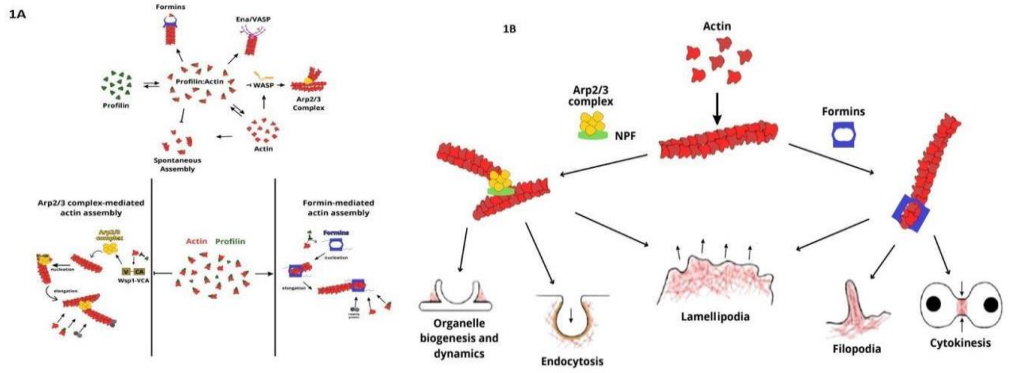
Actin, in its different arrangements (Figure 1), plays many roles in eukaryotic cells. It ranges from cell shaping, motility, signal transduction, cell contraction, and even within the nucleus[11,12,13]. A substantial number of accessory proteins, known as nucleator proteins, regulate actin dynamics to facilitate these functions effectively. Currently, there are three major actin nucleator networks, the Arp2/3 complex, the Rho/formins-mediated and also the Ena/VASP proteins, each of them mediating actin nucleation by distinct mechanisms, yielding the capability to build different larger actin arrangements, to perform other tasks within cells and also mediating external interactions and signal transduction. In addition, the WASP (WH2 domain-containing nucleators) can interact and modulate the Arp2/3 network (Figure 1B).

The Arp2/3 complex can polymerize branched actin filaments that sustain interactions at the branching point. Formins can build long, straight actin filaments and remain bound at the growing end. Ena/VASP proteins present the EVH1 and EVH2 signature domains and a proline-rich central region. These nucleators play a role in fibroblast migration, axon guidance, and T cell polarization and are important for the actin-based motility of the intracellular pathogen *Listeria monocytogenes*. They associate with barbed ends of actin filaments to avoid filament capping; and can reduce the Arp2/3-dependent actin filament branching by binding to Profilin, as well as they are modulated by the PKA/PKG serine/threonine kinases[14].

Also, The Wiskott-Aldrich syndrome protein (WASP) family includes several members, WASP, N-WASP, WAVE, WASH, WHAMM, JMY, and WHIMP, which are ubiquitous regulators of actin dynamics by interactions via the

signature domain WH2which allows the interaction with Arp2/3 complex (Figure 1A). Despite these three networks' pivotal roles, we will focus on the biology of metazoan formins for this review.

Figure 1. The actin nucleators: 1A. The three major actin nucleator networks. 1B. How Formins and ARP2/3 complex assemble actin filaments in different structures.

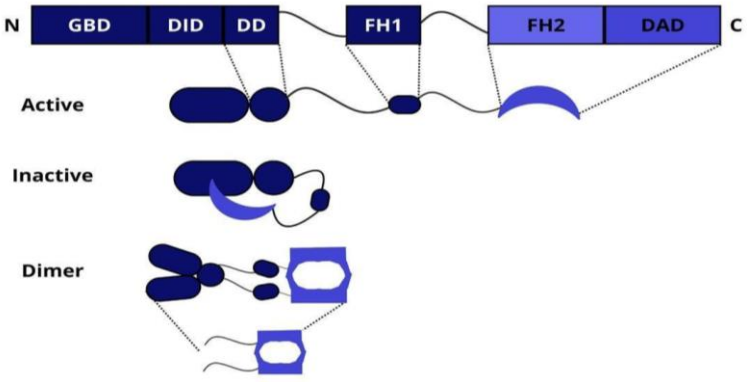


Formins were named after the limb deformities seen in mutant mice at the limb deformity locus, which affects the *Fmn1* and the *gremlin* genes. The next formin to be discovered was *diaphanous* (Dia), one of the most represented formins playing roles in DCs, as shown in this review. After that, several actin regulatory proteins resembling formins were found from yeast to mammals, and nowadays, the formin superfamily, has nine subtypes: Dia/DIAPH (Diaphanous); Daam (Disheveled-associated activators of morphogenesis); FMNL/FRL (Formin-related proteins identified in leukocytes); INF (inverted formins); FHOD (Formin homology domain containing proteins); GRID2IP (Delphilin); FMN (the founding family of 'formins'); MWHF (Multiple wing hairs formins) and PHCF (PH-domain-containing formins)[15].

Only the two last ones do not have known human isoforms, accounting for fifteen human known genes encoding formins. Considering alternative splicing and variant isoforms, the number becomes more significant. The phylogenetic signature of a formin is the presence of FH1 and FH2 domains (Formin homology domains), being the FH2 domain the one accountable for actin nucleation and the other ones for the regulation and interactions with other proteins as depicted on Figure 2.

Due to its molecular peculiarities, different subtypes of formins perform multiple roles within a cell. Besides their wide range of functions, formins are also related to diseases, as nicely reviewed by Valencia & Quinlan, highlighting the biological relevance and characteristics of metazoan formins[16].

Figure 2. The molecular organization of an active formin and its domains (on top). If inactive, the diaphanous inhibitory domain (DID) and the diaphanous auto-regulatory domain (DAD) are bound. A dimer modulates the actin nucleation. FH2 domains bind to actin monomers to initiate filament assembly. While other domains are regulatory.



Kinetically speaking, actin polymerization is an unfavorable process because actin is generally associated to proteins, such as profilin, limiting its availability (Figure 1); besides that, actin monomers "naturally" bind to each other in a very low speed (spontaneous nucleation). Thus, for the cell to rapidly and dynamically build different actin arrangements "on demand" it is necessary to break this unfavorable state by interacting with nucleators such as formins (Figure 1 and 2). Formins were shown to bind and stay bound to the "barbed ends" of a growing actin filament, which is capable of polymerizing at a rate ten times faster than the other end, called the "pointed end".

Thus, they increase the polymerization speed and compete with capping proteins to regulate the growing length and number of filaments (Figure 1). The time that a formin remains bound to the filament is an important feature called "processivity," which enormously helps the actin dynamics under different circumstances, coordinating the formation of many noticeably short linear filaments, a few long ones, and everything in between.

This refined molecular structure and function of formins (Figure 1 and 2) require the modulation and interaction with other proteins through its domains, allowing the correct space and time of actin polymerization. The well-known small GTPase Rho binds to GBD domains in mDia1 formins. Rho-associated kinase ROCK also controls specific phosphorylation and function of different formins, mainly in the FHOD subtype of formins. It was also proposed that formins can select specific isoforms of actin (six actin isoforms in humans) to different cell types and functions of the actin cytoskeleton. This is the case of Delphilin which selects specific actin isoforms in Purkinje cells.

Other activities of formins beyond cytoskeleton remodeling, are known, such as the formin INF2, which plays a role in “severing” filaments besides elongating them. Thus, INF2 contributes to the formation of larger and complex actin structures. Formins also bind to microtubule binding proteins, connecting the actin and tubulin cytoskeletons. Specific formins can exhibit additional conserved domains such as PDZ, PH and WH2 domains, arguing for more specificity of partners in the actin regulation, nucleation dynamics, and spatial distribution.

Based on the biological significance of DCs and formins depicted above, in this narrative review, we gathered the most significant data on the functions of formins in Dendritic cells, aiming to understand how these proteins participate in the ability of DCs to perform their wide array of tasks in acquired immune responses, or activation state (“resting” to “mature”) as well as in the uptaking of pathogens and antigenic presentation. Besides, to explore how formins could contribute to their therapeutical use as anti-tumoral vaccines, in migration ability and eventually playing novel roles, such as biomarkers or biosensors. To do so, we performed a series of searches in the NLM database using the PubMed search tool (https://pubmed.ncbi.nlm.nih.gov/).

The queries combined "Dendritic Cells" with keywords: “formin”; “Diaphanous related formins”; “mDia”; “DIAPH1”; “DIAPH2” using “AND” as a Boolean term. We have applied the “Free Full Text” filter only, without temporal restriction, to allow the temporal understanding of the known roles of formins in

Dendritic cells. As a note, due to the large number of metazoan formins, its subtypes, isoforms, and aliases, in this review, we have used the name and "acronym" of formins present in the selected articles. Eventually, after the critical reading of the articles, when necessary, we performed additional searches with specific purposes to contribute to the final text. Duplicates and non-English, Spanish, or Portuguese articles were excluded. The following section discusses the major aspects of Formins in the biology of DCs.

FORMINS BELONG TO THE "TOOL KIT" OF DENDRITIC CELLS

The articles chosen for discussion are summarized in TABLES 1 and 2. The immunoglobulin-like lectin receptor CD169 (Siglec-1) mediates the capture of HIV-1 by activated dendritic cells (DCs). In activated DCs, following the challenge with VLPs (HIV-viral like particles), there is a nanoclustering of Siglec-1, at specific sites in the plasma membrane, even though there is no direct interaction of Siglec-1 with the cytoskeleton[17]. Such constraint results in a better capture of the virus if compared to inactive (or resting) DCs. This "constraint" of the receptor at specific membrane sites was shown to be mediated by Rho-ROCK activation and a formin-dependent actin polymerization. A confinement and enrichment of Siglec-1 and actin filaments distribution near cell edges is observed, suggesting that the Siglec-1 nanoclustering could be actin-regulated, what was reinforced by the fact that Cytochalasin D, which inhibits actin polymerization, treatment is capable to disrupted this pattern, dispersing Siglec-1 throughout the cytoplasm. Additionally, it was revealed that formin inhibition by SMIFH2 disrupted the spatial distribution Siglec-1 near cell edges, becoming dispersed as its density decreased. The studies also revealed, by a semi 3D environment, that Siglec-1 was constrained and colocalized to actin in the cell rear (in a formin-dependent trailing edge actin arrangement) instead of the leading edge of lamellipodium of mature DCs activated by the CCL19 chemokine (Arp2/3-dependent actin polymerization-dependent). Taken together, those data reveal that a formin-dependent cortical actin distribution modulates the nanoscale clustering of Siglec-1 in mature instead of resting DCs.

After the search that maturation of DCs increases the activity of the formin regulators Rho-A small GTPases, in polarized regions at the plasma membrane, with formin-dependent actin filamentous bundles[18], it was also seen that Siglec-1 colocalizes with both actin and phosphorylated ERM (pERM), an actin-associated marker downstream the activation of RhoA. Furthermore, there was an increase in pERM levels in mDCs if compared to iDCs (resting cells) and, most importantly, a polarization of Siglec-1 distribution in the basal membrane of mDCs and abundance of filopodia enriched with pERM form.

The use of the pharmacological inhibitor of RhoA/Rock pathway CT04 caused a decrease in the polarized distribution of pERM and disruption of Siglec-1 nanoclusters polarization to levels seen on immature DCs (iDCs). Thus, the nanoclustering of Siglec-1 in mDCs happens following the increase of RhoA activation.

The inhibition of pERM also caused similar disruption in the nanoclustering of Siglec-1, and hence, strongly suggests that the spatial distribution and confinement of Siglec-1, in polarized regions, is interdependent of two necessary mechanisms, formin-activation and ERM phosphorylation mediated by the RhoA/ROCK pathway. The inhibition of these two pathways affected the capture of VLPs (HIV-viral like particles). These two pathways contribute or facilitate the capture of VLPs, acting as avid docking sites to viral ligands, indicating a pivotal role of formins and RhoA/ROCK pathway in the actin polymerization machinery and nanoclustering of Siglec-1, consequently affecting the most effective mechanism of viral capture with a potential use of Siglec-1 for clinical purposes.

Additional report showed the role of the formin FHOS (formin homolog overexpressed in spleen)[19] as a member of the IRAP-interactome. IRAP (insulin regulated aminopeptidase) is a type II transmembrane protein, a significant component of Glut4 storage vesicles, GSVs. IRAP plays a role in the intracellular trafficking of several proteins, since either deletion or depletion of IRAP impacts on endocytic trafficking and consequently on the cross-presentation of antigens by dendritic cells and priming of the adaptive immune responses as well as in the

control of innate and adaptive immune receptor signaling and modulation of inflammatory responses[20]. FHOS interacts with IRAP in DCs to anchor endosomes carrying TLR9/ligands to the cell periphery, avoiding their fusion to lysosomes[21]. In the absence of IRAP, and upon activation of DCs by CpG activation, TLR9 signaling was enhanced and led to a higher production of proinflammatory cytokines and type 1 IFN, especially in bone marrow-derived DCs (BMDCs), as well as in conventional splenic and plasmacytoid DCs. Similarly, knockdown by siRNA of FHOD4 caused an aberrant TLR9 trafficking and increased proinflammatory cytokines under the same sort of stimulation by CpG. These results reveal that IRAP recruits FHOD4 to TLR9-containing endosomes, and the formin might be one of the factors contributing to the actin polymerization around endosomes. Such interactions might cause a delay in the transport of TLR9-containing endosomes to lysosomes, thus limiting antigen processing and activation of further immune responses[22]. In DCs, IRAP controls TLR9 activation by delaying the targeting of TLR9-ligand complex to lysosomes, and that involves the participation of dynamic cytoskeleton polymerization around the endosomal compartment. The authors gathered a significant amount of evidences showing that, besides its aminopeptidase activity, IRAP controls endosomal trafficking and claim the necessity of future experiments to address how cell surface receptors and downstream signaling pathways regulate IRAP-mediated trafficking in immune systems, since other studies revealed that IRAP trafficking can be affected by the activation of TLR4 and FCFRs in dendritic cells, and that might also involve the active polymerization of actin by formins[23].

Another meta-analysis and machine learning report from the transcriptome of macrophages, NKs and DCs exposed to *Leishmania spp* found 703 differentially expressed genes (DEGs), and by co-expression analysis, seven “hub genes” were related to signaling pathways, the formin DIAPH1 was amongst them[24]. DIAPH1 acts in cell adhesion, motility, vesicular trafficking, and cytokinesis[25]. In this way, the functions of these hub genes and the activation of signaling pathways pose

valuable information about the molecular basis underlying the activation of immune responsive cells under infection by *Leishmania* and its potential therapeutic use.

Further study addresses the role of Slit2N in the infection by HIV-1 in CD4(+) T-cells by an infectious synapse with DCs[26]. DCs are the first line of defense in sexually acquired HIV-1 infections. At the molecular level, this direct contact between DCs and CD4(+) T-cells occurs by actin-rich membrane extensions, facilitating the viral transmission. For such extensions and contacts to occur, it is necessary that several actin polymerization molecules play a role, such as c-Src (tyrosine Kinase) and Cdc42 (Rho GTPase). Downstream of cdc2 activation, WASP (Wiskott-Aldrich syndrome protein) is activated and modulates the interaction with the actin cytoskeleton via the Arp2/3 complex. An alternative method uses the formin Diaphanous 2 (Dia2/DIAPH2) to elongate filopodia filled with newly formed virions in activated DCs (called viral filopodia). In this scenario, Slit2N, a member of the Slit family of large and secreted glycoproteins, is a ligand for Robo (Roundabout) receptors. Slit2 is cleaved into two fragments in vivo, and the N-terminal fragment (Slit2N) is the active one in the known responses. Using Robo-1 immunoprecipitation of DC lysates, followed by mass spectrometry, one of the most interactive proteins was Fli1 (Flightless-1, initially found in Drosophila), which interacts with formins. Shrivastava et. al, sought[26] for the role of Fli1 in the HIV-1 viral filopodia, since a great deal of actin dynamics is present. Fli1, present in DCs, interacts with Robo1 and is localized along the viral filopodia with higher concentration at their tips. The knockdown of Fli1 showed that a sufficient expression level of Fli1 is necessary for the HIV transmission in such viral synapses. The missing point was how Slit2N links to Fli1 and the cytoskeleton in those viral synapses. Formins are natural candidates to fulfill such a role since, at least, three formins are known to interact with Fli1, which are DIAPH1 and 2 and mDia1[27]. HIV-1 activates Cdc42 and induces membrane extensions in immature dendritic cells to facilitate cell-to-cell virus propagation[28]. The incubation of Slit2N DCs with or without HIV-1, followed by actin immunoprecipitation and western blotting, testing for DIAPH2 and Fli-1 interactions to actin, were done. The immuno

precipitation after HIV infection revealed an enhanced ligation of actin to both Fli1 and DIAPH2, however, in control and untreated DCs, no effect was seen by confocal microscopy, similar behavior was observed; thus, it seems that Slit2N inhibits HIV-1 enhanced association of actin with DIAPH2 and Fli-1 in dendritic cells. Taken together, these results imply the Slit- Robo pathway as limiting factors for HIV infection and that formins are partners in such modulation of viral transmission.

In another study, reviewing phagocytosis, the relationship between actin and glycocalyx, in the control of membrane proteins and lipids mobility, as well as in providing mechanical forces to propel these cells towards phagocytic targets in either macrophages or DCs, was discussed. The authors comment that phagocytic cells are never completely resting because they are constantly under a basal stimulus, such as shearing or adhesion forces, chemokine gradients, and growth factors. As they receive such stimuli, cells form ruffles extending outward of the membrane. For that, it is necessary to engage the actin polymerization by both Arp2/3 and Rho/formin/myosin complexes, which can assemble different f-actin branched and contractile mesh structures at the membrane skeleton. These two actin-remodeling networks dynamically interact and balance each other, mainly by competing for actin monomers and by eliciting inhibitors. If shifting in favor of one network, there is an impact over membrane tension, cell shape and migration[30]. For example, if inhibiting the formin-mediated network, there is an increase in the lateral diffusion of transmembrane pickets, showing that formins are suitable for tethering such pickets. These immobile pickets obstruct the diffusion of non-tethered membrane proteins and lipids. Conversely, if the balance is shifted to the f-actin polymerization by the Arp2/3 complex, a higher mobility and lateral diffusion is seen, thus prompting cells for the aggregation of receptors and phagocytosis. Another critical aspect of controlling the balance of actin-remodeling networks can be seen during cell migration. Limiting the Arp2/3 branching at the front of the cell and increasing the stability at the rear by the Rho/formin/myosin network helps to improve the speed and persistence of cell migration when

stimulated by LPS[31]. The authors also discussed that this bistable balance of the two actin-remodeling networks, seen in phagocytosis, could be extrapolated to other immune processes and also to migrating cells, all of them likely entail a shift from Rho/formin/myosin actin mesh to a branched actin protrusion mediated by the Arp2/3 complex, causing alterations in the mobility of membrane proteins. Such changes in the cytoskeleton architecture are associated with the displacement of glycocalyx, facilitating the segregation of phosphoinositide species. The depletion of actin at these sites of segregated phosphoinositides is essential to allow the arrival of endomembrane vesicles to the plasma membrane. These findings on a "balance" of the two networks bring more complexity to the role of formins in different biological processes and cell types.

A study by Tanizaki et. al., shows the requirement of the formin mDia1 for adhesion, migration, and T-Cell activation by Dendritic cells, which is essential for initiating acquired immune responses[32]. The proper actin nucleation and polymerization are critical steps in triggering the immune response and are mediated by an enormous group of proteins, including the mammalian Diaphanous-related formin (mDia). Bone marrow-derived DCs (BDMCs) from mDia1-deficient mice (mDia1$^{-/-}$) exhibited impaired DC adhesion. Both in vitro and in vivo assays in mDia1$^{-/-}$ genetic background DCs showed suppression of migration. Also, the T-cell interaction in lymph nodes in those deficient mice-derived DCs was impaired attenuating the DC-dependent delayed hypersensitivity. Those data reinforce the essential role of the actin cytoskeleton polymerization and remodeling in various aspects of acquired immune responses such as antigen acquisition, cell migration, and sustained cell-cell and cell-ECM (extracellular matrix) contacts. The formation of actin oligomers is an essential step before polymerization by either the Rho/formin mediated network, especially mDia, or by the Arp2/3 complex, depending on the availability of effector and modulatory proteins. mDia family of formins belong to the Rho GTPase-dependent pathway acting as effectors on the cytoskeleton. There are three mDia isoforms 1, 2 and 3. mDia1 rapidly catalyzes the elongation of long and straight actin filaments and participates in the

establishment of cell polarity, morphogenesis, and cytokinesis. The authors determined the mRNA expression of the three isoforms in dermal and splenic DCs, and as expected, all three isoforms were detected. Following the LPS activation in BMDCs, no alterations were seen in the expression level of mDia isoforms. BMDCs from deficient mDia1$^{-/-}$ mice, as also expected, did not express mDia1; however, mDia2 expression was higher than in wild-type cells. The number of DCs from different sources and subsets were counted, and any significant alterations were seen in mDia1$^{-/-}$ mice-derived cells, suggesting that mDia1 is unrelated to the development and maturation of DCs. The authors addressed the impact of the mDia1 deficiency on in vivo DC mobilization by topical stimulation of mouse footpads with FITC. DCs in the skin uptake FITC and migrate towards popliteal and inguinal lymph nodes (LNs) as MHC class II FITC cells. The accumulation of these cells in the LNs was lower in mDia1$^{-/-}$ mice. Trans well assay of mDia1$^{-/-}$ mouse ear epidermal cells showed impairment of chemotaxis in trans wells with smaller pore size, suggesting that mDia1 is crucial to cell polarity-dependent transmigration. By evaluating the invasion and directional movement of cells in trans wells coated with Matrigel, there was a decrease in the lower chambers in mDia1$^{-/-}$ cells. Using the TAXIScan, the migration of BMDCs towards a gradient of CCL21 chemokine was also impaired in mDia1$^{-/-}$ BDMCs. This is suggestive that mDia1 in DCs is pivotal for migration into the ECM.

The expression of endogenous mDia1 is diffuse throughout the cytoplasm and also strongly localized at the leading edge in BDMCs, while this pattern is disrupted in mDia1$^{-/-}$cells. By analyzing the colocalization of mDia1 and f-actin, followed by a time course analysis after spreading out cells, it was possible to see that mDia1$^{-/-}$ BDMCs stayed round in shape and did not exhibit any membrane protrusions; filopodia could be seen from early to late time points of incubation, however, at lower counts. The 3-dimensional live-imaging of DCs using 2-photon microscopy revealed that mDia1 expression in DCs is required to maintain sustained interactions with T cells. mDia1 deficiency also attenuates the T cell-stimulatory capacity of DCs in vitro since lower proliferation of T-cells and lower

levels of IFN was seen after mDia1$^{-/-}$ BDMCs stimulus. The expression of DC maturation markers such as MHC class II, CD40 and CD54 were also lower in mDia1$^{-/-}$ BDMCs. This was suggestive that DCs undergo further maturation in sustained contact with T-cells in a mDia1-dependent way, as the deficiency leads to impairment of T-cell stimulatory capacity. Altogether, these results reveal the importance of mDia1 at all steps of DC functions and, consequently, for the acquired immune response effectiveness.

Regarding HIV infection, a study place DCs as distinct cells, as they are the first point of contact at the genital mucosa. At the primary steps of the immune response, DCs act in small groups and make numerous and repetitive contacts to communicate with appropriate CD4 T-cells. The authors hypothesized that HIV-1 virus hijacks DCs as a strategy to spread out. Imaging of HIV in infected DCs revealed that virions are forming on the tips of long filopodia. These filopodia pivoted and moved such virions in trajectories, leading to numerous contacts with CD4 T-cells, allowing the virus to spread more efficiently. These are called “viral filopodia” (VF), as priorly mentioned. The extension of these VF is dependent on Diaph2, as seen by knocking down of this protein through the expression of shRNA clones targeting its mRNA. This was not seen for Wasp. When Diaph2 was knocked down, VF lengths and velocity were affected, even creating shorter and static filopodia. Also, lower HIV transfer was seen after Diaph2 knockdown. These results indicate that HIV viral filopodia formation depends on Diaph2, a classic marker of long filopodia.

The patrolling role of DCs in peripheral tissues for the antigen presentation and triggering acquired immune responses was pursued in another article[34]. The authors show that two members of the Ena/VASP family of proteins interact with formins and the Arp2/3 complex, impacting on the efficiency of migration of dendritic cells. The proteins of the Ena/VASP family act as actin polymerases, very much like formins (i.e. mDia1) and the Arp2/3 complex, being considered as a third family of actin nucleators (Figure 1)[35]. Studies have shown that Ena/VASP-family members act as a “regulatory hub” by interacting with the formin mDia1 and the

Arp2/3 activator WRC (WAVE), via the Ena/VASP homology 1 domain (EVH1)[36]. Ena/VASP proteins would then work as a "fine tuning" of the actin dynamics by coordinating mDia1 to polymerize linear actin filaments while regulating Arp2/3 members to form branched filaments of actin. The loss of Evl and VASP affects the processes of macropinocytosis, spreading, and migration of DCs. Double knockouts of these two proteins combined with the inhibition of the Arp2/3 complex have a major impact on the ability of DCs to migrate. The formin mDia1 also interacts with these two Ena/Vasp members. The authors claim that DCs use these three different actin nucleation/polymerization machineries for efficient cell migration, placing the Ena/Vasp proteins as "hubs or connecting scaffolds", defining the way to go further in actin dynamics, as its loss impairs DC migration. The authors point out that understanding the timing of events and determining if there are specificities in such a "hub" in iDCs and mDCs is mandatory.

Another study about the formins mDia1 and FMNL1 revealed their role during the uptake of *Borrelia* (which causes Lyme disease) by macrophages and also dendritic cells[37]. Macrophages and DCs are the first cells of the immune system to sense the infection. A "coiling type" phagocytosis is responsible for uptaking borreliae by unilateral pseudopods, which can uptake and enwrap spirochetes. Even though the participation of the Arp2/3 complex in such phagocytosis was known, the authors show the participation of the two formins (mDia1 and FMNL1) by live-cell imaging and immunofluorescence, amongst other techniques. There is an enhancement of these formins in those pseudopods uptaking borreliae. The knockdown of both, by siRNA, decreased the formation of pseudopods during infection. FMNL1 is an effector from Cdc42 and Rac1, and it is upregulated during the differentiation of monocytes to macrophages[38]. Alternative splicing can lead to different isoforms and functions in phagocytosis of distinct targets if compared to mDia1. FMNL1 is recruited to phagocytic cups dependent on Cdc-42. The authors suggest that FMNL1 and mDia1 are novel regulators of spirochete uptake by human immune cells.

Finally, it was demonstrated that the formin Daam1 mediates the uptake of *B. burgdorfery* by neuroglial cells in a similar way, as *B. burgdorferi* was also enwrapped by Daam1-enriched coiling pseudopods. A report showed a decrease in the *B. burgdorfery* uptake after pre-treatment with anti-Daam1 antibody, indicating that Daam1 is required for borrelial phagocytosis. Furthermore, Daam1 colocalized to the *B. burgdorferi* surface, as seen by confocal microscopy, that. These results show that coiling phagocytosis is a mechanism for borrelial internalization by neuroglial cells mediated by Daam1 and also other formins[39].

Table 1. Summary of the functions of formins in Dendritic cells described in the selected articles.

REF #	FORMIN	ROLE in DCs	REMARKS
17	Formins in general	Formins along RhoA/ROCK pathway mediate the actin-dependent nanoclustering of Siglec-1 at the basal membrane od mature DCs	The correct formin-mediated nanoclustering of Siglec-1 significantly affects the efficiency of HIV-1 viral capture.
19	FHOS and FHOD4	IRAP recruits formins to endosomal compartments delaying TLR9 vesicles trafficking to lysosomes	IRAP controls TLR9 activation by delaying the targeting of TLR9-ligand complex to lysosomes and influencing immune responses in activated DCs
21	DIAPH 1	DIAPH1 is a hub gene differentially expressed following *Leishmania* infection in DCs, NKs and macrophages	The expression data must be explored molecularly to understand the role of DIAPH1 as a hub gene in immune cell response to *Leishmania* infection.
26	DIAPH 2	Partner of the Slit-Robo pathway to coordinate actin polymerization in DCs infected by HIV-1 and its transmission in viral synapses	DIAPH2, decreased filopodial extensions on dendritic cells and inhibited cell-to-cell transmission of HIV-1 in a Robo1-dependent manner.

DISCUSSION

In this review, we gathered the most prominent roles of formins in the context of Dendritic cell biology. It is not surprising their key role as cytoskeleton remodeling factors for all eukaryotic cells. The numerous molecules, which interact directly or indirectly with the cytoskeleton, increase the complexity of cell biology. The complexity should be considered for every cell type and function. In Dendritic

cells, as the first line of defense in the immune system, this relationship with formins becomes even more significant. The cytoskeleton dynamics can contribute to DCs functions, ranging from “change of state” (from resting to activated DCs), efficiency in uptaking pathogens, trafficking and transmission of pathogens, antigen presentation, migration efficiency, and DC-ECM interactions. Those are the most significant behaviors that Dendritic cells must perform to induce correct immune responses.

Table 2**.** Summary of the functions of formins in Dendritic cells described in the selected articles.

REF #	FORMIN	ROLE in DCs	REMARKS
29	Formins in general	Rho/formin/myosin mediated actin in balance with Arp2/3 im DCs	Shifting to different networks impacts membrane properties
32	mDia1	mDia1 formin is required for adhesion, migration and T-Cell activation in Dendritic cells	mDia1$^{-/-}$ BDMCs, affects all functions of DCs,
33	DIAPH2	Long viral filopodial formation depended on the formin diaphanous 2	HIV corrupts DC to CD4 T cell interactions
34	mDia1DIAPH1	Form an interactome wirh Ena/VASP and coordinates actin remodeling and migration	DCs use different actin polymerization machineries for cell migration,
37	FMNL1 and mDia1	Primary human macrophages engage FMNL1 and mDia formins in the uptake of Borreliae by pseudopods	Coiling phagocytosis involves mDia1 and FMNL1
38	FMNL1	the formin FMNL1 is a key regulator of podosomes and is required for normal macrophage migration	Suppression of FMNL1 decreases in macrophage migration
39	Daam1	Human neuroglial cells internalize Borrelia burgdorferi by coiling phagocytosis mediated by Daam1	Daam1 mediates the coiling phagocytosis of borrelia

As summarized in TABLES 1 and 2, we can highlight the role of “general formins” (FH2 domain-containing ones) in docking and uptaking HIV by DCs in a formin-mediated nanoclustering of Siglec-1. They could act, also, as potential biomarkers of inactive (resting DCs) and active or mature DCs[17]. DIAPH2 demonstrated analogous behavior by interacting with Fli-1 in the formation of "viral

synapses" through a Slit/Robo dependent pathway, thereby influencing the efficiency of HIV uptake and transmission, as well as the activation of specific T-cells[16]. DIAPH2 is the only actin nucleator involved in the formation of the long Viral Filopodia (VFs) and contributes to the HIV infection to spread out more efficiently by corrupting DCs to elongate these VFs embedded with virions[33]. DIAPH1, another DRF, plays a role in response to pathogens, such as *Leishmania*. Using transcriptomics, the gene encoding *DIAPH1* works as a "hub gene" differentially expressed in macrophages, NKs, and DCs infected by *Leishmania*, being central in the signaling pathways and hence with potential therapeutic use [24]. DIAPH1 and mDia1 form an interactome with the Ena/Vasp-mediated actin remodeling in the migration of DCs. In this case, Ena/Vasp is a connecting scaffold to select the most effective actin nucleator network for efficient cell migration[34]. mDia1, a member of the DRF family in metazoans, is necessary for dendritic cells to adhere, migrate, and activate T-cells, what was demonstrated by alterations in all functions of DCs in mDia1$^{-/-}$ BDMCs, showing that this formin is essential for the initiation of acquired immune responses[32]. mDia1 forms an actin pool at the rear end of immature DCs (iDCs) to facilitate faster migration in a RhoA dependent signaling pathway. Once the Arp2/3 complex moves the cell front at lower speeds, mDia1 can be suggested as a marker for DC functional state (iDCs/mDCs)[31]. Another role of mDia1 was in the extension of pseudopods and uptake of *Borrelia* (Lyme disease) by macrophages and other phagocytic cells such as DCs. In this case, mDia1 acts along with another formin the FMNL1 and were considered as new regulators of "coiling phagocytosis", a stereotypical actin-rich unilateral pseudopod that efficiently captures spirochetes by immune and microglial cells cells[37,39].

Two formins of the FHOD-subtype, FHOS and FHOD4, are part of the IRAP interactome in DCs. Both formins are recruited by IRAP, which controls TLR9-ligand endosomal trafficking from membrane to lysosomes by dynamic cytoskeleton polymerization around them. Thus, these actin nucleators participate

in the engagement of both adaptive and innate immune responses and in the production of proinflammatory mediators[19].

Finally, another ordinary function of formins in DCs involves a bistable balance between the Rho/formin/myosin actin nucleation network and the Arp2/3 complex to modulate lateral diffusion and mobility of transmembrane proteins and lipids, by shifting from one network to another, and that influences different cell changes in shape, membrane composition and migration[29].

Figure 3. Diagram of formin functions in Dendritic Cells. **A.** Immature DCs showing formin functions. **B.** Mature (mDCs) exhibiting its filopodia, pseudopods and showing the immune system activators, like pathogens, as well as the expressed formins and their participation for each of these cell extensions. Besides it is shown the interaction of DCs with T-Cells and ECM (extracellular matrix).

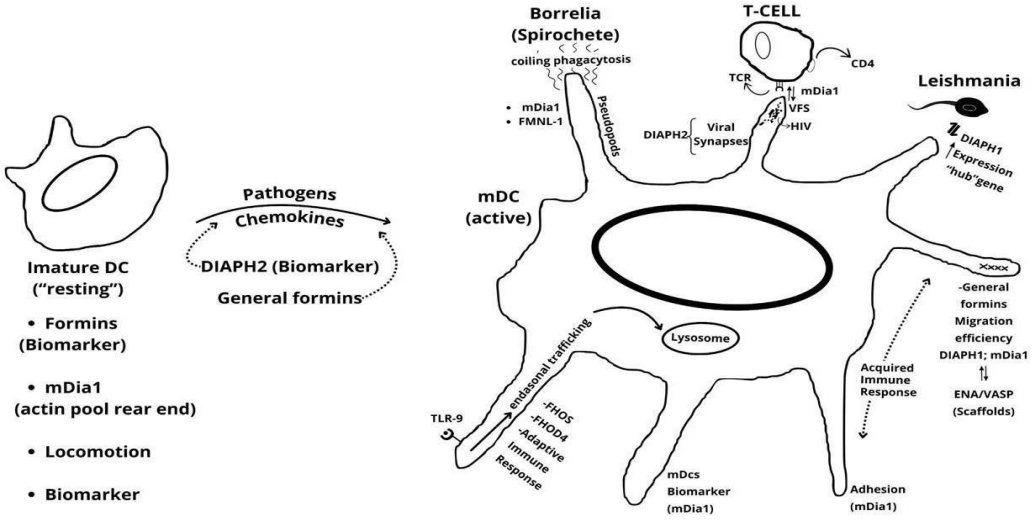


Figure 3 depicts the primary functions discussed in this review and poses several relevant questions for further study: Are other formins involved in dendritic cells (DCs)? This question is based, primarily, on the fact that most data were obtained from DRF subtype (Diaphanous Related Formins), mainly mDia1 and DIAPH, and FHOS/FHOD subtype. The functions of formins described here as "formins in general" might be applicable to other subtypes, such as FMNs and FMNLs that possess the FH2 actin nucleator domain and are found in leukocytes. The inhibitor SMIFH2, which targets FH2-containing formins, disrupted their activity. Can formins serve as biomarkers for DC maturation? Can formins improve

therapeutic uses of DC such as antitumor vaccines or antivirals? since formins interact with the Siglec-1 in HIV infected cells. Could it also be true with other pathogens (i.e., *Borrelia, Leishmania*) or stimuli by chemokines? A comparable inquiry may be posed for mDia1, given that its knockout in bone marrow-derived dendritic cells affected both their locomotion and activation status. Could formins work as biomarkers in protocols during personalized immunotherapies?

All those questions need further investigation, at the molecular level, and bring the perspective of new prominent roles of formins and the cytoskeleton to the biology of dendritic cells with potential therapeutic uses.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Claudio Roberto Simon (PhD) idealized the review, performed the database searches, and wrote the majority of the manuscript.
Saulo Fernando (PhD): wrote a section of the manuscript, contributed with the interpretations and critically review the text.
Márcia Antoniazi Michelin (PhD): wrote a section of the manuscript and contributed to the critical review and interpretation of the data.

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