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INSIGHTS

Talin1 sets the stage for dendritic cell activation

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In this issue of *JEM*, Lim et al. (https://doi.org/10.1084/jem.20191810) provide exciting new evidence that talin1 plays an essential role in dendritic cell (DC) maturation and activation. Using conditional knockout mice, they demonstrate that talin1 promotes the formation of a preassembled TLR–Myddosome signaling complex in steady-state DCs but not macrophages. This may explain why DCs respond faster and more vigorously to TLR ligand binding than their closely related macrophages.

Dendritic cells (DCs) and macrophages represent a heterogeneous family of mononuclear phagocytes that play essential roles in regulating innate and adaptive immune responses. They are strategically positioned at epithelial borders to the environment, including the skin, and sense invading pathogens through recognition by various pattern recognition receptors like TLRs. While macrophages help maintain tissue homeostasis and eliminate pathogens after phagocytosis and TLR activation, DCs have the unique capacity to balance tolerance and immunity. Both in the steady state as well as during infection and inflammation, DCs migrate to tissue-draining LNs to present phagocytosed self- or microbial antigens to naive T cells for the induction of appropriate regulatory and effector T cell responses. The latter is achieved by distinct TLR activation of DCs, which not only enhances their surface MHC and co-stimulatory molecule expression for efficient T cell stimulation, but also triggers a specific pro-inflammatory cytokine profile to ensure adequate T cell polarization. Thus, the ability of DCs to regulate immunity critically depends on their migration and maturation to deliver peripheral antigens as well as information about the nature of the pathogenic threat to T cells. In this issue, Lim et al. (2020) unravel a pivotal function of talin1, a central adapter molecule of integrin signaling, in orchestrating both DC migration and maturation/activation.

Integrins are transmembrane receptors that have so far mostly been recognized for their role in cell–extracellular matrix (ECM) adhesion and cell–cell contacts. Moreover, they are dynamically coupled to the actomyosin cytoskeleton by talin1 and facilitate "haptokinetic" (adhesion driven) cell migration (Lämmermann et al., 2008). Specifically, integrins anchor membrane protrusions pushed out by F-actin polymerization at the cell front to an extracellular substrate. Subsequent myosin II–mediated contraction of the actin network inflicts retrograde pulling forces via talin1, which enable forward locomotion of the cell body (Calderwood and Ginsberg, 2003). Notably, leukocytes, including DCs, require integrin-mediated adhesion only to cross tissue barriers like, for example, continuous endothelial linings during their extravasation into inflamed tissues (Lämmermann et al., 2008). In addition, on their way to skin-draining LNs, Langerhans cells (LCs)—the unique DC population in the epidermis—up-regulate α6 integrin to bind to its ligand laminin and allow passage through the basement membrane into the dermis (Price et al., 1997). In agreement with this concept, using DC-specific talin1 knockout mice (*Tln1*$^{f/f}$*CD11c*-Cre mice), Lim et al. (2020) find that talin1-deficient LCs accumulate in the epidermis under both steady-state and inflammatory conditions. This result confirms earlier work from the same laboratory demonstrating that the methyltransferase

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Ezh2 controls LC transmigration across the basement membrane through direct methylation of the adapter molecule talin1, which disrupts its binding to F-actin and thereby functionally enhances the cellular disassembly of focal adhesions (Gunawan et al., 2015; Loh et al., 2018).

Among skin DCs, LCs are unique in their expression of multiple epithelial adhesion-associated molecules like E-cadherin, EpCAM, and others, which allow LCs to attach themselves to the surrounding keratinocytes (Clausen and Stoitzner, 2015). In fact, during their mobilization, LCs not only undergo the phenotypic and functional maturation program described above (up-regulation of MHCII, co-stimulatory molecules, and pro-inflammatory cytokines), but also a transformation process called epithelial-to-mesenchymal transition (EMT).

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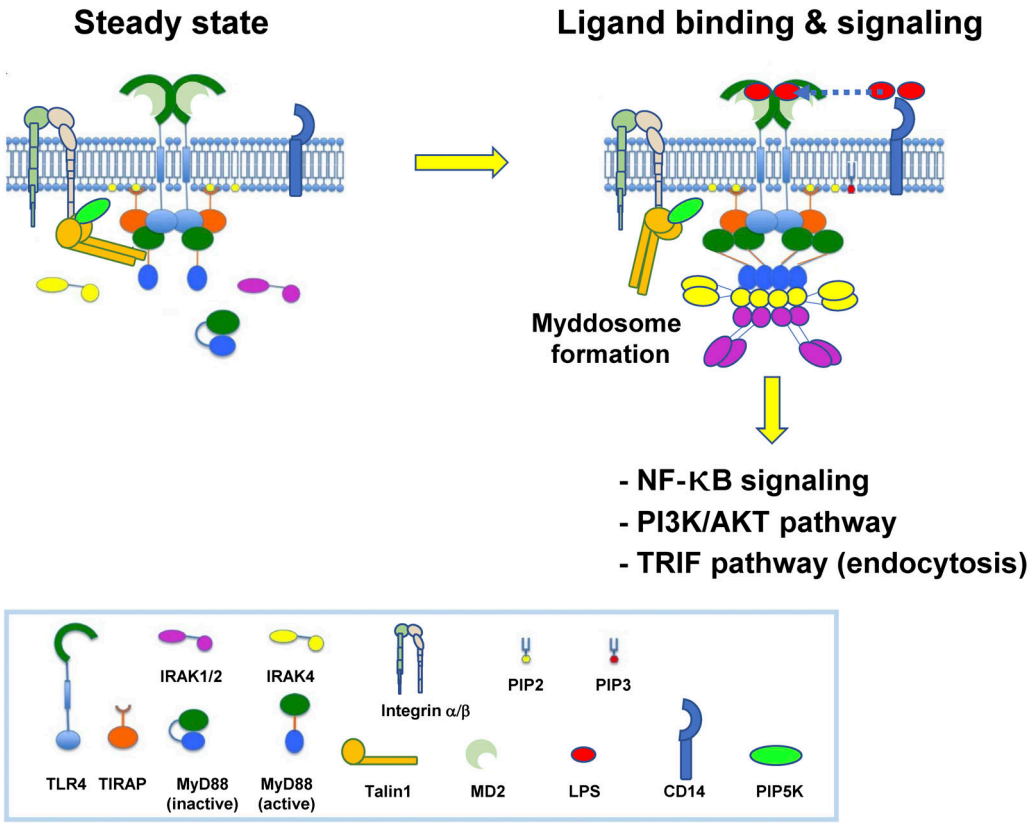

Talin1 interacts with MyD88 and PIP5K to enable the preassembly of TLR4 complexes in steady-state DCs (left). Local production of PIP2 by PIP5K then recruits TIRAP to the preassembled complexes, which are required for TLR4 downstream signaling events upon LPS binding (right). These include Myddosome formation leading to NF-κB activation, as well as TLR4 endocytosis and triggering of TRIF-dependent pathways during DC activation (right). Moreover, elevated PIP2 levels adjacent to TLR4 also serve as a substrate for PI3K to synthesize PIP3, which mediates the recruitment and activation of AKT (cell survival). Modified from Lim et al. (2020).

EMT involves cytoskeletal rearrangements driven by the down-regulation of epithelial markers facilitating adhesion and up-regulation of mesenchymal markers (e.g., N-cadherin, matrix metalloproteinases [MMPs], and integrins) promoting migration, as seen in cancer development during metastatic transformation (Konradi et al., 2014). Intriguingly, Lim et al. (2020) observe that talin1-deficient LCs, despite similar surface expression in the steady state, fail to down-regulate E-cadherin upon activation. Although the lack of E-cadherin alone does not trigger EMT and LC emigration from the skin (Brand et al., 2020), conversely, the inability to down-regulate E-cadherin could prevent talin1-deficient LCs to disengage from the keratinocytes and acquire a motile phenotype, contributing to their inability to leave the epidermis. Whether talin1 also influences EMT in a broader sense during LC mobilization remains to be investigated, although the up-regulation of MMP2/9 was not affected by the lack of talin1 (yet insufficient to rescue their inability to cross the basement membrane).

Interestingly, Lim et al. (2020) also noticed a compromised migration of talin1-deficient dermal DCs to skin-draining LNs in the steady state and during inflammation following LPS treatment. This observation is rather unexpected since dermal DCs, which do not have to overcome the basement membrane, are thought to reach the LNs by talin1/integrin-independent "flowing and squeezing" migration (Lämmermann et al., 2008). While these inconsistent results may be explained by the footpad injection of a high number of talin1-deficient bone marrow–derived DCs (BMDCs) in the previous report, which may have compensated for their compromised LN migration, the current study does not reveal the underlying mechanism for their attenuated migration because LPS-stimulated BMDCs lacking talin1 fail to migrate toward a CCL19 gradient in vitro, despite efficient up-regulation of the CCL19 receptor CCR7. On the other hand, talin1-deficient dermal DCs and BMDCs (as well as LCs) exhibit a reduced up-regulation of the maturation markers MHCII and CD86 upon TLR4 (LPS) activation. Moreover, in the absence of talin1, LCs and BMDCs display a diminished expression of a broad range of pro-inflammatory cytokines after LPS stimulation due to impaired activation of NF-κB and upstream signaling pathways (PI3K and MAPK). These data demonstrate that talin1 controls efficient TLR4-mediated phenotypic and functional DC maturation via NF-κB activation, although the reason for their disabled migration remains unclear.

It had previously been shown that LPS signaling through TLR4 induces the formation of oligomeric signaling platforms called the Myddosome and Triffosome (Gay et al., 2014). Notably, Lim et al. (2020) now establish that talin1 is required to form a preassembled MyD88-dependent TLR complex via direct interactions with MyD88 and

PIP5K in steady-state DCs but not macrophages. Intriguingly, preassembly of the TLR–Myddosome complex may occur in LCs as well despite their common origin from embryonic macrophage precursors (Kaplan, 2017). This novel mechanism not only explains the rapid response of LCs and DCs to TLR ligands as compared to macrophages, but it also provides a mechanistic link for the crosstalk between TLRs and the PI3K–AKT pathways. PIP5K-driven PIP2 production adjacent to TLRs both enhances the recruitment of TIRAP and serves as a substrate for PI3K to synthesize PIP3, which is required for the recruitment and activation of AKT. In addition, the integrin binding and activation capacity of talin1 is essential for the preassembly of the TLR complex and subsequent activation upon ligand binding, suggesting that the extracellular domain of active integrin may contribute to the stabilization of preassembled TLR complexes and TLR ligand binding. Hence, this study by Lim et al. (2020) represents an important conceptual shift in the preconceived idea of how talin1 and TLRs function in DCs, which is not only relevant for understanding innate immune responses in the initial phase of microbial infection, but also has a profound impact on the formation of adaptive immune responses and the final clearance of microbes. The relevant unresolved question remains whether Ezh2-mediated talin1 methylation is involved in this novel regulatory mechanism. Moreover, aside from MyD88-dependent TLRs, it remains elusive whether talin1 orchestrates other innate immune receptors. Notably, talin1 is dispensable for the assembly of the MyD88-independent TLR3/TRIF signalosome and does not regulate the downstream TBK1–IRF3 pathway leading to type I IFN production upon poly(I:C) treatment.

Having established the novel regulatory function of talin1 for LC and DC migration and activation, Lim et al. (2020) probed its physiological relevance in vivo. For one, they investigated a model of cutaneous bacterial infection with the common commensal and opportunistic pathogen *Staphylococcus aureus*, a gram$^+$ bacterium whose cell wall components trigger DC activation via the TLR2 signaling cascade. In agreement with the severely impaired talin1-deficient LC and dermal DC migration and activation, *Tln1*$^{f/f}$*CD11c*-Cre mice mount significantly compromised Th1/Th17 responses upon epicutaneous infection with *S. aureus*. At the same time, these data also prove that talin1 is essential for effective MyD88-dependent TLR signaling in general. Next, the authors interrogated murine contact hypersensitivity (CHS), a non-infectious skin inflammation model, in which the effector T cell response is largely orchestrated by skin DCs (Clausen and Stoitzner, 2015). Beyond the functional specialization of epidermal LCs and the different dermal DC subsets in CHS, which is still controversially discussed, the dose of contact sensitizer (hapten) topically applied onto the skin represents another critical factor for the magnitude of the ear swelling reaction (Noordegraaf et al., 2010; Romani et al., 2010). In line with these previous reports, *Tln1*$^{f/f}$*CD11c*-Cre mice develop similar and attenuated CHS reactions compared to controls upon sensitization with a high and a low hapten dose, respectively, indicating that the high hapten concentration compensates for any migratory and functional defects of talin1-deficient skin DCs. Consistent with the tolerogenic properties of LCs in certain situations (Gomez de Aguero et al., 2012), which depend on their efficient migration out of the skin, talin1-deficient LCs fail to tolerize antigen-specific T cells in a tolerogenic CHS protocol. While tolerization with the weak hapten dinitrothiocyanobenzene is able to suppress dinitrofluorobenzene-induced ear swelling in control mice, *Tln1*$^{f/f}$*CD11c*-Cre mice mount a robust CHS reaction under these conditions. In conclusion, these findings establish that talin1 governs the pro- and anti-inflammatory functions of LCs and dermal DCs, which makes it an attractive target for immunotherapeutic intervention. For example, specifically interfering with LC migration and activation by pharmacological inhibition of talin1 may enhance protective immunity in cutaneous leishmaniasis (Kautz-Neu et al., 2011).

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