Trpml controls actomyosin contractility and couples migration to phagocytosis in fly macrophages.

Summary

Phagocytes use their actomyosin cytoskeleton to migrate as well as to probe their environment by phagocytosis or macropinocytosis. Although migration and extracellular material uptake have been shown to be coupled in some immune cells, the mechanisms involved in such coupling are largely unknown. By combining time-lapse imaging with genetics, we here identify the lysosomal Ca2+ channel Trpml as an essential player in the coupling of cell locomotion and phagocytosis in hemocytes, the Drosophila macrophage-like immune cells. Trpml is needed for both hemocyte migration and phagocytic processing at distinct subcellular localizations: Trpml regulates hemocyte migration by controlling actomyosin contractility at the cell rear, whereas its role in phagocytic processing lies near the phagocytic cup in a myosin-independent fashion. We further highlight that Vamp7 also regulates phagocytic processing and locomotion but uses pathways distinct from those of Trpml. Our results suggest that multiple mechanisms may have emerged during evolution to couple phagocytic processing to cell migration and facilitate space exploration by immune cells.

Deterministic actin waves as generators of cell polarization cues.

Summary

Dendritic cells “patrol” the human body to detect pathogens. In their search, dendritic cells perform a random walk by amoeboid migration. The efficiency of pathogen detection depends on the properties of the random walk. It is not known how the dendritic cells control these properties. Here, we quantify dendritic cell migration under well-defined 2-dimensional confinement and in a 3-dimensional collagen matrix through recording their long-term trajectories. We find 2 different migration states: persistent migration, during which the dendritic cells move along curved paths, and diffusive migration, which is characterized by successive sharp turns. These states exhibit differences in the actin distributions. Our theoretical and experimental analyses indicate that this kind of motion can be generated by
spontaneous actin polymerization waves that contribute to dendritic cell polarization and migration. The relative distributions of persistent and diffusive migration can be changed by modification of the molecular actin filament nucleation and assembly rates. Thus, dendritic cells can control their migration patterns and adapt to specific environments. Our study offers an additional perspective on how dendritic cells tune their searches for pathogens.


Actomyosin-driven force patterning controls endocytosis at the immune synapse.

*Nature communications* : 2870 :

**Summary**

An important channel of cell-to-cell communication is direct contact. The immune synapse is a paradigmatic example of such type of interaction: it forms upon engagement of antigen receptors in lymphocytes by antigen-presenting cells and allows the local exchange of molecules and information. Although mechanics has been shown to play an important role in this process, how forces organize and impact on synapse function is unknown. We find that mechanical forces are spatio-temporally patterned at the immune synapse: global pulsatile myosin II-driven tangential forces are observed at the synapse periphery while localised forces generated by invadosome-like F-actin protrusions are detected at its centre. Noticeably, we observe that these force-producing actin protrusions constitute the main site of antigen extraction and endocytosis and require myosin II contractility to form. The interplay between global and local forces dictated by the organization of the actomyosin cytoskeleton therefore controls endocytosis at the immune synapse.

Juan José Sáez, Ana-Maria Lennon-Duménil, María-Isabel Yuseff (2019 Jun 1)

Studying MHC Class II Presentation of Immobilized Antigen by B Lymphocytes.

*Methods in molecular biology (Clifton, N.J.)* : 419-437 :

**Summary**

The ability of B lymphocytes to capture external antigens (Ag) and present them as peptide fragments, loaded on major histocompatibility complex (MHC) class II molecules, to CD4 T cells is a crucial part of the adaptive immune response. This allows for T-B cooperation, a cellular communication that is required for B cells to develop into germinal centers (GC) and form mature high affinity antibody producing cells and to further develop B cell memory. MHC class II antigen presentation by B lymphocytes is a multistep process involving (1) Recognition and capture of external Ag by B lymphocytes through their B cell receptor (BCR), (2) Ag processing, which comprises the degradation of Ag in internal compartments within the B cell and loading of the corresponding peptide fragments on MHC class II molecules, and (3) Presentation of MHCII-peptide complexes to CD4 T cells. Here, we describe how to study
the biochemical and morphological changes that occur in B lymphocytes at these three major levels.

Hélène D Moreau, Carles Blanch-Mercader, Rafaele Attia, Mathieu Maurin, Zahraa Alraies, Doriane Sanséau, Odile Malbec, Maria-Graciela Delgado, Philippe Bouso, Jean-François Joanny, Raphaël Voituriez, Matthieu Piel, Ana-Maria Lennon-Duménil (2019 Apr 16)

**Macropinocytosis Overcomes Directional Bias in Dendritic Cells Due to Hydraulic Resistance and Facilitates Space Exploration.**

*Developmental cell*: 171-188.e5 : DOI : S1534-5807(19)30235-7

**Summary**

The migration of immune cells can be guided by physical cues imposed by the environment, such as geometry, rigidity, or hydraulic resistance (HR). Neutrophils preferentially follow paths of least HR in vitro, a phenomenon known as barotaxis. The mechanisms and physiological relevance of barotaxis remain unclear. We show that barotaxis results from the amplification of a small force imbalance by the actomyosin cytoskeleton, resulting in biased directional choices. In immature dendritic cells (DCs), actomyosin is recruited to the cell front to build macropinosomes. These cells are therefore insensitive to HR, as macropinocytosis allows fluid transport across these cells. This may enhance their space exploration capacity in vivo. Conversely, mature DCs down-regulate macropinocytosis and are thus barotactic. Modeling suggests that HR may help guide these cells to lymph nodes where they initiate immune responses. Hence, DCs can either overcome or capitalize on the physical obstacles they encounter, helping their immune-surveillance function.

Daisuke Inoue, Dorian Obino, Judith Pineau, Francesca Farina, Jérémie Gaillard, Christophe Guerin, Laurent Blanchoin, Ana-Maria Lennon-Duménil, Manuel Théry (2019 Mar 24)

**Actin filaments regulate microtubule growth at the centrosome.**

*The EMBO journal*: DOI : e99630

**Summary**

The centrosome is the main microtubule-organizing centre. It also organizes a local network of actin filaments. However, the precise function of the actin network at the centrosome is not well understood. Here, we show that increasing densities of actin filaments at the centrosome of lymphocytes are correlated with reduced amounts of microtubules. Furthermore, lymphocyte activation resulted in disassembly of centrosomal actin and an increase in microtubule number. To further investigate the direct crosstalk between actin and microtubules at the centrosome, we performed reconstitution assays based on (i) purified centrosomes and (ii) on the co-micropatterning of microtubule seeds and actin filaments. These two assays demonstrated that actin filaments constitute a physical barrier blocking elongation of nascent microtubules. Finally, we showed that cell adhesion and cell spreading lead to lower densities of centrosomal actin, thus resulting in higher microtubule growth. We therefore propose a novel mechanism, by which the number of centrosomal
microtubules is regulated by cell adhesion and actin-network architecture.

Year of publication 2018

Dorian Obino, Luc Fetler, Andrea Soza, Odile Malbec, Juan José Saez, Mariana Labarca, Claudia Oyanadel, Felipe Del Valle Batalla, Nicolas Goles, Aleksandra Chikina, Danielle Lankar, Fabián Segovia-Miranda, Camille Garcia, Thibaut Léger, Alfonso Gonzalez, Marion Espéli, Ana-Maria Lennon-Duménil, Maria-Isabel Yuseff (2018 Dec 13)

**Galectin-8 Favors the Presentation of Surface-Tethered Antigens by Stabilizing the B Cell Immune Synapse.**

*Cell reports*: 3110-3122.e6 : [DOI: S2211-1247(18)31815-1]

**Summary**

Complete activation of B cells relies on their capacity to extract tethered antigens from immune synapses by either exerting mechanical forces or promoting their proteolytic degradation through lysosome secretion. Whether antigen extraction can also be tuned by local cues originating from the lymphoid microenvironment has not been investigated. We here show that the expression of Galectin-8, a glycan-binding protein found in the extracellular milieu, which regulates interactions between cells and matrix proteins, is increased within lymph nodes under inflammatory conditions where it enhances B cell arrest phases upon antigen recognition in vivo and promotes synapse formation during BCR recognition of immobilized antigens. Galectin-8 triggers a faster recruitment and secretion of lysosomes toward the B cell-antigen contact site, resulting in efficient extraction of immobilized antigens through a proteolytic mechanism. Thus, extracellular cues can determine how B cells sense and extract tethered antigens and thereby tune B cell responses in vivo.


**Innate Immune Signals Induce Anterograde Endosome Transport Promoting MHC Class I Cross-Presentation.**

*Cell reports*: 3568-3581 : [DOI: S2211-1247(18)31312-3]

**Summary**

Both cross-presentation of antigens by dendritic cells, a key pathway triggering T cell immunity and immune tolerance, and survival of several pathogens residing in intracellular vacuoles are intimately linked to delayed maturation of vesicles containing internalized antigens and microbes. However, how early endosome or phagosome identity is maintained is incompletely understood. We show that Toll-like receptor 4 (TLR4) and Fc receptor ligation
induces interaction of the GTPase Rab14 with the kinesin KIF16b mediating plus-end-directed microtubule transport of endosomes. As a result, Rab14 recruitment to phagosomes delays their maturation and killing of an internalized pathogen. Enhancing anterograde transport by overexpressing Rab14, promoting the GTP-bound Rab14 state, or inhibiting retrograde transport upregulates cross-presentation. Conversely, reducing Rab14 expression, destabilizing Rab14 endosomes, and inhibiting anterograde microtubule transport by KIF16b knockdown compromise cross-presentation. Therefore, regulation of early endosome trafficking by innate immune signals is a critical parameter in cross-presentation by dendritic cells.


Summary

Thanks to the power of Drosophila genetics, this animal model has been a precious tool for scientists to uncover key processes associated to innate immunity. The fly immune system relies on a population of macrophage-like cells, also referred to as hemocytes, which are highly migratory and phagocytic, and can easily be followed in vivo. These cells have shown to play important roles in fly development, both at the embryonic and pupal stages. However, there is no robust assay for the study of hemocyte migration in vitro, which limits our understanding of the molecular mechanisms involved. Here, we contribute to fill this gap by showing that hemocytes adopt a polarized morphology upon ecdysone stimulation, allowing the study of the cytoskeleton rearrangements and organelle reorganization that take place during the first step of cell locomotion.

Felipe Del Valle Batalla, Ana-María Lennon-Dumenil, María-Isabel Yuseff (2018 Jun 24) 
Tuning B cell responses to antigens by cell polarity and membrane trafficking. 
Molecular immunology : 140-145 : DOI : S0161-5890(18)30214-1

Summary

The capacity of B lymphocytes to produce specific antibodies, particularly broadly neutralizing antibodies that provide immunity to viral pathogens has positioned them as valuable therapeutic targets for immunomodulation. To become competent as antibody secreting cells, B cells undergo a series of activation steps, which are triggered by the recognition of antigens frequently displayed on the surface of other presenting cells. Such antigens elicit the formation of an immune synapse (IS), where local cytoskeleton rearrangements coupled to mechanical forces and membrane trafficking orchestrate the extraction and processing of antigens in B cells. In this review, we discuss the molecular mechanisms that regulate polarized membrane trafficking and mechanical properties of the
immune synapse, as well as the potential extracellular cues from the environment, which may impact the ability of B cells to sense and acquire antigens at the immune synapse. An integrated view of the diverse cellular mechanisms that shape the immune synapse will provide a better understanding on how B cells are efficiently activated.

Hélène D Moreau, Matthieu Piel, Raphaël Voituriez, Ana-Maria Lennon-Duménil (2018 May 22)
Integrating Physical and Molecular Insights on Immune Cell Migration.
*Trends in immunology* : 632-643 : [DOI : S1471-4906(18)30084-X](https://doi.org/S1471-4906(18)30084-X)

**Summary**

The function of most immune cells depends on their ability to migrate through complex microenvironments, either randomly to patrol for the presence of antigens or directionally to reach their next site of action. The actin cytoskeleton and its partners are key conductors of immune cell migration as they control the intrinsic migratory properties of leukocytes as well as their capacity to respond to cues present in their environment. In this review we focus on the latest discoveries regarding the role of the actomyosin cytoskeleton in optimizing immune cell migration in complex environments, with a special focus on recent insights provided by physical modeling.

Pablo J Sáez, Juan C Sáez, Ana-María Lennon-Duménil, Pablo Vargas (2018 May 2)
Role of calcium permeable channels in dendritic cell migration.
*Current opinion in immunology* : 74-80 : [DOI : S0952-7915(18)30016-5](https://doi.org/S0952-7915(18)30016-5)

**Summary**

Calcium ion (Ca) is an essential second messenger involved in multiple cellular and subcellular processes. Ca can be released and sensed globally or locally within cells, providing complex signals of variable amplitudes and time-scales. The key function of Ca in the regulation of acto-myosin contractility has provided a simple explanation for its role in the regulation of immune cell migration. However, many questions remain, including the identity of the Ca stores, channels and upstream signals involved in this process. Here, we focus on dendritic cells (DCs), because their immune sentinel function heavily relies on their capacity to migrate within tissues and later on between tissues and lymphoid organs. Deciphering the mechanisms by which cytoplasmic Ca regulate DC migration should shed light on their role in initiating and tuning immune responses.

Cesar Oyarce, Sebastián Cruz-Gomez, Felipe Galvez-Cancino, Pablo Vargas, Hélène D Moreau, Natalia Diaz-Valdivia, Jorge Diaz, Flavio Andres Salazar-Onfray, Rodrigo Pacheco, Ana Maria Lennon-Dumenil, Andrew F G Quest, Alvaro Lladser (2018 Jan 13)
Caveolin-1 Expression Increases upon Maturation in Dendritic Cells and
Promotes Their Migration to Lymph Nodes Thereby Favoring the Induction of CD8 T Cell Responses.

> **Summary**

Dendritic cell (DC) trafficking from peripheral tissues to lymph nodes (LNs) is a key step required to initiate T cell responses against pathogens as well as tumors. In this context, cellular membrane protrusions and the actin cytoskeleton are essential to guide DC migration towards chemotactic signals. Caveolin-1 (CAV1) is a scaffolding protein that modulates signaling pathways leading to remodeling of the actin cytoskeleton and enhanced migration of cancer cells. However, whether CAV1 is relevant for DC function and specifically for DC migration to LNs is unknown. Here, we show that CAV1 expression is upregulated in DCs upon LPS- and TNF-α-induced maturation. CAV1 deficiency did not affect differentiation, maturation, or the ability of DCs to activate CD8 T cells. However, CAV1-deficient (CAV1) DCs displayed reduced trafficking to draining LNs in control and inflammatory conditions. CAV1 DCs showed reduced directional migration in CCL21 gradients in transwell assays without affecting migration velocity in confined microchannels or three-dimensional collagen matrices. In addition, CAV1 DCs displayed reduced activation of the small GTPase Rac1, a regulator of actin cytoskeletal remodeling, and lower numbers of F-actin-forming protrusions. Furthermore, mice adoptively transferred with peptide-pulsed CAV1 DCs showed reduced CD8 T cell responses and antitumor protection. Our results suggest that CAV1 promotes the activation of Rac1 and the formation of membrane protrusions that favor DC chemotactic trafficking toward LNs where they can initiate cytotoxic T cell responses.

**Year of publication 2017**

Hélène D Moreau, Philippe Bousso, Ana-Maria Lennon-Duménil (2017 Mar 4)

**Microchannels for the Study of T Cell Immunological Synapses and Kinapses.**

> **Summary**

T Cells can form very stable (synapses) or very transient and migratory (kinapses) contacts with antigen-presenting cells. Here, we describe how microchannels can be used to conveniently study the distinct dynamics of T cells during antigen recognition. Microchannels provide a controlled confined environment that promotes T cell migration and recapitulates kinapse and synapse behaviors when coated with appropriate pMHC molecules. We also depict the advantages of this in vitro approach for addressing mechanistic issues and for analysis.

**Year of publication 2016**

Paolo Pierobon, Ana-Maria Lennon-Duménil (2016 Dec 22)
To use or not to use the force: How B lymphocytes extract surface-tethered antigens.

*The Journal of cell biology*: DOI: jcb.201612043

**Summary**

Using an exquisite cell imaging approach based on DNA nanosensors, Spillane and Tolar (2016. J. Cell Biol. https://doi.org/10.1083/jcb.201607064) explore how the physical properties of antigen-presenting cell surfaces affect how B cells internalize surface-tethered antigens. Soft and flexible surfaces promote mechanical force-mediated antigen extraction, whereas stiff surfaces lead to enzyme-mediated antigen release before subsequent internalization.