Année de publication : 2020

Laurene Aoun, Alexander Farutin, Nicolas Garcia-Seyda, Paulin Nègre, Mohd Suhail Rizvi, Sham Tlili, Solene Song, Xuan Luo, Martine Biarnes-Pelicot, Rémi Galland, Jean-Baptiste Sibarita, Alphée Michelot, Claire Hivroz, Salima Rafai, Marie-Pierre Valignat, Chaouqi Misbah, Olivier Theodoly (2020 Sep 4)

Amoeboid Swimming Is Propelled by Molecular Paddling in Lymphocytes.

Biophysical journal : 1157-1177 : DOI : S0006-3495(20)30604-4

Résumé

Mammalian cells developed two main migration modes. The slow mesenchymatous mode, like crawling of fibroblasts, relies on maturation of adhesion complexes and actin fiber traction, whereas the fast amoeboid mode, observed exclusively for leukocytes and cancer cells, is characterized by weak adhesion, highly dynamic cell shapes, and ubiquitous motility on two-dimensional and in three-dimensional solid matrix. In both cases, interactions with the substrate by adhesion or friction are widely accepted as a prerequisite for mammalian cell motility, which precludes swimming. We show here experimental and computational evidence that leukocytes do swim, and that efficient propulsion is not fueled by waves of cell deformation but by a rearward and inhomogeneous treadmilling of the cell external membrane. Our model consists of a molecular paddling by transmembrane proteins linked to and advected by the actin cortex, whereas freely diffusing transmembrane proteins hinder swimming. Furthermore, continuous paddling is enabled by a combination of external treadmilling and selective recycling by internal vesicular transport of cortex-bound transmembrane proteins. This mechanism explains observations that swimming is five times slower than the retrograde flow of cortex and also that lymphocytes are motile in nonadherent confined environments. Resultantly, the ubiquitous ability of mammalian amoeboid cells to migrate in two dimensions or three dimensions and with or without adhesion can be explained for lymphocytes by a single machinery of heterogeneous membrane treadmilling.

Eliot Morrison, Tatjana Wegner, Andres Ernesto Zucchetti, Miguel Álvaro-Benito, Ashley Zheng, Stefanie Kliche, Eberhard Krause, Britta Brügger, Claire Hivroz, Christian Freund (2020 Jul 12)

Dynamic palmitoylation events following T-cell receptor signaling.

Communications biology : 368 : DOI : 10.1038/s42003-020-1063-5

Résumé

Palmitoylation is the reversible addition of palmitate to cysteine via a thioester linkage. The reversible nature of this modification makes it a prime candidate as a mechanism for regulating signal transduction in T-cell receptor signaling. Following stimulation of the T-cell receptor we find a number of proteins are newly palmitoylated, including those involved in vesicle-mediated transport and Ras signal transduction. Among these stimulation-dependent palmitoylation targets are the v-SNARE VAMP7, important for docking of vesicular LAT during TCR signaling, and the largely undescribed palmitoyl acyltransferase DHHC18 that is

expressed in two isoforms in T cells. Using our newly developed On-Plate Palmitoylation Assay (OPPA), we show DHHC18 is capable of palmitoylating VAMP7 at Cys183. Cellular imaging shows that the palmitoylation-deficient protein fails to be retained at the Golgi and to localize to the immune synapse upon T cell activation.

Irini Evnouchidou, Pascal Chappert, Samira Benadda, Andres Zucchetti, Mirjana Weimershaus, Marcelle Bens, Vivien Caillens, Despoina Koumantou, Sophie Lotersztajn, Peter van Endert, Jean Davoust, Pierre Guermonprez, Claire Hivroz, David A Gross, Loredana Saveanu (2020 Jun 4)
IRAP-dependent endosomal T cell receptor signalling is essential for T cell responses.
Nature communications : 2779 : DOI : 10.1038/s41467-020-16471-7

Résumé

T cell receptor (TCR) activation is modulated by mechanisms such as TCR endocytosis, which is thought to terminate TCR signalling. Here we show that, upon internalization, TCR continues to signal from a set of specialized endosomes that are crucial for T cell functions. Mechanistically, TCR ligation leads to clathrin-mediated internalization of the TCR-CD3ζ complex, while maintaining CD3ζ signalling, in endosomal vesicles that contain the insulin responsive aminopeptidase (IRAP) and the SNARE protein Syntaxin 6. Destabilization of this compartment through IRAP deletion enhances plasma membrane expression of the TCR-CD3ζ complex, yet compromises overall CD3ζ signalling; moreover, the integrity of this compartment is also crucial for T cell activation and survival after suboptimal TCR activation, as mice engineered with a T cell-specific deletion of IRAP fail to develop efficient polyclonal anti-tumour responses. Our results thus reveal a previously unappreciated function of IRAP-dependent endosomal TCR signalling in T cell activation.

Mélanie Chabaud, Noémie Paillon, Katharina Gaus, Claire Hivroz (2020 Apr 11)
Mechanobiology of antigen-induced T cell arrest.
Biology of the cell : 196-212 : DOI : 10.1111/boc.201900093

Résumé

To mount an immune response, T cells must first find rare antigens present at the surface of antigen-presenting cells (APCs). They achieve this by migrating rapidly through the crowded space of tissues and constantly sampling the surface of APCs. Upon antigen recognition, T cells decelerate and polarise towards the APC, ultimately forming a specialised interface known as the immunological synapse. These conjugates form as the result of the interaction between pairs of receptors/ligands that are under mechanical stress due to the continuously reorganising cell cytoskeleton. In this review, we discuss the involvement of mechanical forces during antigen recognition by migrating T cells. We will explore this question from a conceptual and technical perspective, with the aim of providing new insights into the emerging field of mechanobiology.

Année de publication : 2019

Gehrmann U1,2, Burbage M3, Zueva E3, Goudot C3, Esnault C4, Ye M3, Carpier JM3, Burgdorf N3, Hoyler T3, Suarez G3, Joannas L3, Heurtebise-Chrétien S3, Durand S5,6, Panes R7,8, Bellemare-Pelletier A8, Sáez PJ3, Aprahamian F5,6, Lefevre D5,6, Adoue V9, Zine El Aabidine A4, Muhammad Ahmad M4, Hivroz C3, Joffre O10, Cammas F11,12, Kroemer G5,6,13,14,15, Gagnon E7,8, Andrau JC4, Amigorena S1. (2019 Dec 17)

Critical role for TRIM28 and HP1β/γ in the epigenetic control of T cell metabolic reprograming and effector differentiation.

Proceedings of the National Academy of Sciences : 116 : Proc Natl Acad Sci U S A. 2019 Dec 17;116(51):25839-25849. doi: 10.1073/pnas.1901639116. Epub 2019 Nov 27. : 25839,25849 : DOI : 10.1073/pnas.1901639116

Résumé

Naive $CD4^+$ T lymphocytes differentiate into different effector types, including helper and regulatory cells (Th and Treg, respectively). Heritable gene expression programs that define these effector types are established during differentiation, but little is known about the epigenetic mechanisms that install and maintain these programs. Here, we use mice defective for different components of heterochromatin-dependent gene silencing to investigate the epigenetic control of $CD4^+$ T cell plasticity. We show that, upon T cell receptor (TCR) engagement, naive and regulatory T cells defective for TRIM28 (an epigenetic adaptor for histone binding modules) or for heterochromatin protein 1 β and γ isoforms (HP1β/γ, 2 histone-binding factors involved in gene silencing) fail to effectively signal through the PI3K-AKT-mTOR axis and switch to glycolysis. While differentiation of naive $TRIM28^{-/-}$ T cells into cytokine-producing effector T cells is impaired, resulting in reduced induction of autoimmune colitis, $TRIM28^{-/-}$ regulatory T cells also fail to expand in vivo and to suppress autoimmunity effectively. Using a combination of transcriptome and chromatin immunoprecipitation-sequencing (ChIP-seq) analyses for H3K9me3, H3K9Ac, and RNA polymerase II, we show that reduced effector differentiation correlates with impaired transcriptional silencing at distal regulatory regions of a defined set of Treg-associated genes, including, for example, NRP1 or Snai3. We conclude that TRIM28 and HP1β/γ control metabolic reprograming through epigenetic silencing of a defined set of Treg-characteristic genes, thus allowing effective T cell expansion and differentiation into helper and regulatory phenotypes.

Loredana Saveanu, Andres E Zucchetti, Irini Evnouchidou, Laurence Ardouin, Claire Hivroz (2019 Aug 13)

Is there a place and role for endocytic TCR signaling?

Immunological reviews : 57-74 : DOI : 10.1111/imr.12764

Résumé

T-lymphocyte activation relies on the cognate recognition by the TCR of the MHC-associated

peptide ligand (pMHC) presented at the surface of an antigen-presenting cell (APC). This leads to the dynamic formation of a cognate contact between the T lymphocyte and the APC: the immune synapse (IS). Engagement of the TCR by the pMHC in the synaptic zone induces a cascade of signaling events leading to phosphorylation and dephosphorylation of proteins and lipids, which ultimately shapes the response of T lymphocytes. Although the engagement of the T-cell receptor (TCR) takes place at the plasma membrane, the TCR/CD3 complexes and the signaling molecules involved in transduction of the TCR signal are also present in intracellular membrane pools. These pools, which are both endocytic and exocytic, have tentatively been characterized by several groups including ours. We will herein summarize what is known on the intracellular pools of TCR signaling components. We will discuss their origin and the mechanisms involved in their mobility at the IS. Finally, we will propose several hypotheses concerning the functional role(s) that these intracellular pools might play in T-cell activation. We will also discuss the tools that could be used to test these hypotheses.

Andres Ernesto Zucchetti, Laurence Bataille, Jean-Marie Carpier, Stéphanie Dogniaux, Mabel San Roman-Jouve, Mathieu Maurin, Michael W Stuck, Rosa M Rios, Cosima T Baldari, Gregory J Pazour, Claire Hivroz (2019 Jun 30)

Tethering of vesicles to the Golgi by GMAP210 controls LAT delivery to the immune synapse.

Nature communications : 2864 : DOI : 10.1038/s41467-019-10891-w

Résumé

The T cell immune synapse is a site of intense vesicular trafficking. Here we show that the golgin GMAP210, known to capture vesicles and organize membrane traffic at the Golgi, is involved in the vesicular transport of LAT to the immune synapse. Upon activation, more GMAP210 interact with LAT-containing vesicles and go together with LAT to the immune synapse. Regulating LAT recruitment and LAT-dependent signaling, GMAP210 controls T cell activation. Using a rerouting and capture assay, we show that GMAP210 captures VAMP7-decorated vesicles. Overexpressing different domains of GMAP210, we also show that GMAP210 allows their specific delivery to the immune synapse by tethering LAT-vesicles to the Golgi. Finally, in a model of ectopic expression of LAT in ciliated cells, we show that GMAP210 tethering activity controls the delivery of LAT to the cilium. Hence, our results reveal a function for the golgin GMAP210 conveying specific vesicles to the immune synapse.

Année de publication : 2018

Jean-Marie Carpier, Andres E Zucchetti, Laurence Bataille, Stéphanie Dogniaux, Massiullah Shafaq-Zadah, Sabine Bardin, Marco Lucchino, Mathieu Maurin, Leonel D Joannas, Joao Gamelas Magalhaes, Ludger Johannes, Thierry Galli, Bruno Goud, Claire Hivroz (2018 Feb 15)

Rab6-dependent retrograde traffic of LAT controls immune synapse formation and T cell activation.

The Journal of experimental medicine : 1245-1265 : DOI : 10.1084/jem.20162042

Résumé

The adapter molecule linker for activation of T cells (LAT) orchestrates the formation of signalosomes upon T cell receptor (TCR) stimulation. LAT is present in different intracellular pools and is dynamically recruited to the immune synapse upon stimulation. However, the intracellular traffic of LAT and its function in T lymphocyte activation are ill defined. We show herein that LAT, once internalized, transits through the Golgi-trans-Golgi network (TGN), where it is repolarized to the immune synapse. This retrograde transport of LAT depends on the small GTPase Rab6 and the target soluble -ethylmaleimide-sensitive factor attachment protein receptor (t-SNARE) Syntaxin-16, two regulators of the endosome-to-Golgi/TGN retrograde transport. We also show in vitro in Syntaxin-16- or Rab6-silenced human cells and in vivo in CD4 T lymphocytes of the Rab6 knockout mouse that this retrograde traffic controls TCR stimulation. These results establish that the retrograde traffic of LAT from the plasma membrane to the Golgi-TGN controls the polarized delivery of LAT at the immune synapse and T lymphocyte activation.

Année de publication : 2017

Anna Sawicka, Avin Babataheri, Stéphanie Dogniaux, Abdul I Barakat, David Gonzalez-Rodriguez, Claire Hivroz, Julien Husson (2017 Sep 22)

Micropipette Force Probe to quantify single-cell force generation: application to T cell activation.

Molecular biology of the cell : DOI : mbc.E17-06-0385

Résumé

In response to engagement of surface molecules, cells generate active forces that regulate many cellular processes. Developing tools that permit gathering mechanical and morphological information on these forces is of the utmost importance. Here we describe a new technique, the Micropipette Force Probe, that uses a micropipette as a flexible cantilever that can aspirate at its tip a bead that is coated with molecules of interest and is brought in contact with the cell. This technique simultaneously allows tracking the resulting changes in cell morphology and mechanics as well as measuring the forces generated by the cell. To illustrate the power of this technique, we applied it to the study of human primary T lymphocytes (T cells). It allowed the fine monitoring of pushing and pulling forces generated by T cells in response to various activating antibodies and bending stiffness of the micropipette. We further dissected the sequence of mechanical and morphological events occurring during T cell activation to model force generation and to reveal heterogeneity in the cell population studied. We also report the first measurement of the changes in Young's modulus of T cells during their activation, showing that T cells stiffen within the first minutes of the activation process.

Michael Saitakis, Stéphanie Dogniaux, Christel Goudot, Nathalie Bufi, Sophie Asnacios, Mathieu Maurin, Clotilde Randriamampita, Atef Asnacios, Claire Hivroz (2017 Jun 9)

Different TCR-induced T lymphocyte responses are potentiated by stiffness with variable sensitivity.

eLife : DOI : 10.7554/eLife.23190

Résumé

T cells are mechanosensitive but the effect of stiffness on their functions is still debated. We characterize herein how human primary CD4(+) T cell functions are affected by stiffness within the physiological Young's modulus range of 0.5 kPa to 100 kPa. Stiffness modulates T lymphocyte migration and morphological changes induced by TCR/CD3 triggering. Stiffness also increases TCR-induced immune system, metabolism and cell-cycle-related genes. Yet, upon TCR/CD3 stimulation, while cytokine production increases within a wide range of stiffness, from hundreds of Pa to hundreds of kPa, T cell metabolic properties and cell cycle progression are only increased by the highest stiffness tested (100 kPa). Finally, mechanical properties of adherent antigen-presenting cells modulate cytokine production by T cells. Together, these results reveal that T cells discriminate between the wide range of stiffness values found in the body and adapt their responses accordingly.

Claire Hivroz, Paola Larghi, Mabel Jouve, Laurence Ardouin (2017 Mar 4)

Purification of LAT-Containing Membranes from Resting and Activated T Lymphocytes.

Methods in molecular biology (Clifton, N.J.) : 355-368 : DOI : 10.1007/978-1-4939-6881-7_21

Résumé

In T lymphocytes, the immune synapse is an active zone of vesicular traffic. Directional transport of vesicular receptors and signaling molecules from or to the immune synapse has been shown to play an important role in T-cell receptor (TCR) signal transduction. However, how vesicular trafficking is regulating the activation of T cells is still a burning question, and the characterization of these intracellular compartments remains the first step to understand this process. We describe herein a protocol, which combines a separation of membranes on flotation gradient with an affinity purification of Strep-tagged fusion transmembrane proteins with Strep-Tactin(®) resin, allowing the purification of membranes containing the Strep-tagged molecule of interest. By keeping the membranes intact, this protocol leads to the purification of molecules physically associated with the Strep-tagged protein as well as of molecules present in the same membrane compartment: transmembrane proteins, proteins strongly associated with the membranes, and luminal proteins. The example shown herein is the purification of membrane compartment prepared from T lymphocytes expressing LAT fused to a Strep-tag.

Année de publication : 2016

Asma Beldi-Ferchiou, Marion Lambert, Stéphanie Dogniaux, Frédéric Vély, Eric Vivier, Daniel Olive, Stéphanie Dupuy, Frank Levasseur, David Zucman, Céleste Lebbé, Damien Sène, Claire Hivroz, Sophie Caillat-Zucman (2016 Sep 24)

PD-1 mediates functional exhaustion of activated NK cells in patients with Kaposi sarcoma.

Oncotarget : DOI : 10.18632/oncotarget.12150

Résumé

Programmed Death-1 (PD-1), an inhibitory receptor expressed by activated lymphocytes, is involved in regulating T- and B-cell responses. PD-1 and its ligands are exploited by a variety of cancers to facilitate tumor escape through PD-1-mediated functional exhaustion of effector T cells. Here, we report that PD-1 is upregulated on Natural Killer (NK) cells from patients with Kaposi sarcoma (KS). PD-1 was expressed in a sub-population of activated, mature CD56dimCD16pos NK cells with otherwise normal expression of NK surface receptors. PD-1pos NK cells from KS patients were hyporesponsive ex vivo following direct triggering of NKp30, NKp46 or CD16 activating receptors, or short stimulation with NK cell targets. PD-1pos NK cells failed to degranulate and release IFNγ, but exogenous IL-2 or IL-15 restored this defect. That PD-1 contributed to NK cell functional impairment and was not simply a marker of dysfunctional NK cells was confirmed in PD-1-transduced NKL cells. In vitro, PD-1 was induced at the surface of healthy control NK cells upon prolonged contact with cells expressing activating ligands, i.e. a condition mimicking persistent stimulation by tumor cells. Thus, PD-1 appears to plays a critical role in mediating NK cell exhaustion. The existence of this negative checkpoint fine-tuning NK activation highlights the possibility that manipulation of the PD-1 pathway may be a strategy for circumventing tumor escape not only from the T cell-, but also the NK-cell mediated immune surveillance.

Lionel Guillou, Avin Babataheri, Michael Saitakis, Armelle Bohineust, Stéphanie Dogniaux, Claire Hivroz, Abdul I Barakat, Julien Husson (2016 Sep 9)

T lymphocyte passive deformation is controlled by unfolding of membrane surface reservoirs.

Molecular biology of the cell : DOI : mbc.E16-06-0414

Résumé

T lymphocytes in the human body routinely undergo large deformations, both passively when going through narrow capillaries and actively when transmigrating across endothelial cells or squeezing through tissue. We investigate physical factors that enable and limit such deformations and explore how passive and active deformations may differ. Employing micropipette aspiration to mimic squeezing through narrow capillaries, we find that T lymphocytes maintain a constant volume while increasing their apparent membrane surface area upon aspiration. Human resting T lymphocytes, T lymphoblasts and the leukemic Jurkat T cells all exhibit membrane rupture above a critical membrane area expansion that is

independent of either micropipette size or aspiration pressure. The unfolded membrane matches the excess membrane contained in microvilli and membrane folds, as determined using scanning electron microscopy. In contrast, during transendothelial migration, a form of active deformation, we find that the membrane surface exceeds by a factor of two the amount of membrane stored in microvilli and folds. These results suggest that internal membrane reservoirs need to be recruited, possibly through exocytosis, for large active deformations to occur.

Chantal Lagresle-Peyrou, Sonia Luce, Farid Ouchani, Tayebeh Shabi Soheili, Hanem Sadek, Myriam Chouteau, Amandine Durand, Isabelle Pic, Jacek Majewski, Chantal Brouzes, Nathalie Lambert, Armelle Bohineust, Els Verhoeyen, François-Loïc Cosset, Aude Magerus-Chatinet, Frédéric Rieux-Laucat, Virginie Gandemer, Delphine Monnier, Catherine Heijmans, Marielle van Gijn, Virgil A Dalm, Nizar Mahlaoui, Jean-Louis Stephan, Capucine Picard, Anne Durandy, Sven Kracker, Claire Hivroz, Nada Jabado, Geneviève de Saint Basile, Alain Fischer, Marina Cavazzana, Isabelle André-Schmutz (2016 Jul 14)

X-linked primary immunodeficiency associated with hemizygous mutations in the moesin (MSN) gene.

The Journal of allergy and clinical immunology : DOI : S0091-6749(16)30423-7

Résumé

We investigated 7 male patients (from 5 different families) presenting with profound lymphopenia, hypogammaglobulinemia, fluctuating monocytopenia and neutropenia, a poor immune response to vaccine antigens, and increased susceptibility to bacterial and varicella zoster virus infections.

Roshni Basu, Benjamin M Whitlock, Julien Husson, Audrey Le Floc'h, Weiyang Jin, Alon Oyler-Yaniv, Farokh Dotiwala, Gregory Giannone, Claire Hivroz, Nicolas Biais, Judy Lieberman, Lance C Kam, Morgan Huse (2016 Mar 1)

Cytotoxic T Cells Use Mechanical Force to Potentiate Target Cell Killing.

Cell : 100-10 : DOI : 10.1016/j.cell.2016.01.021

Résumé

The immunological synapse formed between a cytotoxic T lymphocyte (CTL) and an infected or transformed target cell is a physically active structure capable of exerting mechanical force. Here, we investigated whether synaptic forces promote the destruction of target cells. CTLs kill by secreting toxic proteases and the pore forming protein perforin into the synapse. Biophysical experiments revealed a striking correlation between the magnitude of force exertion across the synapse and the speed of perforin pore formation on the target cell, implying that force potentiates cytotoxicity by enhancing perforin activity. Consistent with this interpretation, we found that increasing target cell tension augmented pore formation by perforin and killing by CTLs. Our data also indicate that CTLs coordinate perforin release and

force exertion in space and time. These results reveal an unappreciated physical dimension to lymphocyte function and demonstrate that cells use mechanical forces to control the activity of outgoing chemical signals.