Ester Gea-Mallorquí, Laurent Zablocki-Thomas, Mathieu Maurin, Mabel Jouve, Vasco Rodrigues, Nicolas Ruffin, Philippe Benaroch (2020 Aug 6)  
**HIV-2-Infected Macrophages Produce and Accumulate Poorly Infectious Viral Particles.**  
*Frontiers in microbiology*: 1603 : [DOI: 10.3389/fmicb.2020.01603]

**Summary**

A significant proportion of HIV-2-infected patients exhibit natural virological control that is generally absent from HIV-1-infected patients. Along with CD4 T cells, HIV-1 targets macrophages which may contribute to viral spreading and the latent reservoir. We have studied the relationship between macrophages and HIV-2, focusing on post-entry steps. HIV-2-infected monocyte-derived macrophages (MDMs) produced substantial amounts of viral particles that were largely harbored intracellularly. New viruses assembled at the limiting membrane of internal compartments similar to virus-containing compartments (VCCs) described for HIV-1. VCCs from MDMs infected with either virus shared protein composition and morphology. Strikingly, HIV-2 Gag was mostly absent from the cytosol and almost exclusively localized to the VCCs, whereas HIV-1 Gag was distributed in both locations. Ultrastructural analyses of HIV-2-infected MDMs revealed the presence of numerous VCCs containing both immature and mature particles in the lumen. HIV-2 particles produced by MDMs were poorly infectious in reporter cells and in transmission to activated T cells through a process that appeared independent of BST2 restriction. Rather than being involved in viral spreading, HIV-2-infected macrophages may represent a cell-associated source of viral antigens that can participate in the immune control of HIV-2 infection.

Giulia Maria Piperno, Asma Naseem, Giulia Silvestrelli, Roberto Amadio, Nicoletta Caronni, Karla Evelia Cervantes-Luevano, Nalan Liv, Judith Klumperman, Andrea Colliva, Hashim Ali, Francesca Graziano, Philippe Benaroch, Hans Haecker, Richard N Hanna, Federica Benvenuti (2020 Jul 30)  
**Wiskott-Aldrich syndrome protein restricts cGAS/STING activation by dsDNA immune complexes.**  
*JCI insight*: [DOI: 10.1172/jci.insight.132857]

**Summary**

Dysregulated sensing of self-nucleic acid is a leading cause of autoimmunity in multifactorial and monogenic diseases. Mutations in Wiskott-Aldrich syndrome protein (WASp), a key regulator of cytoskeletal dynamics in immune cells, cause autoimmune manifestations and increased production of type I IFNs by innate cells. Here we show that immune complexes of self-DNA and autoantibodies (DNA-ICs) contribute to elevated IFN levels via activation of the cGAS/STING pathway of cytosolic sensing. Mechanistically, lack of endosomal F-actin nucleation by WASp caused a delay in endolysosomal maturation and prolonged the transit time of ingested DNA-ICs. Stalling in maturation-defective organelles facilitated leakage of DNA-ICs into the cytosol, promoting activation of the TBK1/STING pathway. Genetic deletion
of STING and STING and cGAS chemical inhibitors abolished IFN production and rescued systemic activation of IFN-stimulated genes in vivo. These data unveil the contribution of cytosolic self-nucleic acid sensing in WAS and underscore the importance of WASp-mediated endosomal actin remodeling in preventing innate activation.

Flavien Brouiller, Nicolas Ruffin, Philippe Benaroch (2020 May 2)
[A new population of blood precursors of dendritic cells endowed with specific properties regarding HIV-1].
Medecine sciences : M/S : 316-319 : DOI : 10.1051/medsci/2020050

Summary

Laurent Zablocki-Thomas, Sam A Menzies, Paul J Lehner, Nicolas Manel, Philippe Benaroch (2020 Apr 7)
A genome-wide CRISPR screen identifies regulation factors of the TLR3 signalling pathway.
Innate immunity : 459-472 : DOI : 10.1177/1753425920915507

Summary

A subset of TLRs is specialised in the detection of incoming pathogens by sampling endosomes for nucleic acid contents. Among them, TLR3 senses the abnormal presence of double-stranded RNA in the endosomes and initiates a potent innate immune response via activation of NF-κB and IRF3. Nevertheless, mechanisms governing TLR3 regulation remain poorly defined. To identify new molecular players involved in the TLR3 pathway, we performed a genome-wide screen using CRISPR/Cas9 technology. We generated TLR3 reporter cells carrying a NF-κB-responsive promoter that controls GFP expression. Cells were next transduced with a single-guide RNA (sgRNA) library, subjected to sequential rounds of stimulation with poly(I:C) and sorting of the GFP-negative cells. Enrichments in sgRNA estimated by deep sequencing identified genes required for TLR3-induced activation of NF-κB. Among the hits, five genes known to be critically involved in the TLR3 pathway, including TLR3 itself and the chaperone UNC93B1, were identified by the screen, thus validating our strategy. We further studied the top 40 hits and focused on the transcription factor aryl hydrocarbon receptor (AhR). Depletion of AhR had a dual effect on the TLR3 response, abrogating IL-8 production and enhancing IP-10 release. Moreover, in primary human macrophages exposed to poly(I:C), AhR activation enhanced IL-8 and diminished IP-10 release. Overall, these results reveal AhR plays a role in the TLR3 cellular innate immune response.

Year of publication 2019

Ruffin N1, Gea-Mallorquí E1, Brouiller F1, Jouve M2, Silvin A1,3, See P3, Dutertre CA3,4, Ginhoux
Constitutive Siglec-1 expression confers susceptibility to HIV-1 infection of human dendritic cell precursors.

Proceedings of the National Academy of Sciences : 116 : Proc Natl Acad Sci U S A. 2019 Oct 22;116(43):21685-21693. doi: 10.1073/pnas.1911007116. Epub 2019 Oct 7. : 21685,21693 : DOI: 10.1073/pnas.1911007116

Summary

The human dendritic cell (DC) lineage has recently been unraveled by high-dimensional mapping, revealing the existence of a discrete new population of blood circulating DC precursors (pre-DCs). Whether this new DC population possesses specific functional features as compared to the other blood DC subset upon pathogen encounter remained to be evaluated. A unique feature of pre-DCs among blood DCs is their constitutive expression of the viral adhesion receptor Siglec-1. Here, we show that pre-DCs, but not other blood DC subsets, are susceptible to infection by HIV-1 in a Siglec-1-dependent manner. Siglec-1 mediates pre-DC infection of CCR5- and CXCR4-tropic strains. Infection of pre-DCs is further enhanced in the presence of HIV-2/SIVmac Vpx, indicating that Siglec-1 does not counteract restriction factors such as SAMHD1. Instead, Siglec-1 promotes attachment and fusion of viral particles. HIV-1-infected pre-DCs produce new infectious viral particles that accumulate in intracellular compartments reminiscent of the virus-containing compartment of macrophages. Pre-DC activation by toll-like receptor (TLR) ligands induces an antiviral state that inhibits HIV-1 fusion and infection, but Siglec-1 remains functional and mediates replication-independent transfer of HIV-1 to activated primary T lymphocytes. Altogether, Siglec-1-mediated susceptibility to HIV-1 infection of pre-DCs constitutes a unique functional feature that might represent a preferential relationship of this emerging cell type with viruses.

Clonally Expanded T Cells Reveal Immunogenicity of Rhabdoid Tumors.

Cancer cell : 597-612.e8 : DOI : S1535-6108(19)30482-9

Summary

Rhabdoid tumors (RTs) are genomically simple pediatric cancers driven by the biallelic inactivation of SMARCB1, leading to SWI/SNF chromatin remodeler complex deficiency. Comprehensive evaluation of the immune infiltrates of human and mice RTs, including immunohistochemistry, bulk RNA sequencing and DNA methylation profiling studies showed
a high rate of tumors infiltrated by T and myeloid cells. Single-cell RNA (scRNA) and T cell receptor sequencing highlighted the heterogeneity of these cells and revealed therapeutically targetable exhausted effector and clonally expanded tissue resident memory CD8 T subpopulations, likely representing tumor-specific cells. Checkpoint blockade therapy in an experimental RT model induced the regression of established tumors and durable immune responses. Finally, we show that one mechanism mediating RTs immunogenicity involves SMARCB1-dependent re-expression of endogenous retroviruses and interferon-signaling activation.

Emiliano Roselli, Paula Araya, Nicolás Gonzalo Núñez, Gerardo Gatti, Francesca Graziano, Christine Sedlik, Philippe Benaroch, Eliane Piaggio, Mariana Maccioni (2019 Apr 6)

**TLR3 Activation of Intratumoral CD103 Dendritic Cells Modifies the Tumor Infiltrate Conferring Anti-tumor Immunity.**

*Frontiers in immunology*: 503 : [DOI: 10.3389/fimmu.2019.00503]

**Summary**

An important challenge in cancer immunotherapy is to expand the number of patients that benefit from immune checkpoint inhibitors (CI), a fact that has been related to the pre-existence of an efficient anti-tumor immune response. Different strategies are being proposed to promote tumor immunity and to be used in combined therapies with CI. Recently, we reported that intratumoral administration of naked poly A:U, a dsRNA mimetic empirically used in early clinical trials with some success, delays tumor growth and prolongs mice survival in several murine cancer models. Here, we show that CD103 cDC1 and, to a much lesser extent CD11b cDC2, are the only populations expressing TLR3 at the tumor site, and consequently could be potential targets of poly A:U. Upon poly A:U administration these cells become activated and elicit profound changes in the composition of the tumor immune infiltrate, switching the immune suppressive tumor environment to anti-tumor immunity. The sole administration of naked poly A:U promotes striking changes within the lymphoid compartment, with all the anti-tumoral parameters being enhanced: a higher frequency of CD8 Granzyme B T cells, (lower Treg/CD8 ratio) and an important expansion of tumor-antigen specific CD8 T cells. Also, PD1/PDL1 showed an increased expression indicating that neutralization of this axis could be exploited in combination with poly A:U. Our results shed new light to promote further assays in this dsRNA mimetic to the clinical field.

Vasco Rodrigues, Philippe Benaroch (2019 Mar 23)

**Macrophages hide HIV in the urethra.**

*Nature microbiology*: 556-557 : [DOI: 10.1038/s41564-019-0418-5]

**Summary**
Year of publication 2018

Anna Baranska, Alaa Shawket, Mabel Jouve, Myriam Baratin, Camille Malosse, Odessa Voluzan, Thien-Phong Vu Manh, Frédéric Fiore, Marc Bajénoff, Philippe Benaroch, Marc Dalod, Marie Malissen, Sandrine Henri, Bernard Malissen (2018 Mar 8)

Unveiling skin macrophage dynamics explains both tattoo persistence and strenuous removal.
*The Journal of experimental medicine*: 1115-1133 : [DOI: 10.1084/jem.20171608](https://doi.org/10.1084/jem.20171608)

Summary

Here we describe a new mouse model that exploits the pattern of expression of the high-affinity IgG receptor (CD64) and allows diphtheria toxin (DT)-mediated ablation of tissue-resident macrophages and monocyte-derived cells. We found that the myeloid cells of the ear skin dermis are dominated by DT-sensitive, melanin-laden cells that have been missed in previous studies and correspond to macrophages that have ingested melanosomes from neighboring melanocytes. Those cells have been referred to as melanophages in humans. We also identified melanophages in melanocytic melanoma. Benefiting of our knowledge on melanophage dynamics, we determined the identity, origin, and dynamics of the skin myeloid cells that capture and retain tattoo pigment particles. We showed that they are exclusively made of dermal macrophages. Using the possibility to delete them, we further demonstrated that tattoo pigment particles can undergo successive cycles of capture-release-recapture without any tattoo vanishing. Therefore, congruent with dermal macrophage dynamics, long-term tattoo persistence likely relies on macrophage renewal rather than on macrophage longevity.

Year of publication 2017

Vasco Rodrigues, Nicolas Ruffin, Mabel San-Roman, Philippe Benaroch (2017 Dec 19)

Myeloid Cell Interaction with HIV: A Complex Relationship.
*Frontiers in immunology*: 1698 : [DOI: 10.3389/fimmu.2017.01698](https://doi.org/10.3389/fimmu.2017.01698)

Summary

Cells of the myeloid lineage, particularly macrophages, serve as primary hosts for HIV, along with CD4 T lymphocytes. Macrophages are present in virtually every tissue of the organism, including locations with negligible T cell colonization, such as the brain, where HIV-mediated inflammation may lead to pathological sequelae. Moreover, infected macrophages are present in multiple other tissues. Recent evidence obtained in humanized mice and macaque models highlighted the capacity of macrophages to sustain HIV replication in the absence of T cells. Combined with the known resistance of the macrophage to the cytopathic effects of HIV infection, such data bring a renewed interest in this cell type both as a vehicle for viral spread as well as a viral reservoir. While our understanding of key processes of HIV infection of macrophages is far from complete, recent years have nevertheless brought important insight into the uniqueness of the macrophage infection. Productive infection of
macrophages by HIV can occur by different routes including from phagocytosis of infected T cells. In macrophages, HIV assembles and buds into a peculiar plasma membrane-connected compartment that preexists to the infection. While the function of such compartment remains elusive, it supposedly allows for the persistence of infectious viral particles over extended periods of time and may play a role on viral transmission. As cells of the innate immune system, macrophages have the capacity to detect and respond to viral components. Recent data suggest that such sensing may occur at multiple steps of the viral cycle and impact subsequent viral spread. We aim to provide an overview of the HIV-macrophage interaction along the multiple stages of the viral life cycle, extending when pertinent such observations to additional myeloid cell types such as dendritic cells or blood monocytes.

Jérémie Decalf, Marion Desdouits, Vasco Rodrigues, François-Xavier Gobert, Matteo Gentili, Santy Marques-Ladeira, Célia Chamontin, Marylène Mougel, Bruna Cunha de Alencar, Philippe Benaroch (2017 May 12)

Sensing of HIV-1 Entry Triggers a Type I Interferon Response in Human Primary Macrophages.
Journal of virology : DOI : e00147-17

Summary

Along with CD4(+) T lymphocytes, macrophages are a major cellular source of HIV-1 replication and a potential viral reservoir. Following entry and reverse transcription in macrophages, cloaking of the viral cDNA by the HIV-1 capsid limits its cytosolic detection, enabling efficient replication. However, whether incoming HIV-1 particles are sensed by macrophages prior to reverse transcription remains unclear. Here, we show that HIV-1 triggers a broad expression of interferon (IFN)-stimulated genes (ISG) in monocyte-derived macrophages within a few hours after infection. This response does not require viral reverse transcription or the presence of HIV-1 RNA within particles, but viral fusion is essential. This response is elicited by viruses carrying different envelope proteins and thus different receptors to proceed for viral entry. Expression of ISG in response to viral entry requires TBK1 activity and type I IFNs signaling. Remarkably, the ISG response is transient but affects subsequent viral spread. Together, our results shed light on an early step of HIV-1 sensing by macrophages at the level of entry, which confers an early protection through type I IFN signaling and has potential implications in controlling the infection.IMPORTANCE HIV infection is restricted to T lymphocytes and macrophages. HIV-1-infected macrophages are found in many tissues of infected patients, even under antiretroviral therapy, and are considered a viral reservoir. How HIV-1 is detected and what type of responses are elicited upon sensing remain in great part elusive. The kinetics and localization of the production of cytokines such as interferons in response to HIV is of critical importance to understanding how the infection and the immune response are established. Our study provides evidence that macrophages can detect HIV-1 as soon as it enters the cell. Interestingly, this sensing is independent of the presence of viral nucleic acids within the particles but requires their fusion with the macrophages. This triggers a low interferon response, which activates an antiviral program protecting cells against further viral challenge and thus potentially limiting the spread of the infection.
Mapping the human DC lineage through the integration of high-dimensional techniques.
*Science (New York, N.Y.)* : [DOI: eaag3009](https://doi.org/eaag3009)

**Summary**

Dendritic cells (DC) are professional antigen-presenting cells that orchestrate immune responses. The human DC population comprises two main functionally specialized lineages, whose origins and differentiation pathways remain incompletely defined. Here, we combine two high-dimensional technologies—single-cell messenger RNA sequencing (scmRNAseq) and cytometry by time-of-flight (CyTOF)—to identify human blood CD123(+)CD33(+)CD45RA(+) DC precursors (pre-DC). Pre-DC share surface markers with plasmacytoid DC (pDC) but have distinct functional properties that were previously attributed to pDC. Tracing the differentiation of DC from the bone marrow to the peripheral blood revealed that the pre-DC compartment contains distinct lineage-committed subpopulations, including one early uncommitted CD123(high) pre-DC subset and two CD45RA(+)CD123(low) lineage-committed subsets exhibiting functional differences. The discovery of multiple committed pre-DC populations opens promising new avenues for the therapeutic exploitation of DC subset-specific targeting.

Year of publication 2016

**TLR3-Induced Maturation of Murine Dendritic Cells Regulates CTL Responses by Modulating PD-L1 Trafficking.**
*PLoS one* : e0167057 : [DOI: 10.1371/journal.pone.0167057](https://doi.org/10.1371/journal.pone.0167057)

**Summary**

Targeting TLR3 through formulations of polyI:C is widely studied as an adjuvant in cancer immunotherapy. The efficacy of such targeting has been shown to increase in combination with anti-PD-L1 treatment. Nevertheless, the mechanistic details of the effect of polyI:C on DC maturation and the impact on T-DC interactions upon PD-L1 blockade is largely unknown. Here we found that although DC treatment with polyI:C induced a potent inflammatory response including the production of type I interferon, polyI:C treatment of DCs impaired activation of peptide specific CD8+ T cells mainly due to PD-L1. Interestingly, we found that PD-L1 trafficking to the cell surface is regulated in two waves in polyI:C-treated DCs. One
induced upon overnight treatment and a second more rapid one, specific to polyI:C treatment, was induced upon CD40 signaling leading to a further increase in surface PD-L1 in DCs. The polyI:C-induced cell surface PD-L1 reduced the times of contact between DCs and T cells, potentially accounting for limited T cell activation. Our results reveal a novel CD40-dependent regulation of PD-L1 trafficking induced upon TLR3 signaling that dictates its inhibitory activity. These results provide a mechanistic framework to understand the efficacy of anti-PD-L1 cancer immunotherapy combined with TLR agonists.

Summary

HIV type 1 (HIV-1) infects CD4(+) T lymphocytes and tissue macrophages. Infected macrophages differ from T cells in terms of decreased to absent cytopathicity and for active accumulation of new progeny HIV-1 virions in virus-containing compartments (VCC). For these reasons, infected macrophages are believed to act as “Trojan horses” carrying infectious particles to be released on cell necrosis or functional stimulation. Here we explored the hypothesis that extracellular ATP (eATP) could represent a microenvironmental signal potentially affecting virion release from VCC of infected macrophages. Indeed, eATP triggered the rapid release of infectious HIV-1 from primary human monocyte-derived macrophages (MDM) acutely infected with the CCR5-dependent HIV-1 strain. A similar phenomenon was observed in chronically infected promonocytic U1 cells differentiated to macrophage-like cells (D-U1) by costimulation with phorbol esters and urokinase-type plasminogen activator. Worthy of note, eATP did not cause necrotic, apoptotic, or pyroptotic cell death, and its effect on HIV-1 release was suppressed by Imipramine (an antidepressant agent known to inhibit microvesicle formation by interfering with membrane-associated acid sphingomyelinase). Virion release was not triggered by oxidized ATP, whereas the effect of eATP was inhibited by a specific inhibitor of the P2X7 receptor (P2X7R). Thus, eATP triggered the discharge of virions actively accumulating in VCC of infected macrophages via interaction with the P2X7R in the absence of significant cytopathicity. These findings suggest that the microvesicle pathway and P2X7R could represent exploitable targets for interfering with the VCC-associated reservoir of infectious HIV-1 virions in tissue macrophages.
Benaroch, Jorge Geffner, Matías Ostrowski (2015 May 6)

**Rab27a controls HIV-1 assembly by regulating plasma membrane levels of phosphatidylinositol 4,5-bisphosphate.**

*The Journal of cell biology* : 435-52 : [DOI : 10.1083/jcb.201409082]

**Summary**

During the late stages of the HIV-1 replication cycle, the viral polyprotein Pr55(Gag) is recruited to the plasma membrane (PM), where it binds phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) and directs HIV-1 assembly. We show that Rab27a controls the trafficking of late endosomes carrying phosphatidylinositol 4-kinase type 2 α (PI4KIIα) toward the PM of CD4(+) T cells. Hence, Rab27a promotes high levels of PM phosphatidylinositol 4-phosphate and the localized production of PI(4,5)P2, therefore controlling Pr55(Gag) membrane association. Rab27a also controls PI(4,5)P2 levels at the virus-containing compartments of macrophages. By screening Rab27a effectors, we identified that Slp2a, Slp3, and Slac2b are required for the association of Pr55(Gag) with the PM and that Slp2a cooperates with Rab27a in the recruitment of PI4KIIα to the PM. We conclude that by directing the trafficking of PI4KIIα-positive endosomes toward the PM, Rab27a controls PI(4,5)P2 production and, consequently, HIV-1 replication.