Année de publication : 2019

Paola Bonaventura, Tala Shekarian, Vincent Alcazer, Jenny Valladeau-Guilemond, Sandrine Valsesia-Wittmann, Sebastian Amigorena, Christophe Caux, Stéphane Depil (2019 Feb 26)
Cold Tumors: A Therapeutic Challenge for Immunotherapy.
Frontiers in immunology : 168 : DOI : 10.3389/fimmu.2019.00168

Résumé

Therapeutic monoclonal antibodies targeting immune checkpoints (ICPs) have changed the treatment landscape of many tumors. However, response rate remains relatively low in most cases. A major factor involved in initial resistance to ICP inhibitors is the lack or paucity of tumor T cell infiltration, characterizing the so-called « cold tumors. » In this review, we describe the main mechanisms involved in the absence of T cell infiltration, including lack of tumor antigens, defect in antigen presentation, absence of T cell activation and deficit of homing into the tumor bed. We discuss then the different therapeutic approaches that could turn cold into hot tumors. In this way, specific therapies are proposed according to their mechanism of action. In addition, »supra-physiological » therapies, such as T cell recruiting bispecific antibodies and Chimeric Antigen Receptor (CAR) T cells, may be active regardless of the mechanism involved, especially in MHC class I negative tumors. The determination of the main factors implicated in the lack of preexisting tumor T cell infiltration is crucial for the development of adapted algorithms of treatments for cold tumors.

Marine Gros, Sebastian Amigorena (2019 Feb 13)
Regulation of Antigen Export to the Cytosol During Cross-Presentation.
Frontiers in immunology : 41 : DOI : 10.3389/fimmu.2019.00041

Résumé

Cross-priming refers to the induction of primary cytotoxic CD8 T cell responses to antigens that are not expressed in antigen presenting cells (APCs) responsible for T cell priming. Cross-priming is achieved through cross-presentation of exogenous antigens derived from tumors, extracellular pathogens or infected neighboring cells on Major Histocompatibility Complex (MHC) class I molecules. Despite extensive research efforts to understand the intracellular pathways involved in antigen cross-presentation, certain critical steps remain elusive and controversial. Here we review recent advances on antigen cross-presentation, focusing on the mechanisms involved in antigen export to the cytosol, a crucial step of this pathway.

Véronique Adoue, Bénédicte Binet, Agathe Malbec, Joanna Fourquet, Paola Romagnoli, Joost P M van Meerwijk, Sebastian Amigorena, Olivier P Joffre (2019 Feb 10)
The Histone Methyltransferase SETDB1 Controls T Helper Cell Lineage Integrity by Repressing Endogenous Retroviruses.

Immunity : 629-644.e8 : DOI : S1074-7613(19)30003-2

Résumé

Upon activation, naive CD4 T cells differentiate into distinct T cell subsets via processes reliant on epigenetically regulated, lineage-specific developmental programs. Here, we examined the function of the histone methyltransferase SETDB1 in T helper (Th) cell differentiation. Setdb1 naive CD4 T cells exhibited exacerbated Th1 priming, and when exposed to a Th1-instructive signal, Setdb1 Th2 cells crossed lineage boundaries and acquired a Th1 phenotype. SETDB1 did not directly control Th1 gene promoter activity but relied instead on deposition of the repressive H3K9me3 mark at a restricted and cell-type-specific set of endogenous retroviruses (ERVs) located in the vicinity of genes involved in immune processes. Refined bioinformatic analyses suggest that these retrotransposons regulate Th1 gene cis-regulatory elements or act as Th1 gene enhancers. Thus, H3K9me3 deposition by SETDB1 ensures Th cell lineage integrity by repressing a repertoire of ERVs that have been exapted into cis-regulatory modules to shape and control the Th1 gene network.

Année de publication : 2018

Paula Michea, Floriane Noël, Eve Zakine, Urszula Czerwinska, Philémon Sirven, Omar Abouzid, Christel Goudot, Alix Scholer-Dahirel, Anne Vincent-Salomon, Fabien Reyal, Sebastian Amigorena, Maude Guillot-Delost, Elodie Segura, Vassili Soumelis (2018 Jul 18)

Adjustment of dendritic cells to the breast-cancer microenvironment is subset specific.

Nature immunology : 885-897 : DOI : 10.1038/s41590-018-0145-8

Résumé

The functions and transcriptional profiles of dendritic cells (DCs) result from the interplay between ontogeny and tissue imprinting. How tumors shape human DCs is unknown. Here we used RNA-based next-generation sequencing to systematically analyze the transcriptomes of plasmacytoid pre-DCs (pDCs), cell populations enriched for type 1 conventional DCs (cDC1s), type 2 conventional DCs (cDC2s), CD14 DCs and monocytes-macrophages from human primary luminal breast cancer (LBC) and triple-negative breast cancer (TNBC). By comparing tumor tissue with non-invaded tissue from the same patient, we found that 85% of the genes upregulated in DCs in LBC were specific to each DC subset. However, all DC subsets in TNBC commonly showed enrichment for the interferon pathway, but those in LBC did not. Finally, we defined transcriptional signatures specific for tumor DC subsets with a prognostic effect on their respective breast-cancer subtype. We conclude that the adjustment of DCs to the tumor microenvironment is subset specific and can be used to predict disease outcome. Our work also provides a resource for the identification of potential targets and biomarkers that might improve antitumor therapies.

Tsing-Lee Tang-Huau, Paul Gueguen, Christel Goudot, Mélanie Durand, Mylène Bohec, Sylvain Baulande, Benoit Pasquier, Sebastian Amigorena, Elodie Segura (2018 Jul 4)
Human in vivo-generated monocyte-derived dendritic cells and macrophages cross-present antigens through a vacuolar pathway.
Nature communications : 2570 : DOI : 10.1038/s41467-018-04985-0

Résumé

Presentation of exogenous antigens on MHC-I molecules, termed cross-presentation, is essential for cytotoxic CD8 T cell responses. In mice, dendritic cells (DCs) that arise from monocytes (mo-DCs) during inflammation have a key function in these responses by cross-presenting antigens locally in peripheral tissues. Whether human naturally-occurring mo-DCs can cross-present is unknown. Here, we use human mo-DCs and macrophages directly purified from ascites to address this question. Single-cell RNA-seq data show that ascites CD1c DCs contain exclusively monocyte-derived cells. Both ascites mo-DCs and monocyte-derived macrophages cross-present efficiently, but are inefficient for transferring exogenous proteins into their cytosol. Inhibition of cysteine proteases, but not of proteasome, abolishes cross-presentation in these cells. We conclude that human monocyte-derived cells cross-present exclusively using a vacuolar pathway. Finally, only ascites mo-DCs provide co-stimulatory signals to induce effector cytotoxic CD8 T cells. Our findings thus provide important insights on how to harness cross-presentation for therapeutic purposes.

Andrés Alloatti, Derek C Rookhuizen, Leonel Joannas, Jean-Marie Carpier, Salvador Iborra, Joao G Magalhaes, Nader Yatim, Patrycja Kozik, David Sancho, Matthew L Albert, Sebastian Amigorena (2018 Feb 17)
Correction: Critical role for Sec22b-dependent antigen cross-presentation in antitumor immunity.
The Journal of experimental medicine : 1001 : DOI : 10.1084/jem.2017022902092018c

Résumé

Sebastian Amigorena (2018 Feb 1)
Dendritic Cells on the Way to Glory
The Journal of Immunology : 885-886 : DOI : 10.4049/jimmunol.1701693

Résumé

Pillars of Immunology
The Journal of Immunology

Dendritic Cells on the Way to Glory

Sebastian Amigorena

There was a time when consensus on the existence of dendritic cells (DCs) was tenuous, when many researchers in the field did not believe that "professional" APCs are involved in the priming of T cell responses. Until the late 1980s, the cellular basis that links Ags, which are generally present in tissues (e.g., pathogens or infected cells) to the initiation of immune responses (which occurs in lymph nodes) was unclear. Everybody assumed that soluble Ags drained from tissues to lymph nodes through the lymph, but the question was more delicate for cell-associated Ags. In both cases, the nature of the cells that present Ags to naive T cells for the initiation of T cell immune responses in lymph nodes was an open question.

The 1980s witnessed a change in how we understand the initiation of adaptive immune responses. This change was initiated by the discovery of DCs by Steinman and Cohn (1). It took over 15 y before Steinman proposed a unifying model: DCs pick up Ags by endocytosis and phagocytosis in tissues and then migrate through the lymph to lymph nodes, where they present Ags to naive T cells. This model was based largely on ex vivo experiments showing that, after extraction from tissues (mainly the skin), DCs spontaneously change phenotype, lose endocytic capacities, and gain the ability to costimulate and activate T cells (2). This spontaneous change in phenotype after isolation suggested that DCs existed in two alternative functional states that were rapidly referred to as "immature" and "mature."

Maturation is now known to be a common property of all DC types and subtypes. Indeed, DCs do not represent a homogeneous lineage; instead, they are a set of heterogeneous populations of cells with common characteristics. Spectacular advances in understanding DC ontogeny have been made in the last 10 y, but we still do not fully understand the molecular programs involved. We know that DCs include multiple subpopulations that differentiate in tissues and then migrate to lymph nodes (migratory DCs) or differentiate and reside in lymphoid organs (resident DCs). In the early 1990s, it had already been shown that DCs derive from bone marrow precursors (3), although the precise precursor was still unknown. However, like the primary DCs isolated from tissues (1, 4), in vitro–generated mouse DCs spontaneously matured in culture (5), hampering the molecular characterization of the maturation process.

In this context, the featured article by Sallusto and Lanzavecchia (6), published in 1994, made at least three critical contributions to the field. First, it showed that cells with all of the morphological and functional characteristics of DCs can develop from adult blood monocytes. How and why were these monocyte-derived cells classified as DCs? From very early on, Steinman (2) proposed a functional definition of DCs as cells whose "distinctive role is to initiate T-dependent responses from quiescent lymphocytes." Indeed, the monocyte-derived DCs generated by Sallusto and Lanzavecchia primed resting cord blood T cells to actively proliferate. The monocyte-derived cells were also highly phagocytic and lost their uptake capacities upon maturation while gaining expression of costimulatory molecules and T cell priming capacity. These characteristics very precisely match those of mouse tissue–derived DCs. The finding that DCs can differentiate from monocytes was at that time, and still is, quite confusing.

Are monocyte-derived cells "real" DCs? This question still preoccupies the field, and the debate on the subject is still active. Some researchers believe that the name "DC" should be restricted to cells derived from dedicated precursors, pre-DCs, whereas all cells derived from monocytes should be referred to as monocyte-derived cells (7, 8). This does not exclude that monocytes can differentiate into cells that have macrophage- or DC-like functions. A unifying view, which is gaining increasing experimental support, is that DCs and macrophages have dedicated precursors in adults and embryos, respectively, whereas monocytes can adopt either fate under specific tissue environments, such as inflammation or damage. Alternative development of monocytes into macrophage- or DC-like cells has been shown, but the precise classification of the resulting cells is still debated. Hopefully, identification of the molecular mechanisms that control the two alternative differentiation pathways will clarify the issue.

Second, Sallusto and Lanzavecchia provided a unique culture system to generate immature DCs in vitro, paving the road to understanding the biology of DC maturation. Monocyte-derived human immature DCs presented all of the characteristics previously reported for mouse tissue DCs (i.e., high endocytic capacity and low costimulation). Sallusto and Lanzavecchia showed that TNF-α (an inflammatory cytokine) induced the very same changes in monocyte-derived DCs that were reported previously as a "spontaneous" maturation in mouse DCs (9). Therefore, DC maturation is not only a spontaneous process, but it is a regulated response to specific inflammatory stimuli. These results confirmed the hypothesis that DCs exist under two alternative developmental stages: immature and mature, with distinct markers, morphologies,

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Abbreviation used in this article: DC, dendritic cell.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1701693

Luigia Pace, Christel Goudot, Elina Zueva, Paul Gueguen, Nina Burgdorf, Joshua J. Waterfall, Jean-Pierre Quivy, Geneviève Almouzni, Sebastian Amigorena (2018 Jan 12)
The epigenetic control of stemness in CD8+ T cell fate commitment
Science : 359 : 177-186 : DOI : 10.1126/science.aah6499

Résumé

The epigenetic states and associated chromatin dynamics underlying the initiation and maintenance of memory and effector $CD8^+$ T cells are poorly understood. Pace *et al.* found that mice lacking the histone H3 lysine 9 methyltransferase Suv39h1 had markedly reduced antigen-specific effector $CD8^+$ T cell responses to *Listeria monocytogenes* infection (see the Perspective by Henning *et al.*). Instead, $CD8^+$ T cells in these mice were enriched for genes associated with naïve and memory signatures and showed enhanced memory potential and increased survival capacity. Thus, Suv39h1 marks chromatin through H3K9me3 deposition and silences memory and stem cell programs during the terminal differentiation of effector $CD8^+$ T cells.
Science, this issue p. 177; see also p. 163

After priming, naïve $CD8^+$ T lymphocytes establish specific heritable transcription programs that define progression to long-lasting memory cells or to short-lived effector cells. Although lineage specification is critical for protection, it remains unclear how chromatin dynamics contributes to the control of gene expression programs. We explored the role of gene silencing by the histone methyltransferase Suv39h1. In murine $CD8^+$ T cells activated after *Listeria monocytogenes* infection, Suv39h1-dependent trimethylation of histone H3 lysine 9 controls the expression of a set of stem cell–related memory genes. Single-cell RNA sequencing revealed a defect in silencing of stem/memory genes selectively in *Suv39h1*-defective T cell effectors. As a result, *Suv39h1*-defective $CD8^+$ T cells show sustained survival and increased long-term memory reprogramming capacity. Thus, Suv39h1 plays a critical role in marking chromatin to silence stem/memory genes during $CD8^+$ T effector terminal differentiation.

Année de publication : 2017

Alma-Martina Cepika, Romain Banchereau, Elodie Segura, Marina Ohouo, Brandi Cantarel, Kristina Goller, Victoria Cantrell, Emily Ruchaud, Elizabeth Gatewood, Phuong Nguyen, Jinghua Gu, Esperanza Anguiano, Sandra Zurawski, Jeanine M Baisch, Marilynn Punaro, Nicole Baldwin, Gerlinde Obermoser, Karolina Palucka, Jacques Banchereau, Sebastian Amigorena, Virginia Pascual (2017 Sep 23)
A multidimensional blood stimulation assay reveals immune alterations underlying systemic juvenile idiopathic arthritis.
The Journal of experimental medicine : DOI : jem.20170412

Résumé

The etiology of sporadic human chronic inflammatory diseases remains mostly unknown. To fill this gap, we developed a strategy that simultaneously integrates blood leukocyte responses to innate stimuli at the transcriptional, cellular, and secreted protein levels. When applied to systemic juvenile idiopathic arthritis (sJIA), an autoinflammatory disease of unknown etiology, this approach identified gene sets associated with specific cytokine environments and activated leukocyte subsets. During disease remission and off treatment, sJIA patients displayed dysregulated responses to TLR4, TLR8, and TLR7 stimulation. Isolated sJIA monocytes underexpressed the IL-1 inhibitor aryl hydrocarbon receptor (AHR) at baseline and accumulated higher levels of intracellular IL-1β after stimulation. Supporting the demonstration that AHR down-regulation skews monocytes toward macrophage differentiation, sJIA monocytes differentiated in vitro toward macrophages, away from the dendritic cell phenotype. This might contribute to the increased incidence of macrophage activation syndrome in these patients. Integrated analysis of high-dimensional data can thus unravel immune alterations predisposing to complex inflammatory diseases.

Christel Goudot, Alice Coillard, Alexandra-Chloé Villani, Paul Gueguen, Adeline Cros, Siranush Sarkizova, Tsing-Lee Tang-Huau, Mylène Bohec, Sylvain Baulande, Nir Hacohen, Sebastian Amigorena, Elodie Segura (2017 Sep 21)

Aryl Hydrocarbon Receptor Controls Monocyte Differentiation into Dendritic Cells versus Macrophages.

Immunity : 582-596.e6 : DOI : S1074-7613(17)30374-6

Résumé

After entering tissues, monocytes differentiate into cells that share functional features with either macrophages or dendritic cells (DCs). How monocyte fate is directed toward monocyte-derived macrophages (mo-Macs) or monocyte-derived DCs (mo-DCs) and which transcription factors control these differentiation pathways remains unknown. Using an in vitro culture model yielding human mo-DCs and mo-Macs closely resembling those found in vivo in ascites, we show that IRF4 and MAFB were critical regulators of monocyte differentiation into mo-DCs and mo-Macs, respectively. Activation of the aryl hydrocarbon receptor (AHR) promoted mo-DC differentiation through the induction of BLIMP-1, while impairing differentiation into mo-Macs. AhR deficiency also impaired the in vivo differentiation of mouse mo-DCs. Finally, AHR activation correlated with mo-DC infiltration in leprosy lesions. These results establish that mo-DCs and mo-Macs are controlled by distinct transcription factors and show that AHR acts as a molecular switch for monocyte fate specification in response to micro-environmental factors.

Florence Faure, Mabel Jouve, Isabelle Lebhar-Peguillet, Charlotte Sadaka, Fernando Sepulveda, Olivier Lantz, Stefano Berre, Raphael Gaudin, Silvia Sánchez-Ramón, Sebastian Amigorena (2017 Sep 9)

Blood monocytes sample MelanA/MART1 antigen for long-lasting cross-presentation to CD8(+) T cells after differentiation into dendritic cells.
International journal of cancer : DOI : 10.1002/ijc.31037

Résumé

Human blood monocytes are very potent to take up antigens. Like macrophages in tissue, they efficiently degrade exogenous protein and are less efficient than dendritic cells (DCs) at cross-presenting antigens to CD8(+) T cells. Although it is generally accepted that DCs take up tissue antigens and then migrate to lymph nodes to prime T cells, the mechanisms of presentation of antigens taken up by monocytes are poorly documented so far. In the present work, we show that monocytes loaded in vitro with MelanA long peptides retain the capacity to stimulate antigen-specific CD8(+) T cell clones after 5 days of differentiation into monocytes-derived dendritic cells (MoDCs). Tagged-long peptides can be visualized in electron-dense endocytic compartments distinct from lysosomes, suggesting that antigens can be protected from degradation for extended periods of time. To address the pathophysiological relevance of these findings, we screened blood monocytes from 18 metastatic melanoma patients and found that CD14(+) monocytes from two patients effectively activate a MelanA-specific CD8 T cell clone after in vitro differentiation into MoDCs. This in vivo sampling of tumor antigen by circulating monocytes might alter the tumor-specific immune response and should be taken into account for cancer immunotherapy.

Andrés Alloatti, Derek C Rookhuizen, Leonel Joannas, Jean-Marie Carpier, Salvador Iborra, Joao G Magalhaes, Nader Yatim, Patrycja Kozik, David Sancho, Matthew L Albert, Sebastian Amigorena (2017 Jul 1)
Critical role for Sec22b-dependent antigen cross-presentation in antitumor immunity.
The Journal of experimental medicine : 2231-2241 : DOI : 10.1084/jem.20170229

Résumé

CD8(+) T cells mediate antigen-specific immune responses that can induce rejection of solid tumors. In this process, dendritic cells (DCs) are thought to take up tumor antigens, which are processed into peptides and loaded onto MHC-I molecules, a process called « cross-presentation. » Neither the actual contribution of cross-presentation to antitumor immune responses nor the intracellular pathways involved in vivo are clearly established because of the lack of experimental tools to manipulate this process. To develop such tools, we generated mice bearing a conditional DC-specific mutation in the sec22b gene, a critical regulator of endoplasmic reticulum-phagosome traffic required for cross-presentation. DCs from these mice show impaired cross-presentation ex vivo and defective cross-priming of CD8(+) T cell responses in vivo. These mice are also defective for antitumor immune responses and are resistant to treatment with anti-PD-1. We conclude that Sec22b-dependent cross-presentation in DCs is required to initiate CD8(+) T cell responses to dead cells and to induce effective antitumor immune responses during anti-PD-1 treatment in

mice.

Eik Hoffmann, Anne-Marie Pauwels, Andrés Alloatti, Fiorella Kotsias, Sebastian Amigorena (2017 Feb 28)
Analysis of Phagosomal Antigen Degradation by Flow Organellocytometry.
Bio-protocol : DOI : e2014

Résumé

Professional phagocytes internalize self and non-self particles by phagocytosis to initiate innate immune responses. After internalization, the formed phagosome matures through fusion and fission events with endosomes and lysosomes to obtain a more acidic, oxidative and hydrolytic environment for the degradation of its cargo. Interestingly, phagosome maturation kinetics differ between cell types and cell activation states. This protocol allows to quantify phagosome maturation kinetics on a single organelle level in different types of phagocytes using flow cytometry. Here, ovalbumin (OVA)-coupled particles are used as phagocytosis model system in dendritic cells (DC), which are internalized by phagocytosis. After different time points, phagosome maturation parameters, such as phagosomal degradation of OVA and acquisition of lysosomal proteins (like LAMP-1), can be measured simultaneously in a highly quantitative manner by flow organellocytometry. These read-outs can be correlated to other phagosomal functions, for example antigen degradation, processing and loading in DC.

Andrés Alloatti, Fiorella Kotsias, Eik Hoffmann, Sebastian Amigorena (2017 Feb 28)
Evaluation of Cross-presentation in Bone Marrow-derived Dendritic Cells in vitro and Splenic Dendritic Cells ex vivo Using Antigen-coated Beads.
Bio-protocol : DOI : e2015

Résumé

Antigen presentation by MHC class I molecules, also referred to as cross-presentation, elicits cytotoxic immune responses. In particular, dendritic cells (DC) are the most proficient cross-presenting cells, since they have developed unique means to control phagocytic and degradative pathways. This protocol allows the evaluation of antigen cross-presentation both in vitro (by using bone marrow-derived DC) and ex vivo (by purifying CD8(+) DC from spleen after incorporation of particulate antigen) using ovalbumin (OVA)-coupled particles. Cross-presentation efficiency is measured by three different readouts: the B3Z hybridoma T cell line (Karttunen et al., 1992) and stimulation of antigen-specific CD8(+) T cells (OT-I) (Kurts et al., 1996), either analyzing OT-I activation by CD69 expression or OT-I proliferation after labeling them with carboxyfluorescein succinimidyl ester (CFSE). By using this approach, we could show recently that DCs are able to increase cross-presentation efficiency transiently upon engagement of TLR4 (Alloatti et al., 2015).

Marianne Burbage, Sebastian Amigorena (2017 Feb 24)
Antigen-Primed CD8(+) T Cells Call DCs for Back Up.
Immunity : 163-164 : DOI : S1074-7613(17)30035-3

Résumé

Encounters between naive T lymphocytes and dendritic cells (DCs) bearing adequate co-stimulatory signals are rare. In this issue of Immunity, Brewitz et al. (2017) show that chemokines secreted by CD8(+) T cells recruit myeloid and plasmacytoid DCs that in turn boost CD8(+) T cell activation.