
**A novel combination of chemotherapy and immunotherapy controls tumor growth in mice with a human immune system.**


**Résumé**

Mice reconstituted with a human immune system and bearing human tumors represent a promising model for developing novel cancer immunotherapies. Here, we used mass cytometry and multi-parametric flow cytometry to characterize human leukocytes infiltrating a human breast cancer tumor model in immunocompromised NOD.SCID.γc-null mice reconstituted with a human immune system and compared it to samples of breast cancer patients. We observed highly activated human CD4 and CD8 T cells in the tumor, as well as minor subsets of innate immune cells in both settings. We also report that ICOS CD4 regulatory T cells (Treg) were enriched in the tumor relative to the periphery in humanized mice and patients, providing a target to affect Treg and tumor growth. Indeed, administration of a neutralizing mAb to human ICOS reduced Treg proportions and numbers and improved CD4 + T cell proliferation in humanized mice. Moreover, a combination of the anti-ICOS mAb with cyclophosphamide reduced tumor growth, and that was associated with an improved CD8 to Treg ratio. Depletion of human CD8 T cells or of murine myeloid cells marginally affected the effect of the combination therapy. Altogether, our results indicate that a combination of anti-ICOS mAb and chemotherapy controls tumor growth in humanized mice, opening new perspectives for the treatment of breast cancer. One sentence summary: Targeting ICOS in combination with chemotherapy is a promising strategy to improve tumor immunity in humans.


**Inhibition of PI3K pathway increases immune infiltrate in muscle-invasive bladder cancer.**


**Résumé**

Although immune checkpoint inhibitors have shown improvement in survival in comparison to chemotherapy in urothelial bladder cancer, many patients still fail to respond to these treatments and actual efforts are made to identify predictive factors of response to immunotherapy. Understanding the tumor-intrinsic molecular basis, like oncogenic pathways conditioning the presence or absence of tumor-infiltrating T cells (TILs), should provide a new rationale for improved anti-tumor immune therapies. In this study, we found that urothelial...
bladder cancer from human samples bearing gene mutations was significantly associated with lower expression of a defined immune gene signature, compared to unmutated ones. We identified a reduced 10-gene immune gene signature that discriminates muscle-invasive bladder cancer (MIBC) samples according to immune infiltration and mutation. Using a humanized mouse model, we observed that BKM120, a pan-PI3K inhibitor, significantly inhibited the growth of a human bladder cancer cell line bearing a mutation, associated to increased immune cell infiltration (hCD45+). Using qRT-PCR, we also found an increase in the expression of chemokines and immune genes in mutated tumors from mice treated with BKM120, reflecting an active immune infiltrate in comparison to untreated ones. Moreover, the addition of BKM120 rendered -mutated tumors sensitive to PD-1 blockade. Our results provide a relevant rationale for combination strategies of PI3K inhibitors with immune checkpoint inhibitors to overcome resistance to immune checkpoint inhibitors.

Pierre Guermonprez, Julie Helft (2019 Mar 13)
Inflammasome activation: a monocyte lineage privilege.

Résumé

Pamela Caudana, Nicolas Gonzalo Núñez, Philippe De La Rochere, Anaïs Pinto, Jordan Denizeau, Ruby Alonso, Leticia Laura Niborski, Olivier Lantz, Christine Sedlik, Eliane Piaggio (2019 Jan 18)
IL2/Anti-IL2 Complex Combined with CTLA-4, But Not PD-1, Blockade Rescues Antitumor NK Cell Function by Regulatory T-cell Modulation.
*Cancer immunology research* : 443-457 : DOI : 10.1158/2326-6066.CIR-18-0697

Résumé

High-dose IL2 immunotherapy can induce long-lasting cancer regression but is toxic and insufficiently efficacious. Improvements are obtained with IL2/anti-IL2 complexes (IL2Cx), which redirect IL2 action to CD8 T and natural killer (NK) cells. Here, we evaluated the efficacy of combining IL2Cx with blockade of inhibitory immune pathways. In an autochthonous lung adenocarcinoma model, we show that the IL2Cx/anti-PD-1 combination increases CD8 T-cell infiltration of the lung and controls tumor growth. In the B16-OVA model, which is resistant to checkpoint inhibition, combination of IL2Cx with PD-1 or CTLA-4 pathway blockade reverses that resistance. Both combinations work by reinvigorating exhausted intratumoral CD8 T cells and by increasing the breadth of tumor-specific T-cell responses. However, only the IL2Cx/anti-CTLA-4 combination is able to rescue NK cell antitumor function by modulating intratumoral regulatory T cells. Overall, association of IL2Cx with PD-1 or CTLA-4 pathway blockade acts by different cellular mechanisms, paving the way for the rational design of combinatorial antitumor therapies.
CD16NKG2A Natural Killer Cells Infiltrate Breast Cancer-Draining Lymph Nodes.

Cancer immunology research : 208-218 : DOI : 10.1158/2326-6066.CIR-18-0085

Résumé

Tumor-draining lymph nodes (TD-LNs) are the first site of metastasis of breast cancer. Natural killer (NK) cells that infiltrate TD-LNs [including noninvaded (NI) or metastatic (M)-LN from breast cancer patients] and NK cells from healthy donor (HD)-LN were characterized, and their phenotype analyzed by flow cytometry. Low percentages of tumor cells invaded M-LNs, and these cells expressed ULBP2 and HLA class I molecules. Although NK cells from paired NI and M-LNs were similar, they expressed different markers compared with HD-LN NK cells. Compared with HD-LNs, TD-LN NK cells expressed activating DNAM-1, NKG2C and inhibitory NKG2A receptors, and exhibited elevated CXCR3 expression. CD16, NKG2A, and Nkp46 expression were shown to be increased in stage IIIA breast cancer patients. TD-LNs contained a large proportion of activated CD56CD16 NK cells with high expression of NKG2A. We also showed that a subset of LN NK cells expressed PD-1, expression of which was correlated with Nkp30 and NKG2C expression. LN NK cell activation status was evaluated by degranulation potential and lytic capacity toward breast cancer cells. NK cells from TD-LNs degranulated after coculture with breast cancer cell lines. Cytokine-activated TD-LN NK cells exerted greater lysis of breast cancer cell lines than HD-LN NK cells and preferentially lysed the HLA class I MCF-7 breast cancer cell line. TD-LNs from breast cancer patients, thus, contained activated lytic NK cells. The expression of inhibitory receptor NKG2A and checkpoint PD-1 by NK cells infiltrating breast cancer-draining LNs supports their potential as targets for immunotherapies using anti-NKG2A and/or anti-PD-1.
CD39 expression defines cell exhaustion in tumor-infiltrating CD8+ T cells.
*Cancer research* : [DOI : canres.2684.2016](https://doi.org/canres.2684.2016)

Résumé

The ability of CD8+ T lymphocytes to eliminate tumors is limited by their ability to engender an immunosuppressive microenvironment. Here we describe a subset of tumor-infiltrating CD8+ T cells marked by high expression of the immunosuppressive ATP ecto-nucleotidase CD39. The frequency of CD39highCD8+ T cells increased with tumor growth but was absent in lymphoid organs. Tumor-infiltrating CD8+ T cells with high CD39 expression exhibited features of exhaustion, such as reduced production of TNF and IL-2 and expression of co-inhibitory receptors. Exhausted CD39+CD8+ T cells from mice hydrolyzed extracellular ATP, confirming that CD39 is enzymatically active. Furthermore, exhausted CD39+CD8+ T cells inhibited IFNγ production by responder CD8+ T cells. In specimens from breast cancer and melanoma patients, CD39+CD8+ T cells were present within tumors and invaded or metastatic lymph nodes, but were barely detectable within non-invaded lymph nodes and absent in peripheral blood. These cells exhibited an exhausted phenotype with impaired production of IFNγ, TNF, IL-2 and high expression of co-inhibitory receptors. Although T cell receptor engagement was sufficient to induce CD39 on human CD8+ T cells, exposure to IL-6 and IL-27 promoted CD39 expression on stimulated CD8+ T cells from human or murine sources. Our findings show how the tumor microenvironment drives the acquisition of CD39 as an immune regulatory molecule on CD8+ T cells, with implications for defining a biomarker of T cell dysfunction and a target for immunotherapeutic intervention.

Alice Barbarin, Emilie Cayssials, Florence Jacomet, Nicolas Gonzalo Nunez, Sara Basbous, Lucie Lefèvre, Myriam Abdallah, Nathalie Piccirilli, Benjamin Morin, Vincent Lavoue, Véronique Catros, Eliane Piaggio, André Herbelin, Jean-Marc Gombert (2017 Apr 12)

**Phenotype of NK-Like CD8(+) T Cells with Innate Features in Humans and Their Relevance in Cancer Diseases.**

Résumé

Unconventional T cells are defined by their capacity to respond to signals other than the well-known complex of peptides and major histocompatibility complex proteins. Among the burgeoning family of unconventional T cells, innate-like CD8(+) T cells in the mouse were discovered in the early 2000s. This subset of CD8(+) T cells bears a memory phenotype without having encountered a foreign antigen and can respond to innate-like IL-12 + IL-18 stimulation. Although the concept of innate memory CD8(+) T cells is now well established in mice, whether an equivalent memory NK-like T-cell population exists in humans remains under debate. We recently reported that CD8(+) T cells responding to innate-like IL-12 + IL-18 stimulation and co-expressing the transcription factor Eomesodermin (Eomes) and KIR/NKG2A membrane receptors with a memory/EMRA phenotype may represent a new, functionally distinct innate T cell subset in humans. In this review, after a summary on the known innate CD8(+) T-cell features in the mouse, we propose Eomes together with
KIR/NKG2A and CD49d as a signature to standardize the identification of this innate CD8(+) T-cell subset in humans. Next, we discuss IL-4 and IL-15 involvement in the generation of innate CD8(+) T cells and particularly its possible dependency on the promyelocytic leukemia zinc-finger factor expressing iNKT cells, an innate T cell subset well documented for its susceptibility to tumor immune subversion. After that, focusing on cancer diseases, we provide new insights into the potential role of these innate CD8(+) T cells in a physiopathological context in humans. Based on empirical data obtained in cases of chronic myeloid leukemia, a myeloproliferative syndrome controlled by the immune system, and in solid tumors, we observe both the possible contribution of innate CD8(+) T cells to cancer disease control and their susceptibility to tumor immune subversion. Finally, we note that during tumor progression, innate CD8(+) T lymphocytes could be controlled by immune checkpoints. This study significantly contributes to understanding of the role of NK-like CD8(+) T cells and raises the question of the possible involvement of an iNKT/innate CD8(+) T cell axis in cancer.

Thomas Simon, Julien Pogu, Séverine Rémy, Frédéric Brau, Sylvie Pogu, Maud Maquigneau, Jean-François Fonteneau, Nicolas Poirier, Bernard Vanhove, Gilles Blancho, Eliane Piaggio, Ignacio Anegon, Philippe Blancou (2017 Mar 27)

**Inhibition of effector antigen-specific T cells by intradermal administration of heme oxygenase-1 inducers.**
*Journal of autoimmunity*: 44-55 : DOI : S0896-8411(16)30389-4

**Résumé**

Developing protocols aimed at inhibiting effector T cells would be key for the treatment of T cell-dependent autoimmune diseases including type 1 autoimmune diabetes (T1D) and multiple sclerosis (MS). While heme oxygenase-1 (HO-1) inducers are clinically approved drugs for non-immune-related diseases, they do have immunosuppressive properties when administered systemically in rodents. Here we show that HO-1 inducers inhibit antigen-specific effector T cells when injected intradermally together with the T cell cognate antigens in mice. This phenomenon was observed in both a CD8(+) T cell-mediated model of T1D and in a CD4(+) T cell-dependent MS model. Intradermal injection of HO-1 inducers induced the recruitment of HO-1(+) monocyte-derived dendritic cell (MoDCs) exclusively to the lymph nodes (LN) draining the site of intradermal injection. After encountering HO-1(+)MoDCs, effector T-cells exhibited a lower velocity and a reduced ability to migrate towards chemokine gradients resulting in impaired accumulation to the inflamed organ. Intradermal co-injection of a clinically approved HO-1 inducer and a specific antigen to non-human primates also induced HO-1(+) MoDCs to accumulate in dermal draining LN and to suppress delayed-type hypersensitivity. Therefore, in both mice and non-human primates, HO-1 inducers delivered locally inhibited effector T-cells in an antigen-specific manner, paving the way for repositioning these drugs for the treatment of immune-mediated diseases.

Rodrigo N Ramos, Eliane Piaggio, Emanuela Romano (2017 Mar 19)
Mechanisms of Resistance to Immune Checkpoint Antibodies.
*Handbook of experimental pharmacology* : DOI : 10.1007/164_2017_11

Résumé

Immunothérapie using checkpoint inhibitors has changed the way we treat several aggressive cancers such as melanoma, non-small cell lung and head & neck cancers, among others, with durable responses achieved in the metastatic setting. However, unfortunately, the vast majority of patients do not respond to checkpoint inhibition therapy and a minority of patients, who do respond to treatment, develop secondary resistance and experience relapse by mechanisms still inadequately understood. Emerging evidence shows that alterations in multiple signaling pathways are involved in primary and/or secondary resistance to checkpoint inhibition. In this review we discuss how selected cancer-cell autonomous cues may influence the outcome of cancer immunotherapy, particularly immune checkpoint inhibition.

Année de publication : 2016


Loss of immune tolerance to IL-2 in type 1 diabetes.
*Nature communications* : 13027 : DOI : 10.1038/ncomms13027

Résumé

Type 1 diabetes (T1D) is characterized by a chronic, progressive autoimmune attack against pancreas-specific antigens, effecting the destruction of insulin-producing β-cells. Here we show interleukin-2 (IL-2) is a non-pancreatic autoimmune target in T1D. Anti-IL-2 autoantibodies, as well as T cells specific for a single orthologous epitope of IL-2, are present in the peripheral blood of non-obese diabetic (NOD) mice and patients with T1D. In NOD mice, the generation of anti-IL-2 autoantibodies is genetically determined and their titre increases with age and disease onset. In T1D patients, circulating IgG memory B cells specific for IL-2 or insulin are present at similar frequencies. Anti-IL-2 autoantibodies cloned from T1D patients demonstrate clonality, a high degree of somatic hypermutation and nanomolar affinities, indicating a germinal centre origin and underscoring the synergy between cognate autoreactive T and B cells leading to defective immune tolerance.

Christine Sedlik, Adèle Heitzmann, Sophie Viel, Rafik Ait Sarkouh, Cornélie Batisse, Frédéric Schmidt, Philippe De La Rochere, Nathalie Amzallag, Eduardo Osinaga, Pablo Oppezzo, Otto Pritsch, Xavier Sastre-Garau, Pascale Hubert, Sebastian Amigorena, Eliane Piaggio (2016 Sep 14)
Effective antitumor therapy based on a novel antibody-drug conjugate targeting the Tn carbohydrate antigen.

*Oncoimmunology* : e1171434 : [DOI](https://doi.org/10.1080/2162402X.2016.1171434)

**Résumé**

Antibody-drug conjugates (ADC), combining the specificity of tumor recognition by monoclonal antibodies (mAb) and the powerful cytotoxicity of anticancer drugs, are currently under growing interest and development. Here, we studied the potential of Chi-Tn, a mAb directed to a glyco-peptidic tumor-associated antigen, to be used as an ADC for cancer treatment. First, we demonstrated that Chi-Tn specifically targeted tumor cells in vivo. Also, using flow cytometry and deconvolution microscopy, we showed that the Chi-Tn mAb is rapidly internalized – condition necessary to ensure the delivery of conjugated cytotoxic drugs in an active form, and targeted to early and recycling endosomes. When conjugated to saporin (SAP) or to auristatin F, the Chi-Tn ADC exhibited effective cytotoxicity to Tn-positive tumor cells in vitro, which correlated with the level of tumoral Tn expression. Furthermore, the Chi-Tn mAb conjugated to auristatin F also exhibited efficient antitumor activity in vivo, validating for the first time the use of an anti-Tn antibody as an effective ADC.


Inhibition of the JAK/STAT Signaling Pathway in Regulatory T Cells Reveals a Very Dynamic Regulation of Foxp3 Expression.

*PloS one* : e0153682 : [DOI](https://doi.org/10.1371/journal.pone.0153682)

**Résumé**

The IL-2/JAK3/STAT-5 signaling pathway is involved in the initiation and maintenance of the transcription factor Foxp3 in regulatory T cells (Treg) and has been associated with demethylation of the intronic Conserved Non Coding Sequence-2 (CNS2). However, the role of the JAK/STAT pathway in controlling Foxp3 in the short term has been poorly investigated. Using two different JAK/STAT pharmacological inhibitors, we observed a detectable loss of Foxp3 after 10 min. of treatment that affected 70% of the cells after one hour. Using cycloheximide, a general inhibitor of mRNA translation, we determined that Foxp3, but not CD25, has a high turnover in IL-2 stimulated Treg. This reduction was correlated with a rapid reduction of Foxp3 mRNA. This loss of Foxp3 was associated with a loss in STAT-5 binding to the CNS2, which however remains demethylated. Consequently, Foxp3 expression returns to normal level upon restoration of basal JAK/STAT signaling in vivo. Reduced expression of several genes defining Treg identity was also observed upon treatment. Thus, our results demonstrate that Foxp3 has a rapid turn over in Treg partly controlled at the transcriptional level by the JAK/STAT pathway.

Cira Dansokho, Dylla Ait Ahmed, Saba Aid, Cécile Toly-Ndour, Thomas Chaigneau, Vanessa Calle, Nicolas Cagnard, Martin Holzenberger, Eliane Piaggio, Pierre Aucouturier, Guillaume Dorothée
Regulatory T cells delay disease progression in Alzheimer-like pathology.

**Brain : a journal of neurology** : 1237-51 : [DOI : 10.1093/brain/awv408](http://dx.doi.org/10.1093/brain/awv408)

Résumé

Recent studies highlight the implication of innate and adaptive immunity in the pathophysiology of Alzheimer's disease, and foster immunotherapy as a promising strategy for its treatment. Vaccines targeting amyloid-β peptide provided encouraging results in mouse models, but severe side effects attributed to T cell responses in the first clinical trial AN1792 underlined the need for better understanding adaptive immunity in Alzheimer's disease. We previously showed that regulatory T cells critically control amyloid-β-specific CD4(+) T cell responses in both physiological and pathological settings. Here, we analysed the impact of regulatory T cells on spontaneous disease progression in a murine model of Alzheimer's disease. Early transient depletion of regulatory T cells accelerated the onset of cognitive deficits in APPPS1 mice, without altering amyloid-β deposition. Earlier cognitive impairment correlated with reduced recruitment of microglia towards amyloid deposits and altered disease-related gene expression profile. Conversely, amplification of regulatory T cells through peripheral low-dose IL-2 treatment increased numbers of plaque-associated microglia, and restored cognitive functions in APPPS1 mice. These data suggest that regulatory T cells play a beneficial role in the pathophysiology of Alzheimer's disease, by slowing disease progression and modulating microglial response to amyloid-β deposition. Our study highlights the therapeutic potential of repurposed IL-2 for innovative immunotherapy based on modulation of regulatory T cells in Alzheimer's disease.

Année de publication : 2015

Louis Pérol, Eliane Piaggio (2015 Nov 5)

**New Molecular and Cellular Mechanisms of Tolerance: Tolerogenic Actions of IL-2.**

*Methods in molecular biology (Clifton, N.J.)* : 11-28 : [DOI : 10.1007/978-1-4939-3139-2_2](http://dx.doi.org/10.1007/978-1-4939-3139-2_2)

Résumé

Interleukin-2 (IL-2) is an old molecule with brand new functions. Indeed, IL-2 has been first described as a T-cell growth factor but recent data pointed out that its main function in vivo is the maintenance of immune tolerance. Mechanistically, IL-2 is essential for the development and function of CD4(+) Foxp3(+) regulatory T cells (Treg cells) that are essential players in the control of immune responded to self, tumors, microbes and grafts. Treg cells are exquisitely sensitive to IL-2 due to their constitutive expression of the high affinity IL-2 receptor (IL-2R) and the new paradigm suggests that low-doses of IL-2 could selectively boost Treg cells in vivo. Consequently, a growing body of clinical research is aiming at using IL-2 at low doses as a tolerogenic drug to boost endogenous Treg cells in patients suffering from autoimmune or inflammatory conditions. In this manuscript, we briefly review IL-2(IL-2R) biology and the role of IL-2 in the development, maintenance, and function of Treg cells; and also its effects on other immune cell populations such as CD4(+) T
helper cells and CD8(+) memory T cells. Then, focusing on type 1 diabetes, we review the preclinical studies and clinical trials supporting the use of low-doses IL-2 as a tolerogenic immunotherapy. Finally, we discuss the limitations and future directions for IL-2 based immunotherapy.