HAL
open science

Expanded human CD8+CD45RClowFoxp3+ Tregs secreting IFNγ, IL-10, IL-34 and TGFβinhibit human transplant rejection

Séverine Bézie, Dimitri Meistermann, Laetitia Boucault, Stephanie Kilens, Johanna Zoppi, Elodie Autrusseau, Audrey Donnart, Véronique Daguin, Frédérique Bellier-Waast, Eric Charpentier, et al.

To cite this version:

Séverine Bézie, Dimitri Meistermann, Laetitia Boucault, Stephanie Kilens, Johanna Zoppi, et al.. Expanded human CD8+CD45RClowFoxp3+ Tregs secreting IFNγ, IL-10, IL-34 and TGFβinhibit human transplant rejection. FOCIS, Jun 2017, Chicago, United States. inserm-02159985

HAL Id: inserm-02159985

https://inserm.hal.science/inserm-02159985v1

Submitted on 19 Jun 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Expanded human CD8⁺CD45RC^{low}Foxp3⁺ Tregs secreting IFNγ, IL-10, IL-34 and TGFβ inhibit human transplant rejection

Séverine Bézie[1,2], Dimitri Meistermann[1,2], Laetitia Boucault[1,2], Stéphanie Kilens[1,2], Johanna Zoppi[1,2], Elodie Autrusseau[1,2], Audrey Donnart[4], Véronique Daguin[1,2], Frédérique Bellier-Waast[3], Eric Charpentier[4], Franck Duteille[3], Laurent David[1,2], Ignacio Anegon[1,2] and Carole Guillonneau[1,2]

[1] Centre de Recherche en Transplantation et Immunologie UMR1064, INSERM, Université de Nantes, Nantes, France
[2] Institut de Transplantation Urologie Néphrologie (ITUN), CHU Nantes, Nantes, France
[3] Chirurgie Plastique Reconstructrice et Esthétique, CHU Nantes, Nantes, France
[4] INSERM UMR1087, CNRS UMR6291, Université de Nantes, l'institut du thorax, Nantes, F-44000 France;

Background

Tregs play a critical role in the control of immune responses and tolerance induction; however our understanding of CD8⁺ Tregs is limited while they are particularly promising for therapeutic application. We previously reported that CD8⁺CD45RC^{low} Tregs use IFNγ and IL-34 to suppress donor responses in a rat model of allotransplantation (Guillonneau et al, JCI, 2007; Bezie et al, JCI, 2015). Here, we aim to characterize human CD8⁺ Tregs to assess their potential for cell therapy in transplantation in comparison to CD4⁺CD25^{high}CD127⁻ Tregs .

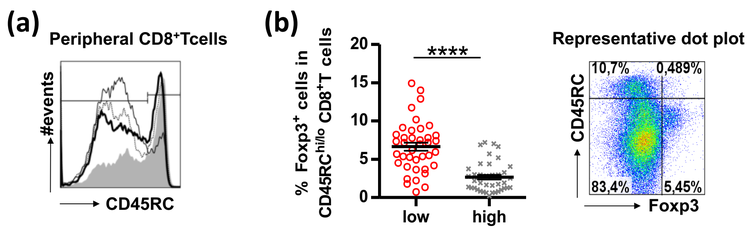
Materials and methods

- All cells were isolated from blood of healthy volunteers.
- Culture: CD8⁺CD45RC^{low} Tregs and CD4⁺CD25^{high}CD127⁻ Tregs were sorted by Facs Aria and plated with allogeneic APCs (Tregs:APC ratio 1:4) or in presence of anti-CD3 (OKT3 clone, coated, 1µg/ml) and anti-CD28 (CD28.2 clone, soluble, 1µg/ml) mAbs in RPMI 1640 medium supplemented with AB serum 10%, IL-2 (1000U/ml) and IL-15 (10ng/ml). Cells were stimulated again with mAbs at day 7, and cytokines were freshly added at day 7, 10 and 12.
- Proteomic analysis: T cells were stimulated 7h with PMA and ionomycin including 4h with BFA before cytokines staining and were analyzed by flow cytometry. Apoptosis was assessed by DAPI/Annexin V staining after 15h culture.
- Transcriptomic analysis: Tregs were sorted, expanded or not for 14 days, and analyzed by 3'DGE RNA-sequencing as compared to non-expanded freshly sorted Tregs.
- Suppression assay: CD4⁺CD25⁻ T cells were sorted by FACS Aria, labeled with CFSE and stimulated by allogeneic APCs in presence or absence of CD8⁺CD45RC^{low} or ^{high} T cells, or CD4⁺CD25^{high}CD127⁻ Tregs. Secretion assay kits from Miltenyi were used to sort CD8⁺CD45RC^{low} Tregs on IFNγ and IL-10 expression. 50µg/ml of blocking Abs or isotypic controls were added at day 0 when indicated. Proliferation was quantified by CFSE dilution in DAPI⁻CD4⁺T cells after 5 days co-culture.
- Skin transplantation model: NSG mice were grafted with 1cm² human skin and injected 4 to 6 weeks later with 5x10⁶ human PBMCs ± a range of expanded CD8⁺Tregs. Allogeneic rejection was assessed by macroscopic observation.

CD8⁺CD45RC^{LOW} T CELLS ARE NATURAL TREGS ACTING THROUGH IFNγ, IL-34,TGFβ and IL-10 SECRETION

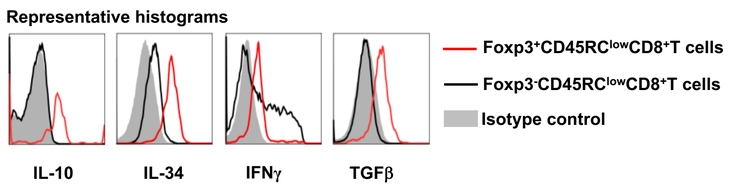
1. Definition

CD45RC marker is differentially represented in blood CD8⁺T cells in healthy volunteers **(a**, overlay of 5 individuals). CD45RC^{low} cells include most of Foxp3⁺ cells **(b)**.



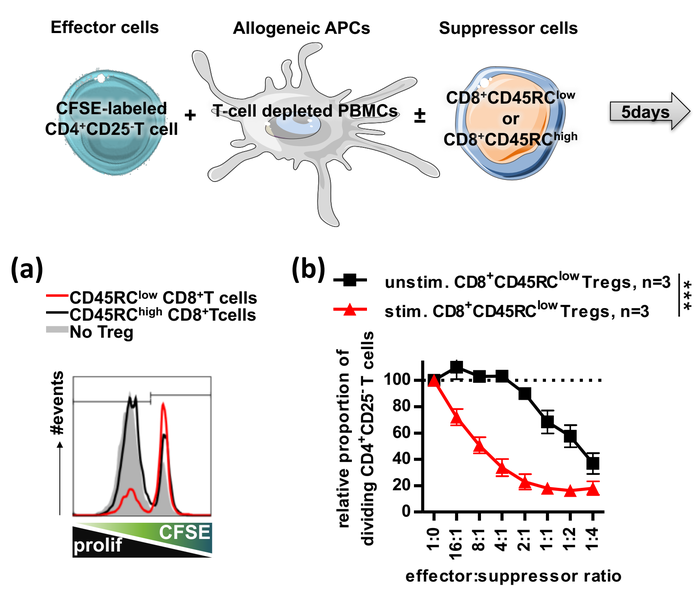
2. Phenotype

Foxp3⁺CD8⁺CD45RC^{low} T cells exclusively express IL-10, IL-34, and TGFβ and low level of IFNγ.



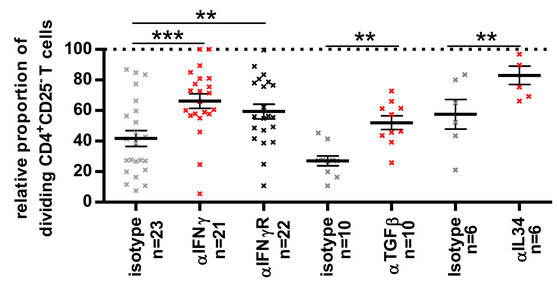
3 . Function

In contrast to CD8⁺CD45RC^{high} T cells, CD8⁺CD45RC^{low} T cells efficiently inhibited responder T cells proliferation in response to allogeneic APCs **(a)** in a dose dependant manner **(b)**.



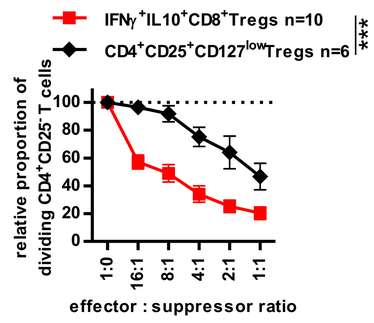
4. Mechanisms

Blocking of IFNγ or its receptor, TGFβ or IL-34 partially restored effector T cells proliferation proving their involvement in Tregs function.



5. Relevance

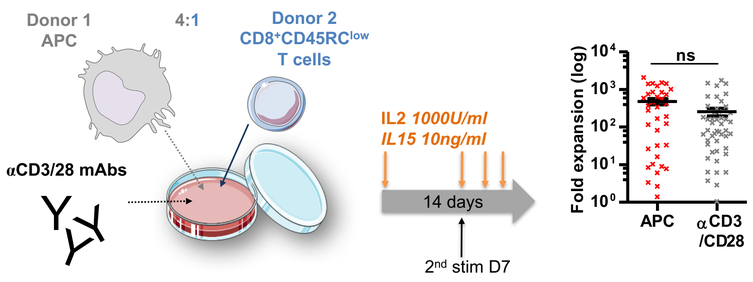
CD8⁺CD45RC^{low}Tregs secreting IL-10 and IFNγ were significantly more efficient to inhibit T cell allo-response than CD4⁺Tregs.



Cell therapy using expanded CD8⁺Tregs inhibits transplant rejection

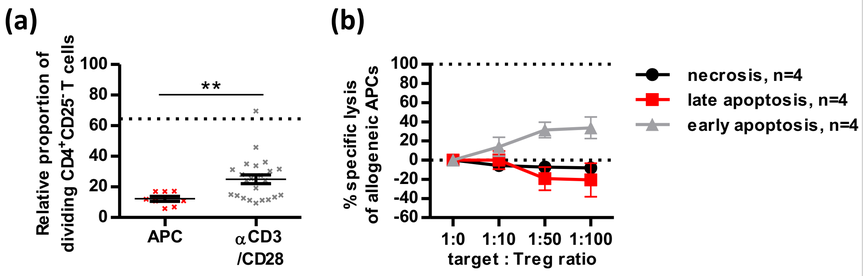
1. Expansion protocol

CD8⁺CD45RC^{low}Tregs were expanded up to 2000 fold in 14 days in presence of allogeneic APCs or anti-CD3/28 mAbs, high dose of IL-2 and IL-15.



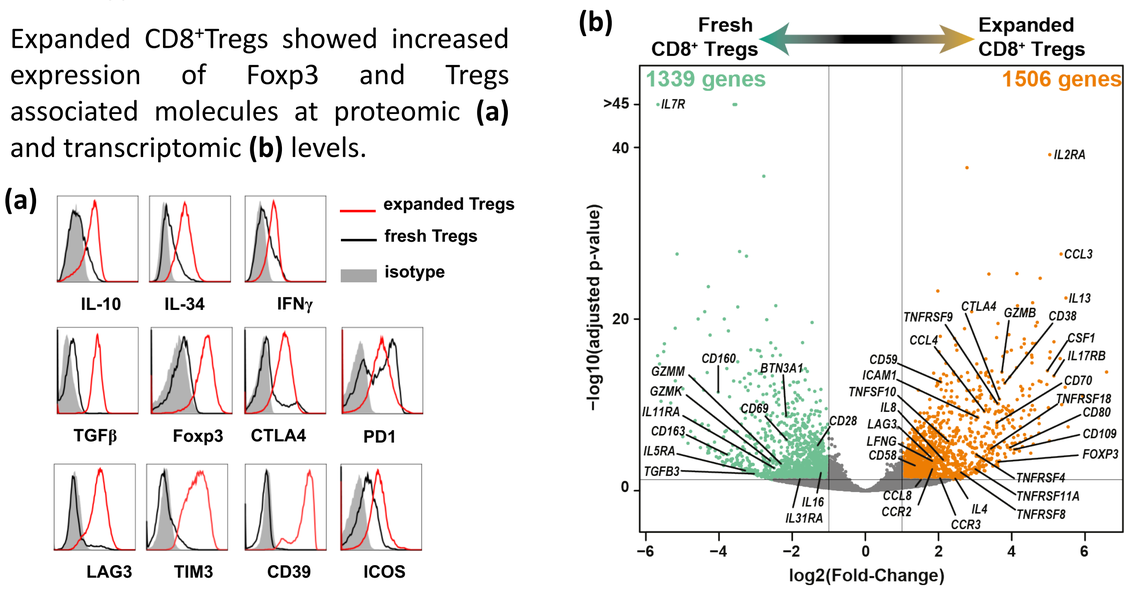
2. Function

Expansion process increased suppressive activity **(a)** but not cytotoxicity **(b)** of Tregs.



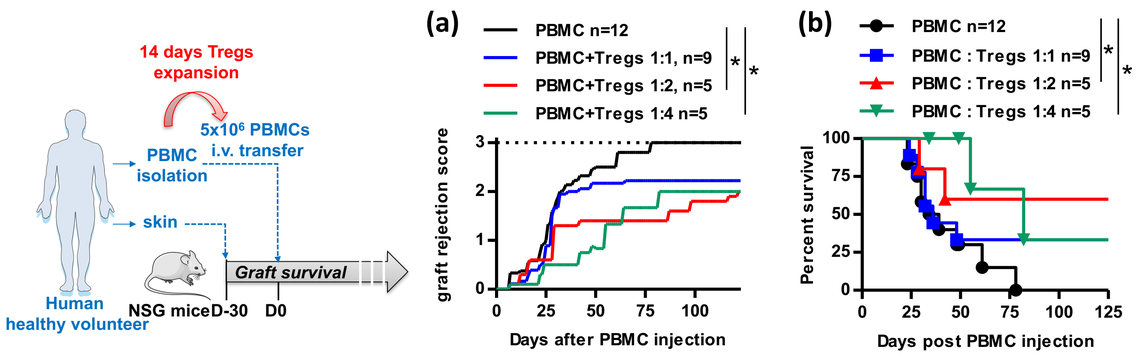
3. Phenotype

Expanded CD8⁺Tregs showed increased expression of Foxp3 and Tregs associated molecules at proteomic **(a)** and transcriptomic **(b)** levels.



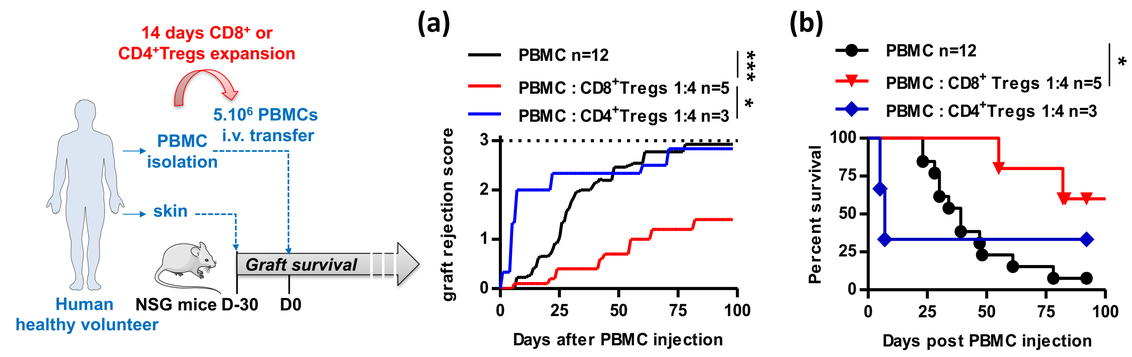
4. SOT

Transfer of expanded CD8⁺Tregs significantly inhibited rejection of human skin graft induced by human allogeneic PBMCs injection in NSG mice, in a dose dependent manner, as monitored by graft rejection score **(a)** and survival **(b)**.



5. Relevance

Expanded CD8⁺Tregs were significantly more efficient than expanded CD4⁺Tregs to inhibit rejection of human skin graft induced by human allogeneic PBMCs injection in NSG mice, as monitored by graft rejection score **(a)** and survival **(b)**.



Conclusions

We report here existence of highly suppressive human CD8⁺CD45RC^{low}Tregs expressing Foxp3 and producing IFNγ, IL-10, IL-34 and TGFβ to mediate their suppressive activity. We show that CD8⁺CD45RC^{low}Tregs are superior to canonical CD4⁺CD25^{high}CD127^{low}Tregs for suppression of allogeneic immune responses in vitro and in vivo to delay human skin rejection in humanized mice. Robustly expanded CD8⁺Tregs displayed a specific gene signature. These results open new possibilities in cell therapy in transplantation and by extension in other diseases.