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.

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 .

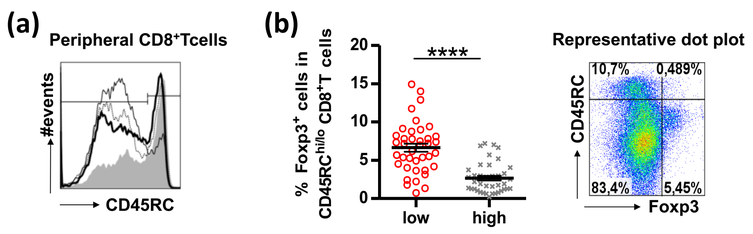
MATERIALS AND METHODS

•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

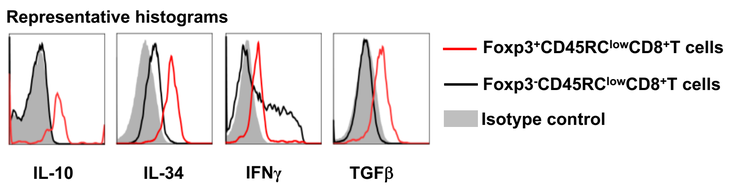
1. Definition

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)**.



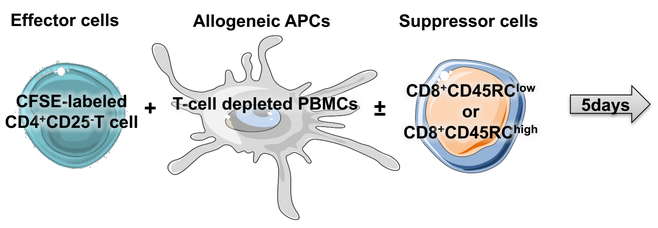
2. Phenotype

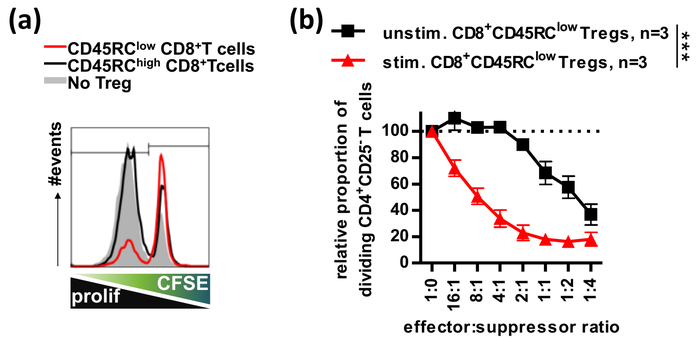
Foxp3⁺CD8⁺CD45RC^{low} T cells exclusively express IL-10, IL-34, and TGFβ and low level of IFNγ.



3 . Function

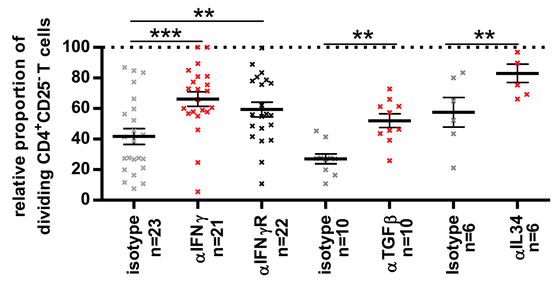
In contrast to CD8⁺CD45RC^{high} T cells, CD8⁺CD45RC^{low} T cells efficiently inhibited responder T cells proliferation in response to allogeneic APCs **(a)** in a dose dependant manner **(b)**.





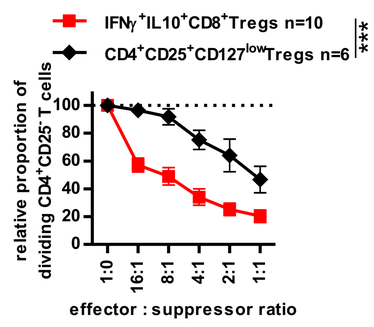
4. Mechanisms

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



5. Relevance

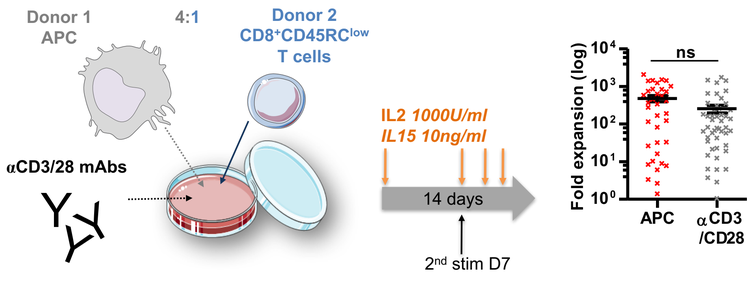
CD8⁺CD45RC^{low}Tregs secreting IL-10 and IFNγ were significantly more efficient to inhibit T cell allo-response than CD4⁺Tregs.



CELL THERAPY USING EXPANDED CD8⁺TREGS INHIBITS TRANSPLANT REJECTION

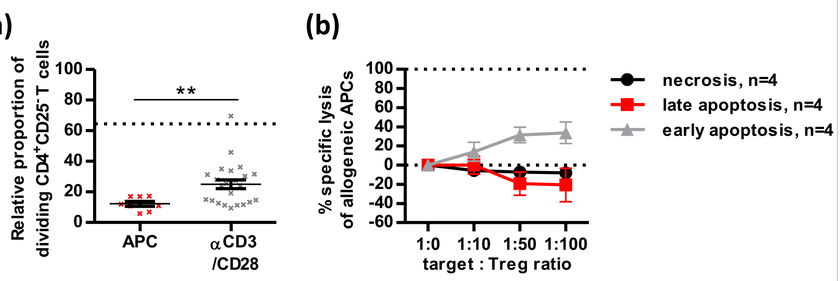
1. Expansion protocol

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.



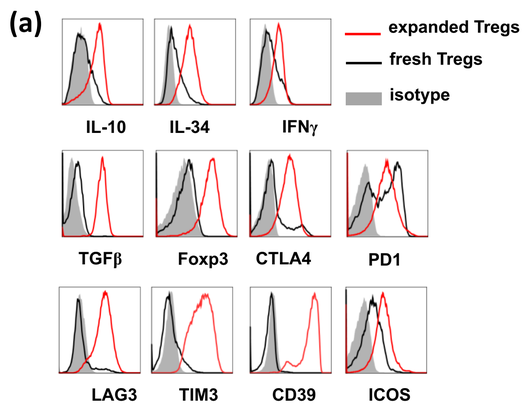
2. Function

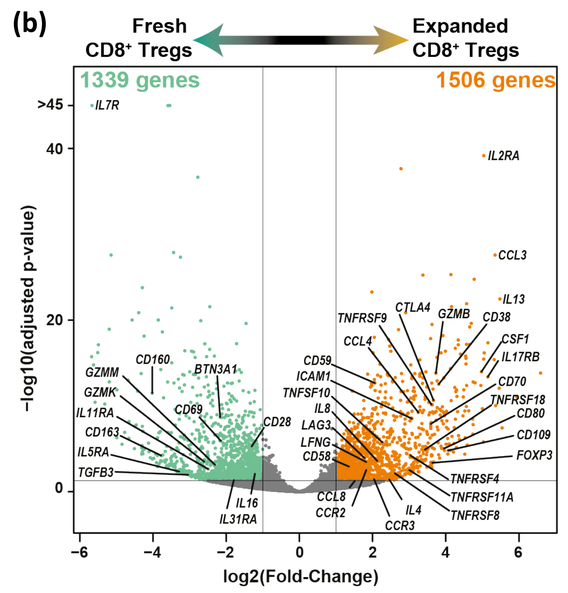
Expansion process increased suppressive activity **(a)** but not cytotoxicity **(b)** of Tregs.



3. Phenotype

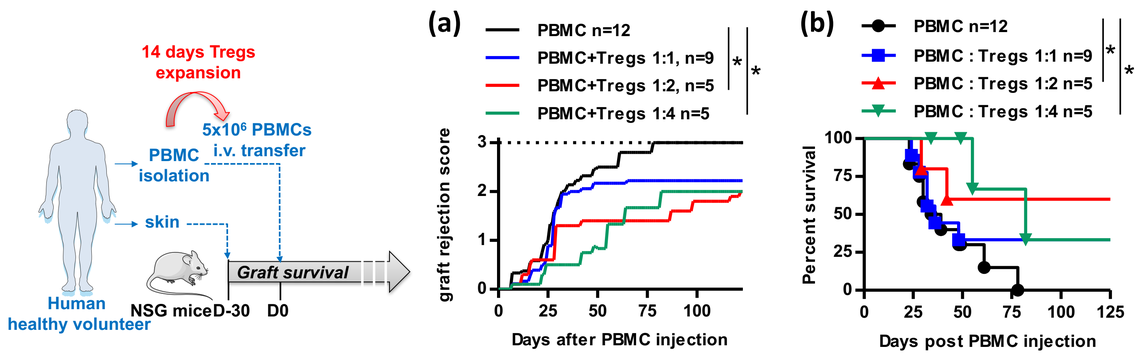
Expanded CD8⁺Tregs showed increased expression of Foxp3 and Tregs associated molecules at proteomic **(a)** and transcriptomic **(b)** levels.





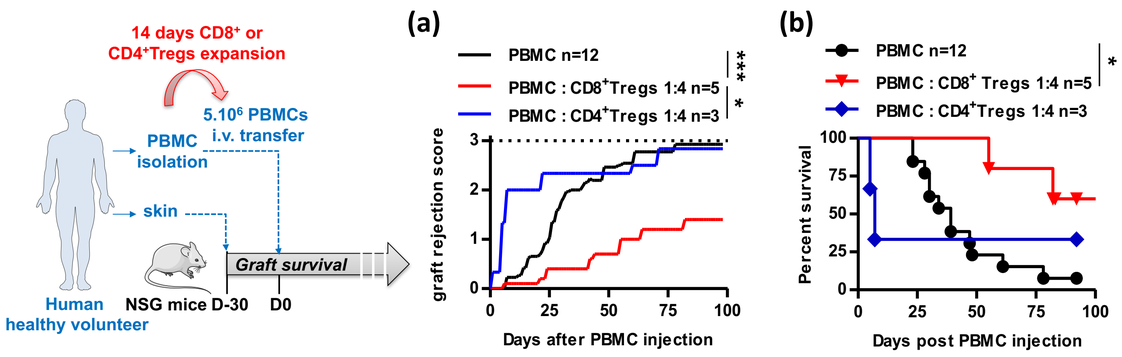
4. SOT

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.