Alteration of CD8$^+$CD45RC$^{int/neg}$ regulatory T cells functions in Multiple Sclerosis and correlates with disease severity

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Background

Autoimmune diseases can develop following pathological activation of autoreactive effector cells and/or, alternatively, after weakening of self-protective regulatory mechanisms. Most of the studies have focused on CD4$^+$ Tregs and the **role of CD8$^+$ Tregs in Multiple Sclerosis (MS) remains largely unexplored**. We previously reported the suppressive properties of rat and human **CD8$^+$CD45RC$^{int/neg}$ Treg cells**, expressing Foxp3 and acting through IFNg, TGFb and IL34 cytokines (Guillonneau, JCI, 2007; Bézie, JCI, 2015, Bézie, Front. Immunol., 2018). Thus, their **potency of suppression** make them strong candidates of disruptive immune tolerance, especially in MS where CD8$^+$ T cells play a major role. **Thus, the overreaching goal of this study is to define the role of CD8$^+$ regulatory T cell in MS pathogenesis**

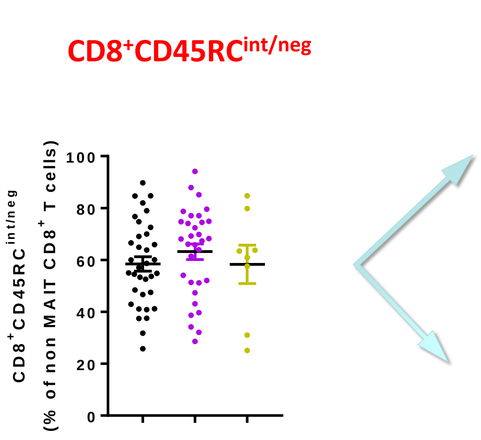
Material & Methods

56 untreated relapsing-remitting MS patients and 52 age- and gender-matched healthy volunteers (HV) were recruited.

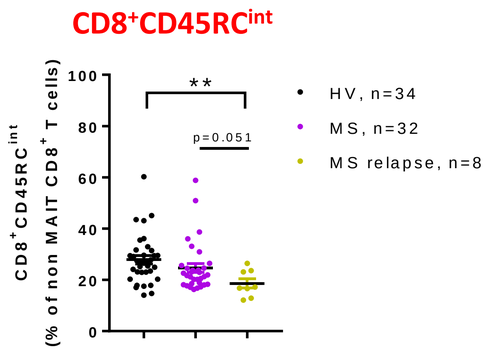
- Tregs were defined as CD3$^+$CD8$^+$ (or CD4$^-$) CD161^{low}Vα7$^-$CD45RC$^{int/neg}$ T cells. MAIT cells were excluded from all analysis.
- Staining: T cells were stimulated or not 5h with PMA+ionomycin including 4h with BFA before FACS depending on the marker investigated.
- Transcriptomic analysis: 3'-Digital Gene Expression Sequencing was performed on unstimulated cells. Analysis was performed using R packages and KEGG, Reactome and Gene Ontology databases for Gene Set Enrichment Analysis (GSEA).

CD8+CD45RC^{INT} Tregs frequency in blood is reduced during exacerbation whereas CD8+CD45RC^{NEG} Tregs are more frequent with enhanced suppressive functions

CD8$^+$CD45RC$^{int/neg}$

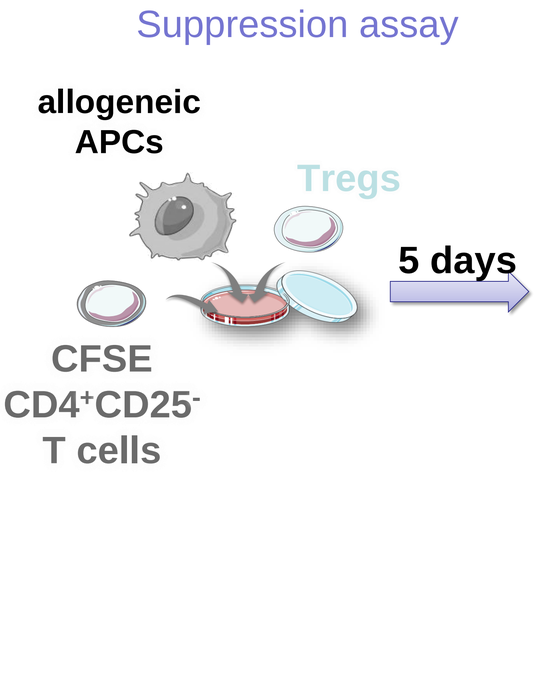


CD8$^+$CD45RC^{int}

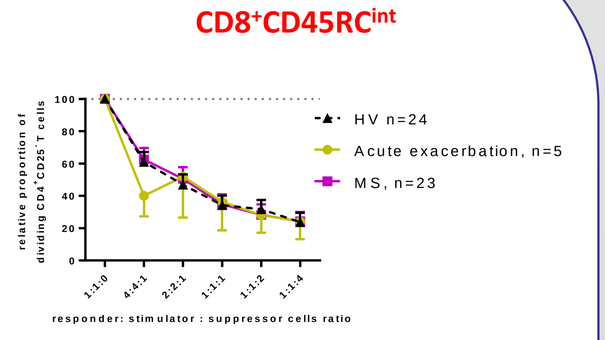


CD8$^+$CD45RC^{neg}

Suppression assay

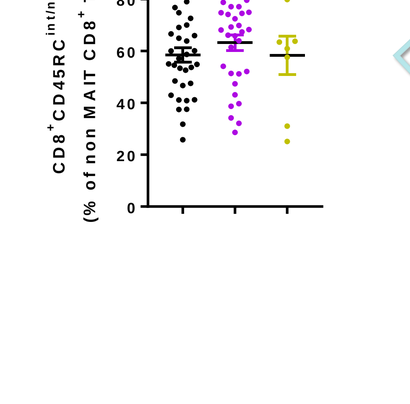


CD8$^+$CD45RC^{int}



CD8$^+$CD45RC^{neg}

In severe patients with a higher Multiple Sclerosis Severity Score, CD8+CD45RC^{INT} Tregs function is impaired



Suppression assay

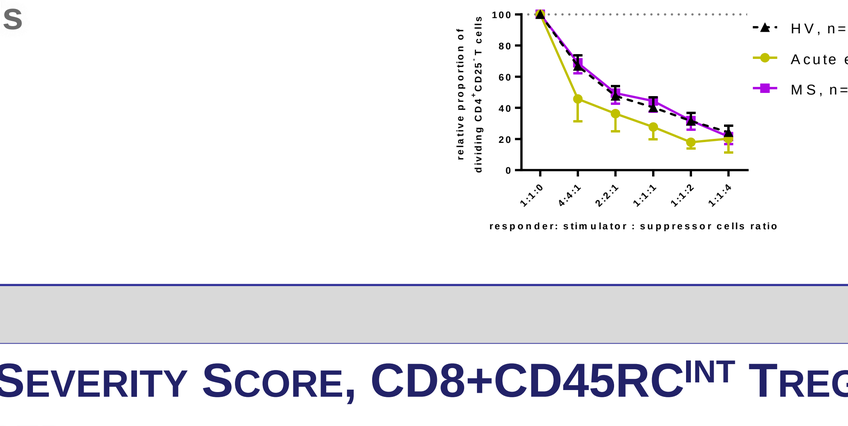
Transcriptomic Analysis (GSEA)

Regulation of Inflammatory response

Cytokine-mediated Signaling Pathway

Immune response pathways are overrepresented in CD8+CD45RC^{int} of MSSS<3,5 patients as compared to MSSS>3,5 patients

Flow Cytometry Analysis



PD-1, and IFNg expression are **decreased in CD8+CD45RC^{int}**

Conclusion

For the first time, we demonstrate an **impairment of CD8+CD45RC^{int} Tregs in MS**.

We propose to define two populations of CD8+CD45RC Tregs:

- **CD8+CD45RC^{int} are dysfunctional in severe MS** patients and **less frequent** during exacerbations
- **CD8+CD45RC^{neg} Tregs react properly to inflammation** with enhanced regulatory functions during exacerbations

We suggest CD8$^+$CD45RC$^{int/neg}$ T cells and subsets may be potential therapeutic targets and prognostic tools in MS

Authors have no financial disclosures that would be a potential conflict of interest with this presentation