



## **B cell differentiation is defective in Multiple Sclerosis patients**

Jérémy Morille, M. Chesneau, F. Lejeune, A. Garcia, L. Berthelot, A. Nicot,  
Sophie Brouard, David-Axel Laplaud, L. Michel

### **► To cite this version:**

Jérémy Morille, M. Chesneau, F. Lejeune, A. Garcia, L. Berthelot, et al.. B cell differentiation is defective in Multiple Sclerosis patients. Labex IGO, Apr 2018, Nantes, France. inserm-02160236

**HAL Id: inserm-02160236**

**<https://inserm.hal.science/inserm-02160236>**

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.



# B cell differentiation is defective in Multiple Sclerosis patients

## Introduction

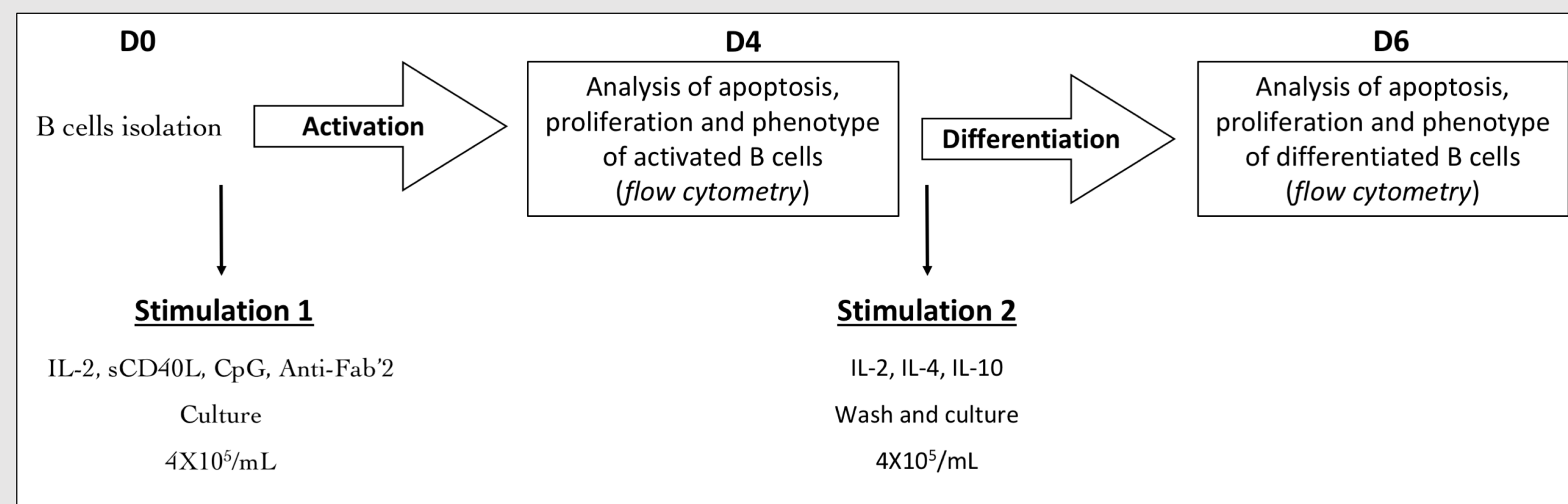
Clinical trials on the efficacy of B-cell depleting therapies in relapsing multiple sclerosis (MS) have suggested that B cells may contribute to MS pathogenesis, potentially through antibody independent mechanisms. Meningeal lymphoid follicle-like structures have been found in some progressive MS patients and meningeal inflammation seem to be observed in all forms of MS. Even if there have been many progresses in the understanding of B cell roles in MS, the exact implication and roles of plasma cells remain badly known.

## Objective

- Characterization of the B cell differentiation profile of MS patients compared to healthy controls (HC)
- Characterization of the frequencies of the different T<sub>FH</sub> subsets in the blood and the cerebrospinal fluid (CSF)

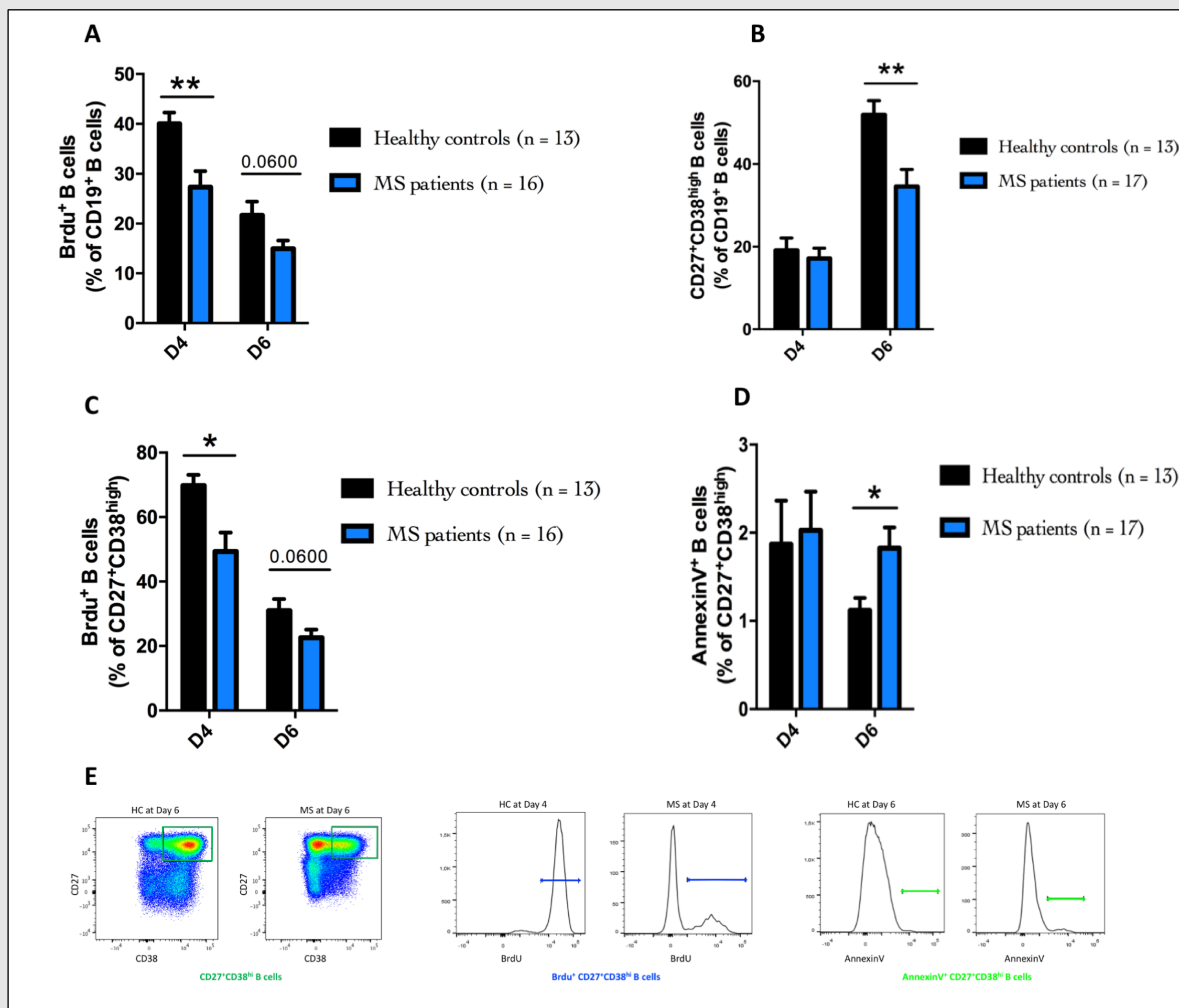
## Methods

- A two-step model of peripheral B cell differentiation



- A ten color analysis of T<sub>FH</sub> phenotype by flow cytometry

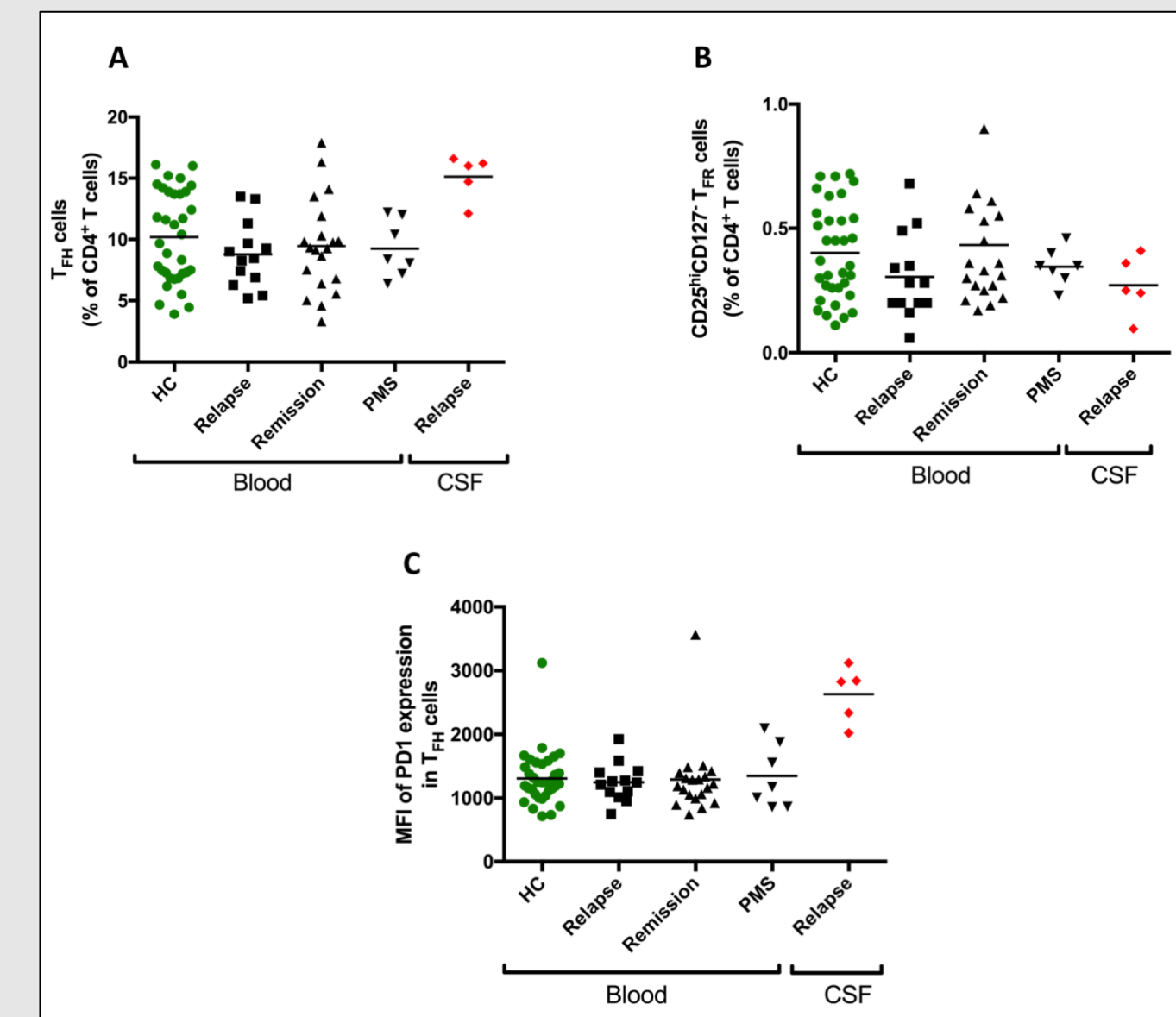
## Characterization of a defective B cell differentiation profile in MS patients



**Figure 1. Analysis of the phenotype, the proliferation and the apoptosis of B cells at day 4 and day 6 of differentiation.**

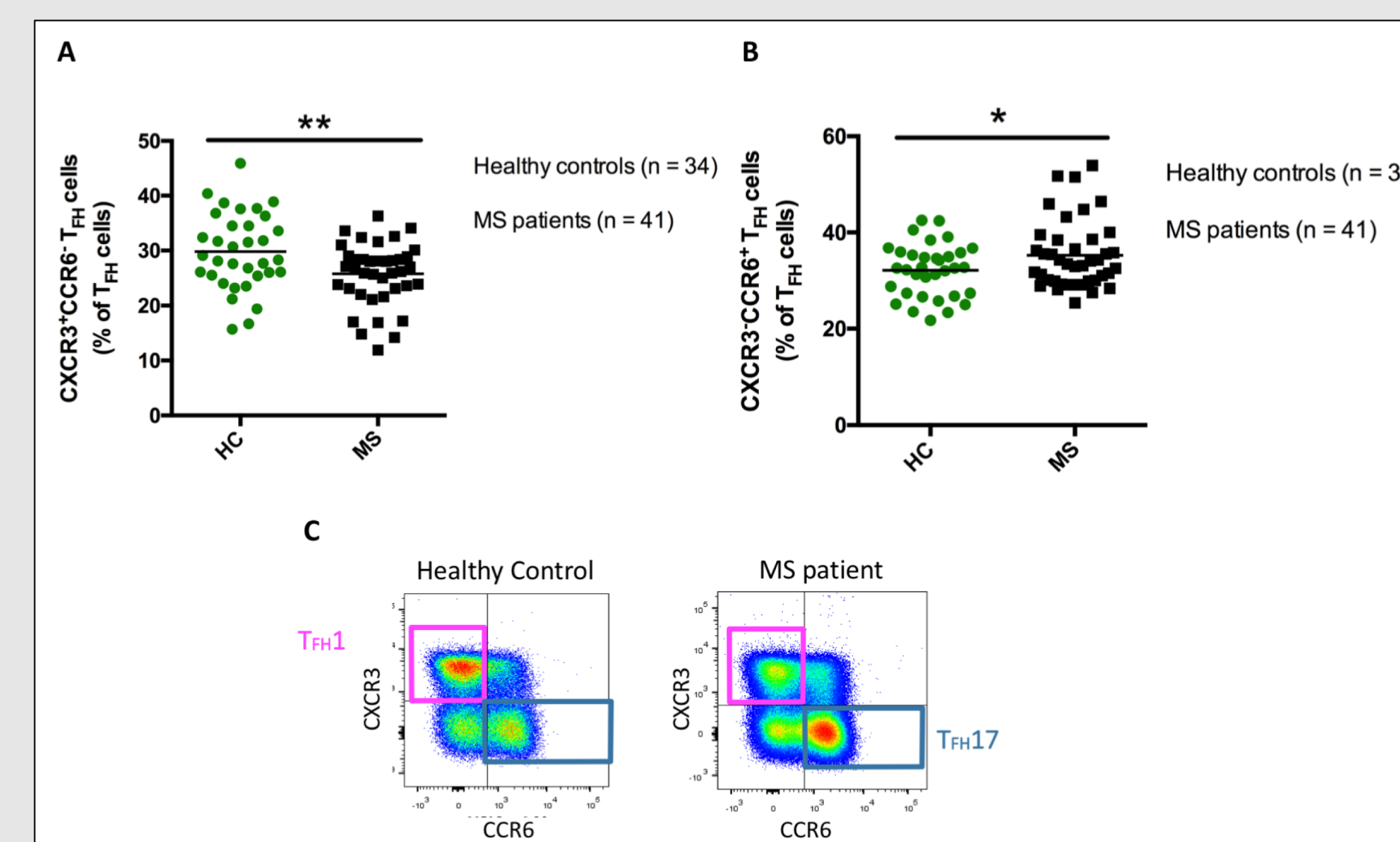
- (A) B cells from MS patients proliferate significantly less ( $27.34 \pm 12.79\%$  vs  $40.05 \pm 8.05\%$  in HC at day 4,  $p < 0.01$ ) compared to B cells from HC at Day 4.
- (B) MS patients harbored a significant lower frequency of CD27<sup>+</sup>CD38<sup>high</sup> differentiated B cells ( $34.58 \pm 16.93\%$  vs  $51.89 \pm 12.09\%$  in HC at day 6,  $p < 0.01$ ) compared to HC at day 6.
- (C) CD27<sup>+</sup>CD38<sup>high</sup> B cells from MS patients proliferate significantly less ( $49.32 \pm 23.48\%$  vs  $69.76 \pm 12.05\%$  in HC at day 4,  $p < 0.05$ ) compared to B cells from HC at day 4.
- (D) CD27<sup>+</sup>CD38<sup>high</sup> B cells from MS patients have a significant higher sensitivity to apoptosis ( $1.82 \pm 0.96\%$  vs  $1.12 \pm 0.51\%$  in HC at day 6,  $p < 0.05$ ) compared to B cells from HC at day 6.
- (E) Example of gating strategy by flow cytometry.

## Phenotype of circulating T<sub>FH</sub> cells in MS patients



**Figure 2. Frequencies of TFH subsets in MS patients groups.**

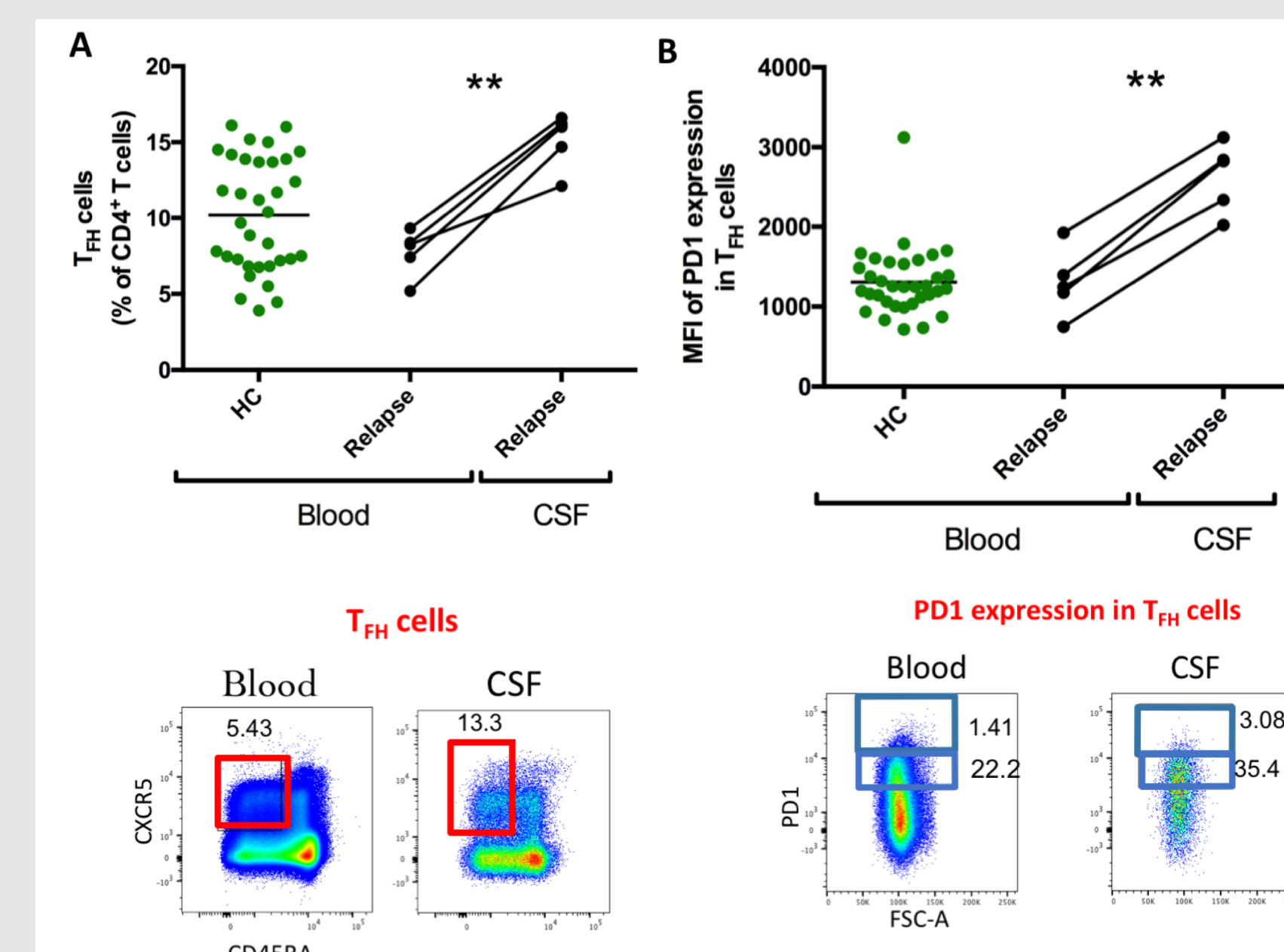
- (A) Frequency of circulating T<sub>FH</sub> cells within RMS patients in relapse, RMS patients in remission and PMS patients. Frequency of CSF-infiltrating T<sub>FH</sub> cells in RMS patients is also shown.
- (B) Frequency of circulating T<sub>FR</sub> cells within RMS patients in relapse, RMS patients in remission and PMS patients. Frequency of CSF-infiltrating T<sub>FR</sub> cells in RMS patients is also shown.
- (C) MFI of the expression of the immunoregulatory molecule PD1 within the circulating T<sub>FH</sub> cells of RMS patients in relapse, RMS patients in remission and PMS patients. The MFI of PD1 within the CSF-infiltrating T<sub>FH</sub> cells in RMS patients is also shown.



**Figure 3. Frequency of circulating T<sub>FH</sub> cells in MS patients and HC.**

- (A) MS patients present a significant higher frequency of T<sub>FH</sub>17 in the blood compared to HC ( $35.2 \pm 1.09\%$  vs  $32.1 \pm 0.9\%$ ,  $p < 0.05$ ).
- (B) MS patients present a significant lower frequency of T<sub>FH</sub>1 cells compared to HC ( $25.79 \pm 0.87\%$  in MS vs  $29.81 \pm 1.19\%$  in HC,  $p < 0.01$ ).
- (C) Example of the gating strategy by flow cytometry.

## Phenotype of CSF-infiltrating T<sub>FH</sub> cells in MS patients



**Figure 4. Frequency of CSF-infiltrating T<sub>FH</sub> cells within RMS patients in relapse**

- (A) MS patients have a significant higher frequency of T<sub>FH</sub> cells within their CSF compared to their blood ( $15.12 \pm 0.81\%$  vs  $7.71 \pm 1.56\%$  in blood,  $p < 0.01$ ).
- (B) MS patients have a significant higher expression of the immunoregulatory molecule PD1 within their CSF-infiltrating T<sub>FH</sub> cells compared to their circulating T<sub>FH</sub> cells ( $2630 \pm 197$  vs  $1297 \pm 190$  in blood for the MFI,  $p < 0.01$ ).

## Conclusion

For the first time, we show that MS patients present a defect in their B cell differentiation. We hypothesize that these plasmablasts are more pro-inflammatory in MS patients, and that the apoptotic cells are probably regulatory plasma cells. To explore this hypothesis, we plan now to perform cytokine analysis (Cytometry and Luminex), transcriptomic analysis, but also to test the supernatant's cytotoxic effects on oligodendrocytes cultures.

We also show that MS patients present an altered T<sub>FH</sub> phenotype in periphery, and for the first time we highlight that T<sub>FH</sub> cells infiltrate the CSF of MS patients with an activated profile. We plan to investigate by immunohistochemistry whether T<sub>FH</sub> cells infiltrate the central nervous system of MS patients. To better understand the involvement of T<sub>FH</sub> cells in the pathophysiology of MS, we also prospect to assess the abilities of T<sub>FH</sub> cells to differentiate B cells by co-culture of autologous T<sub>FH</sub> cells with B cells.