B cell differentiation is defective in Multiple Sclerosis patients

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Introduction

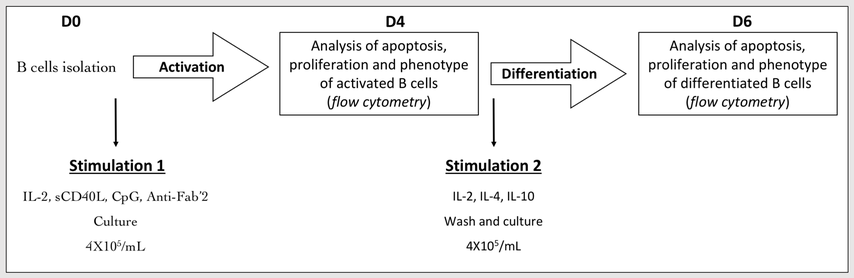
Clinical trials on the efficacy of B-cell depleting therapies in relapsing multiple sclerosis (MS) have suggested that B cells may contribute to MS pathogenesis, potentially through antibody independent mechanisms. Meningeal lymphoid follicle-like structures have been found in some progressive MS patients 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_{FH} 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_{FH} phenotype by flow cytometry

Characterization of a defective B cell differentiation profile in MS patients

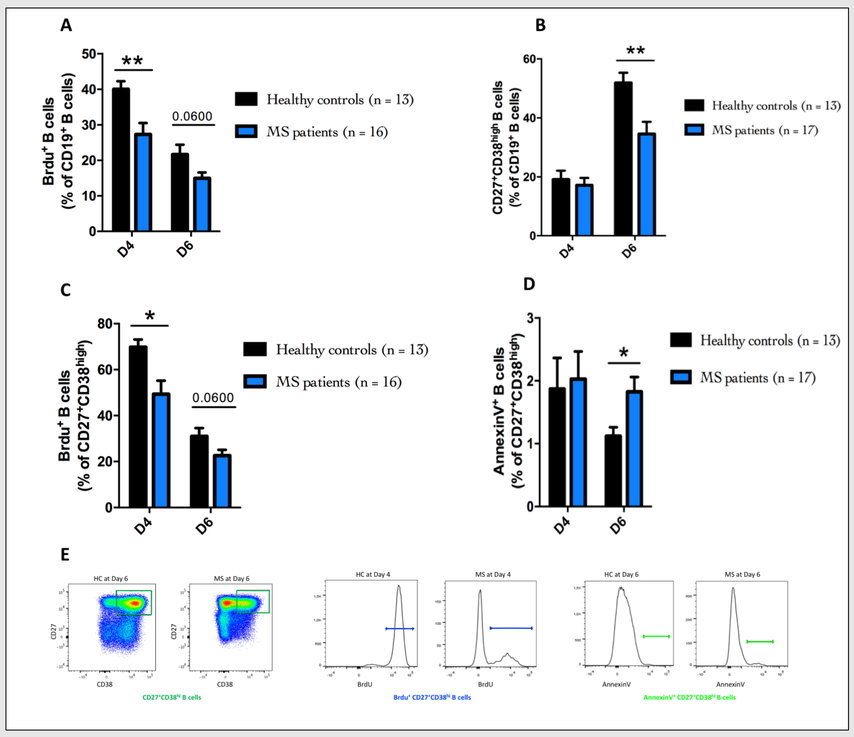


Figure 1. Analysis of the phenotype, the proliferation and the apoptosis of B cells at day 4 and day 6 of differentiation.

(A) B cells from MS patients proliferate significantly less (27.34 ± 12.79% vs 40.05 ± 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 $CD27^+CD38^{high}$ differentiated B cells (34.58 ± 16.93% vs 51.89 ± 12.09% in HC at day 6, $p < 0.01$) compared to HC at day 6.

(C) $CD27^+CD38^{high}$ B cells from MS patients proliferate significantly less (49.32 ± 23.48% vs 69.76 ± 12.05% in HC at day 4, $p < 0.05$) compared to B cells from HC at day 4.

(D) $CD27^+CD38^{high}$ B cells from MS patients have a significant higher sensitivity to apoptosis (1.82 ± 0.96% vs 1.12 ± 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_{FH} cells in MS patients

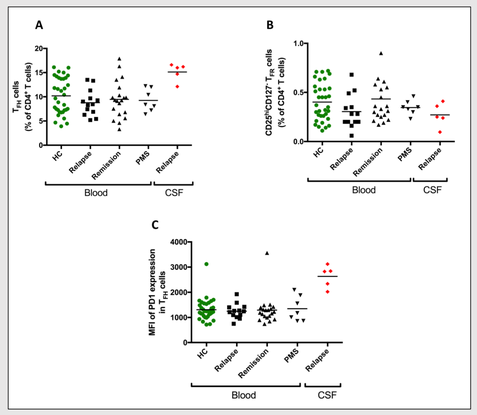


Figure 2. Frequencies of TFH subsets in MS patients groups.

(A) Frequency of circulating T_{FH} cells within RMS patients in relapse, RMS patients in remission and PMS patients. Frequency of CSF-infiltrating T_{FH} cells in RMS patients is also shown.

(B) Frequency of circulating T_{FR} cells within RMS patients in relapse, RMS patients in remission and PMS patients. Frequency of CSF-infiltrating T_{FR} cells in RMS patients is also shown.

(C) MFI of the expression of the immunoregulatory molecule PD1 within the circulating T_{FH} cells of RMS patients in relapse, RMS patients in remission and PMS patients. The MFI of PD1 within the CSF-infiltrating T_{FH} cells in RMS patients is also shown.

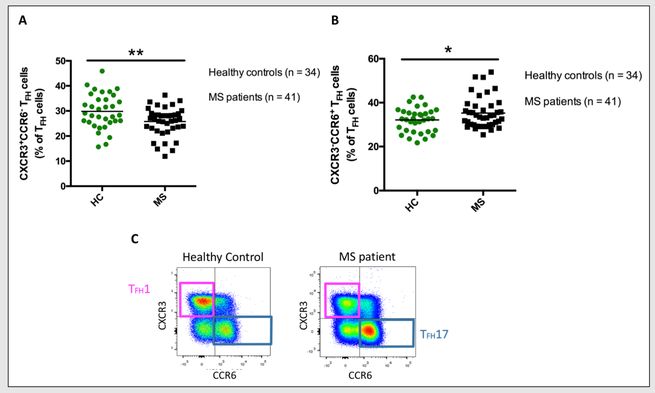


Figure 3. Frequency of circulating T_{FH} cells in MS patients and HC.

(A) MS patients present a significant higher frequency of $T_{FH}17$ in the blood compared to HC (35.2 ± 1.09% vs 32.1 ± 0.9%, $p<0.05$).

(B) MS patients present a significant lower frequency of $T_{FH}1$ cells compared to HC (25.79 ± 0.87% in MS vs 29.81 ± 1.19% in HC, $p<0.01$).

(C) Example of the gating strategy by flow cytometry.

Phenotype of CSF-infiltrating T_{FH} cells in MS patients

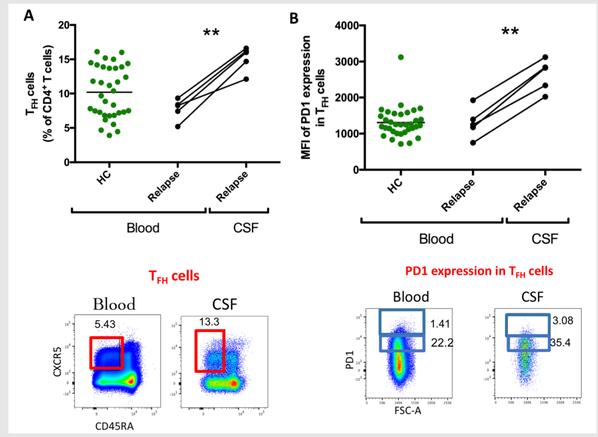


Figure 4. Frequency of CSF-infiltrating T_{FH} cells within RMS patients in relapse

(A) MS patients have a significant higher frequency of T_{FH} cells within their CSF compared to their blood (15.12 ± 0.81% vs 7.71 ± 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_{FH} cells compared to their circulating T_{FH} (2630 ± 197 vs 1297 ± 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_{FH} phenotype in periphery, and for the first time we highlight that T_{FH} cells infiltrate the CSF of MS patients with an activated profile. We plan to investigate by immunohistochemistry wether T_{FH} cells infiltrate the central nervous system of MS patients. To better understand the involvement of T_{FH} cells in the pathophysiology of MS, we also prospect to assess the abilities of T_{FH} cells to differentiate B cells by co-culture of autologous T_{FH} cells with B cells.

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