The smB$^-$ group of Common Variable Immunodeficiency (CVID) patients gathers B cell metabolism defects leading to immunodeficiency

Maria Berman-Riu[1,2], Vanesa Cunill[1,2], Antonio Clemente[3], Antonio López-Gómez[1,2], Jaime Pons[1,2], Joana M Ferrer[1,2]

[1]*Department of Immunology, Son Espases University Hospital, Palma, Spain*
[2]*Human Immunopathology Research Group, Health Research Institute of the Balearic Islands (IdISBa), Palma, Spain*
[3]*GTERi - Group of Innovation in Immunopathology of Infections, Health Research Institute of the Balearic Islands (IdISBa), Palma, Spain*

BACKGROUND AND AIMS

Common Variable Immunodeficiency (CVID) patients are clinically heterogeneous, suffering from infectious and non-infectious complications. CVID hallmark is a deficient memory and antibody-secreting B cell generation leading to hypogammaglobulinemia and poor response to vaccination. CVID patients are classified according to the percentage of switched memory B cell compartment in smB- (<2%) or smB+ (>2%) following the EUROclass classification.

We have recently disclosed specific **alterations** in **mitochondrial function**, **ROS production,** and **autophagy** in B cells from CVID patients that could impact B cell fate and differentiation.

The aim of the present study was to determine whether these alterations are related to the higher **memory B cell** compromise in switched memory B negative (**smB$^-$**) CVID patient subgroup.

METHODS

In vitro cell death (SYTOX$^+$), cells with mitochondrial dysfunction (% Mitotracker Deep Red^{low} and Green^{high} cells), ROS production (% CellROX$^+$ cells), and autophagy (LC3-II levels) in human naïve (CD19$^+$CD27$^-$) and memory (CD19$^+$CD27$^+$) B-cell subpopulations were evaluated by flow cytometry in unstimulated and stimulated PBMCs cultures from smB$^+$ and smB$^-$ CVID patients and controls (Figure 1).

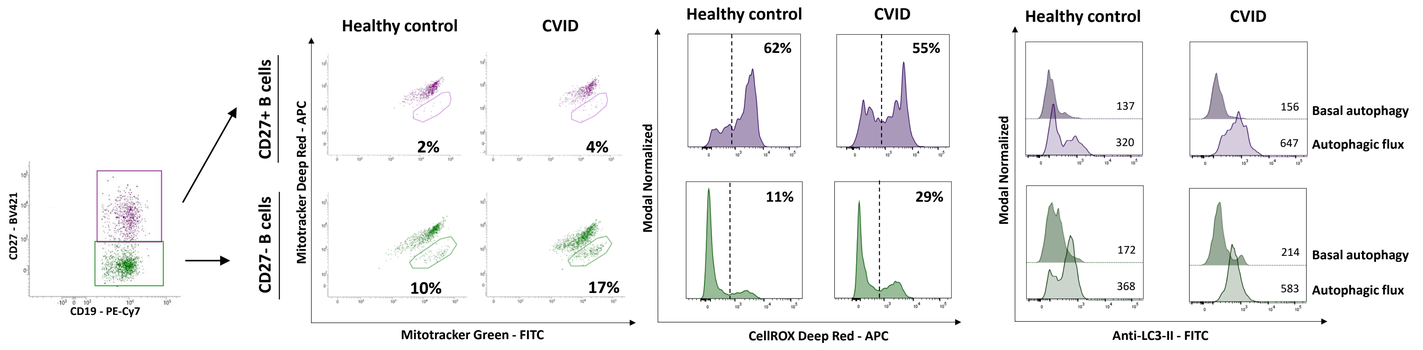

Figure 1. Flow cytometry analysis of viable naïve (green) and memory (purple) B-cell subpopulations with (A) dysfunctional mitochondria, (B) ROS production and (C) autophagy levels from a representative healthy control and CVID patient.

RESULTS

- The percentage of unstimulated naïve CD19$^+$CD27$^-$ B cells with dysfunctional mitochondria was higher in the smB$^-$ CVID group compared to controls. **ROS** basal levels and **autophagy** were more prominent in naïve CD19$^+$CD27$^-$ B cells from both groups of CVID patients compared to controls (Figure 2.A).

- Unstimulated memory CD19$^+$CD27$^+$ B cells from smB$^-$ CVID patients had a trend toward basal **lower ROS** levels and significantly **higher autophagy** compared to both controls and smB$^+$ CVID patients (Figure 2.B). This was also evident after stimulation with anti-CD40, ODN and anti-BCR+IL-21 (lower ROS levels), and with anti-CD40, ODN, and anti-CD40+IL-21 (higher autophagy flux) (Figure 2.B).

- *In vitro* **cell death** of memory CD19$^+$CD27$^+$ B cells from smB$^-$ patients was higher than that of controls and smB$^+$ patients (Figure 3.A).

- **ROS production** negatively correlated with *in vitro* **cell death** of memory CD19$^+$CD27$^+$ B cells from smB$^-$ patients (Figure 3.B).

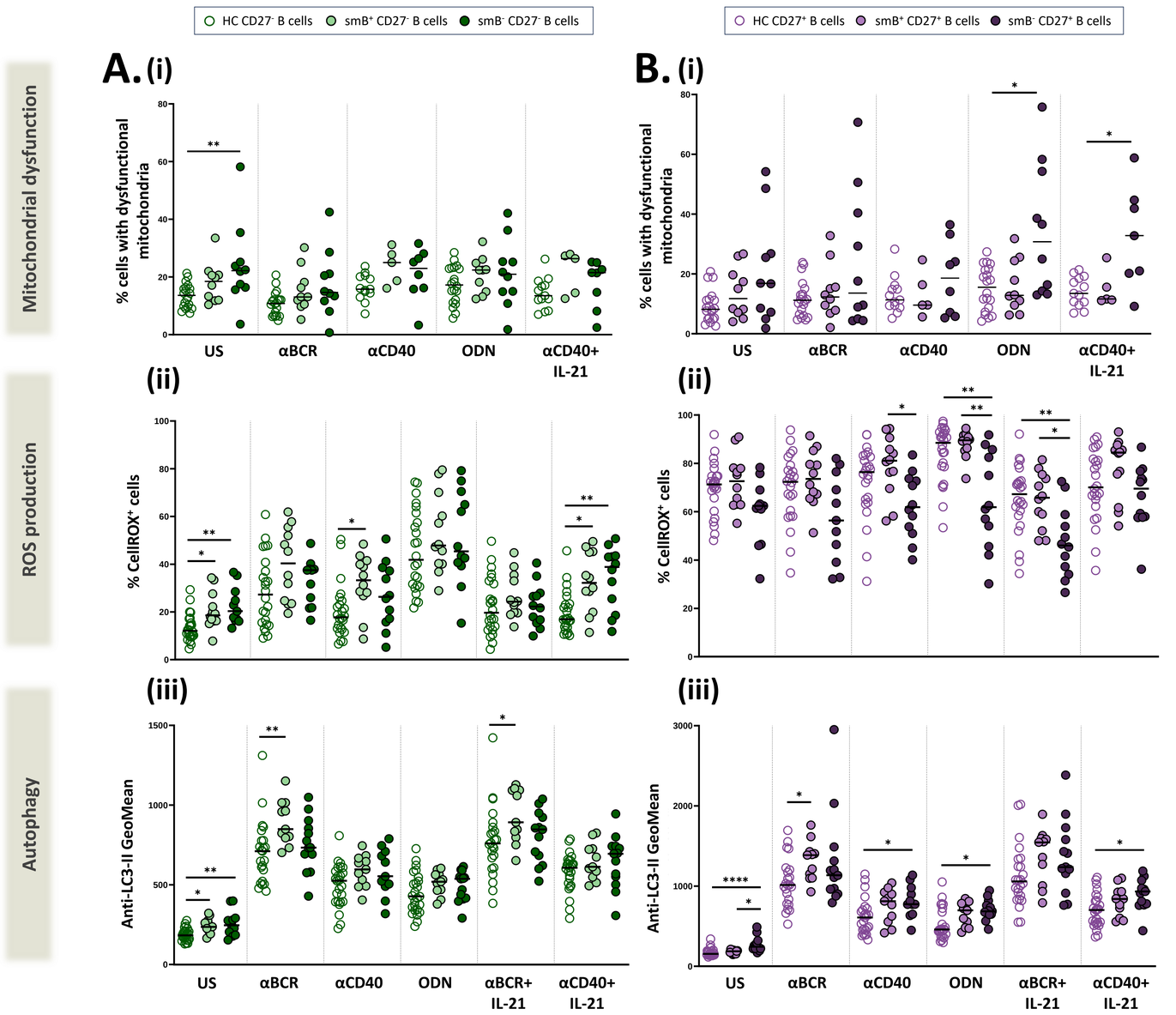

Figure 2. Cells with dysfunctional mitochondria, ROS production, autophagy and in B cell subpopulations from CVID patients and healthy controls. Comparison of B cells with dysfunctional mitochondria, % of CellROX$^+$ and autophagic flux between (A) naïve CD19+CD27− and (B) memory CD19+CD27+ B cells from healthy controls and smB+ and smB- CVID patients after 24 h of culture without (US) or with stimulation (anti-BCR, anti-CD40, CpG-ODN, anti-BCR+IL-21 and anti-CD40+IL-21). Mann–Whitney test p-values: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

CONCLUSIONS

The combination of low levels of **ROS**, which negatively correlate with *in vitro* **cell death**, and uncontrolled **autophagy,** could be the cause of the more compromised memory B cell compartment in smB$^-$ CVID patients.

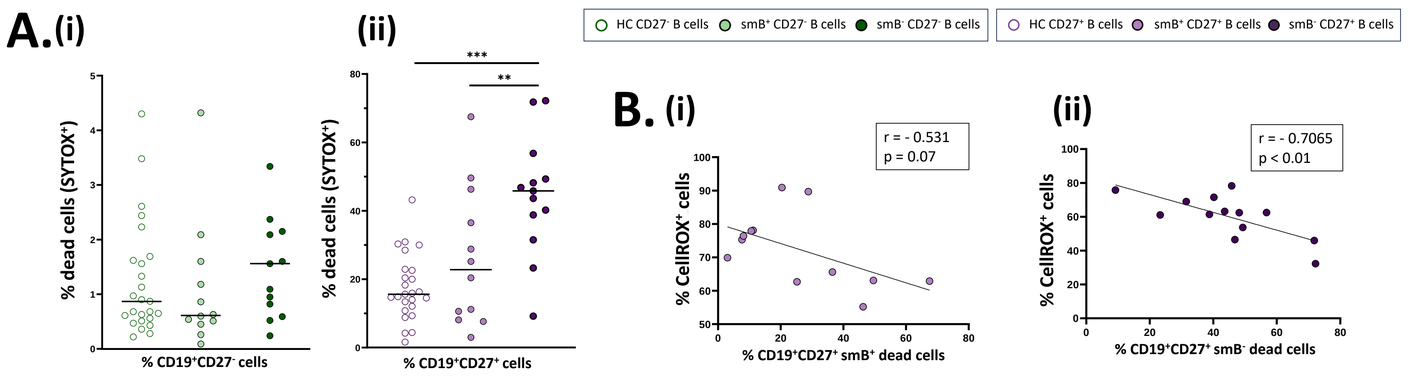

Figure 3. *In vitro* cell death and correlation with ROS production of B cell subpobulations in healthy controls and CVID patients. (A) *In vitro* cell death (% SYTOX$^+$ cells) of (i) naïve and (ii) memory B cells from healthy controls, smB$^+$ and smB$^-$ CVID patients. (B) Correlation between *in vitro* cell death and ROS production on (i) smB$^+$ and (ii) smB$^-$ CVID memory B cells (r: Pearson's correlation coefficient). Mann–Whitney test p-values: **p < 0.01, ***p < 0.001, ****p < 0.0001.

Our previous work: Berman-Riu M, et al. Dysfunctional mitochondria, disrupted levels of reactive oxygen species, and autophagy in B cells from common variable immunodeficiency patients. Front Immunol. 2024

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This work has been funded by the Instituto de Salud Carlos III through the project "PI19/00825" (co-funded by Fondo Europeo de Desarrollo Regional (FEDER)). The work of MB-R has been supported by a grant (JUNIOR19/02) from the Health Research Institute of the Balearic Islands (IdISBa). The work of AC has been funded by the Instituto de Salud Carlos III through a Miguel Servet contract (PI23/00151) and co-funded by the European Union.