Cytotoxic B cells in MS patients

European Charcot Foundation

29th Annual Meeting of the European Charcot Foundation

Vinícius de O. Boldrini[1], Ana M.Marques[1], Raphael P.S. Quintiliano[1], Adriel S. Moraes[1], Carla R. A. V. Stella[1,2], Ana Leda F. Longhini[1,3], Marilia Andrade[1], Alfredo Damasceno[2], Alessandro S. Farias[1], Leonilda M. B. Santos[1]

[1]Autoimmune Research Laboratory – Institute of Biology (IB) – University of Campinas (UNICAMP) – Brazil

[2]Department of Neurology – University of Campinas (UNICAMP) – Brazil

[3]Departament of Immunology and Rheumatology – University of Alabama at Birmingham – USA

BACKGROUND

During relapsing-remitting MS (RRMS), CD8+ T lymphocytes infiltrate into the central nervous system (CNS) being found close to oligodendrocytes and neurons. Moreover, in severe/fatal MS relapses a massive infiltration of $CD8^+$ T cells expressing granzyme B (GzmB) was described in CNS parenchyma, evidencing an aberrant cytotoxic behavior. At the same time, B cells and other subsets were demonstrated to share cytotoxic behavior in several diseases. Since cytotoxicity is thought to be a central mechanism for neurodegeneration, we intend to investigate whether "cytotoxic" B cells occur in RRMS patients.

METHODS

104 RRMS patients (19 Untreated, 15 Glatiramer Acetate [GA], 24 Interferon-β [IFN], 14 Fingolimod [FTY] and 32 Natalizumab [NTZ]), according to the McDonald criteria were recruited in Neurology Clinic at University of Campinas Hospital (UNICAMP). Also, **58 healthy subjects** were included in the control groups. All subjects signed a term of consent approved by the University Committee for Ethical Research (CAAE: 53022516.3.0000.5404).

RESULTS

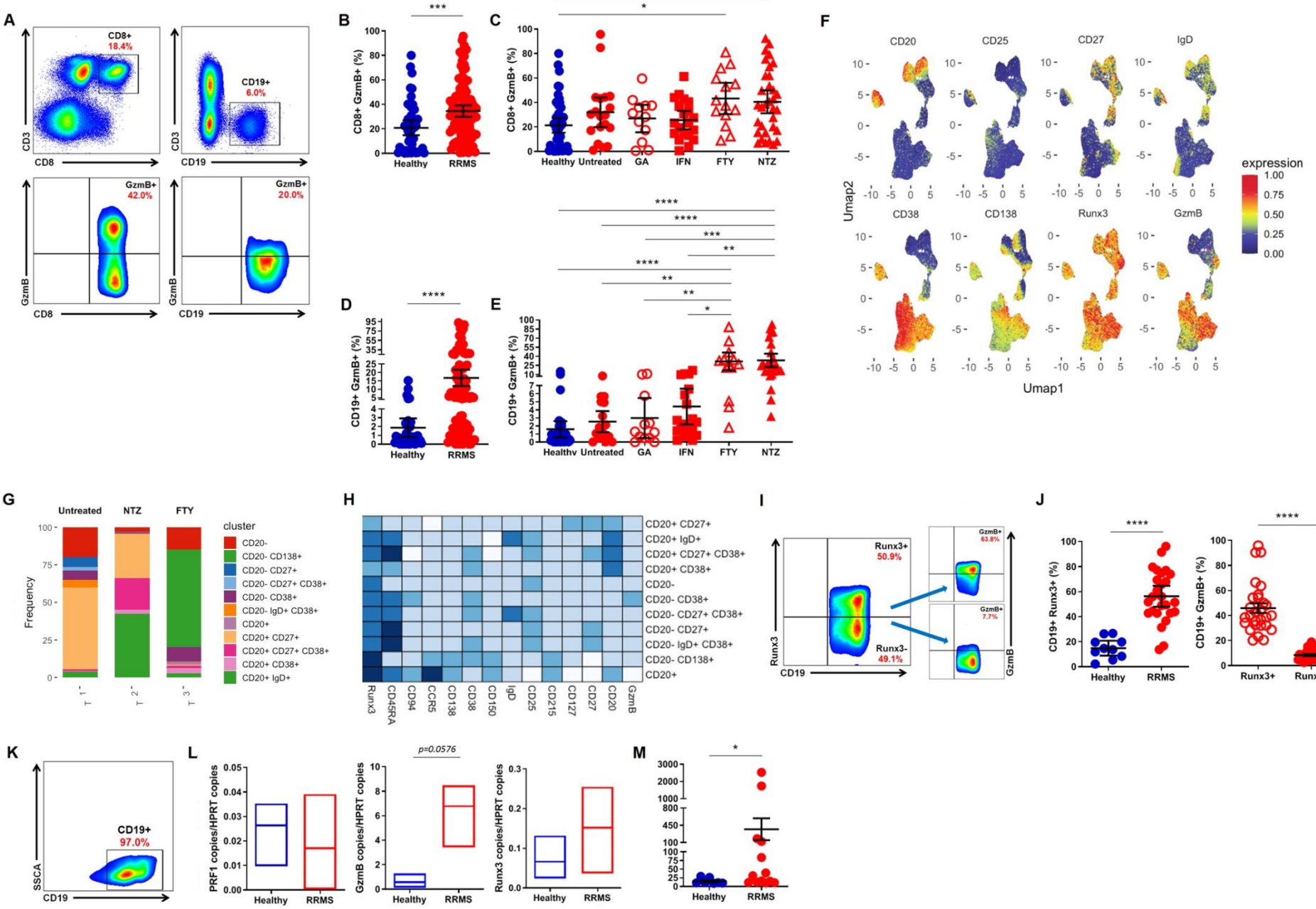

Figure 1: **GzmB-expressing B cells in MS patients. (A)** Gate strategy for $CD8^+GzmB^+$ T lymphocytes and $CD19^+GzmB^+$ B cells. **(B)** Proportion (%) of $CD8^+GzmB^+$ T lymphocytes in RRMS patients (**red**) and healthy donors (**blue**). **(C)** Proportion (%) of $CD8^+GzmB^+$ T lymphocytes in treated RRMS patients (*GA*, *IFN*, *FTY, NTZ*) (**red**), untreated MS (**red**) and healthy donors (**blue**). **(D)** Proportion (%) of $CD19^+GzmB^+$ B cells in RRMS patients (**red**) and healthy donors (**blue**). **(E)** Proportion (%) of $CD19^+GzmB^+$ B cells in treated RRMS patients (*GA*, *IFN*, *FTY, NTZ*) (**red**), untreated RRMS (**red**) and healthy donors (blue). **(F)** UMAP gated in $CD19^+$ B cells from RRMS patients with different conditions non identified and based on the arcsine-transformed expression of the markers. **(G)** Barplot representing the frequency of each subpopulation in $CD19^+$ B cells. **(H)** Heatmap of the expression of the markers in subpopulations manually identified in $CD19^+$ B cells. **(I)** Gate strategy for GzmB-derived $CD19^+Runx3^+$ B cells. **(J)** Proportion (%) of $CD19^+Runx3^+$ B cells in RRMS patients (**red**) and healthy donors (**blue**) and proportion (%) of GzmB-derived $CD19^+Runx3^+$ or $CD19^+Runx3^-$ B cells in RRMS patients (**red**). **(K)** Gate strategy for isolated $CD19^+$ B cells. **(L)** Quantitative PCR for *PRF1*, *GzmB* and *Runx3* in isolated B cells from RRMS patients (**red**) and healthy donors (**blue**). **(M)** Concentration (pg/mL) of GzmB-derived from $CD19^+$ B cells supernatants from RRMS patients (**red**) and healthy donors (**blue**). *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

CONCLUSION

Increased percentage of $CD19^+GzmB^+$ B cells was observed in FTY and NTZ subgroups when compared to GA, IFN and untreated RRMS patients. Moreover, using high-dimensional FACS (UMAP), we observed that $CD19^+CD138^+$ plasma cells, but not $CD19^+CD20^+$ B cells, seem to represent a main subset of B cells involved in GzmB-expression. Also, Runx3, a master regulator of cytotoxic activity classically described in $CD8^+$ T lymphocytes, seems to be associated with GzmB expression in $CD19^+$ B cells in MS patients. Thus, in addition to antigen presentation and cytokine production, ectopic cytotoxicity may represent a novel antibody-independent mechanism derived from B cells with possible implications for MS pathophysiology. Further investigations, in larger cohorts, may elucidate the eventual clinical/therapeutic relevance of "cytotoxic" B cells during MS.