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Marginal Zone-Like B Cells Drive Inflammation and Undergo Long-Term Depletion After Cladribine in MS

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Background

Multiple sclerosis (MS) is increasingly seen as B-cell-driven. While cladribine induces lasting remission, its impact on specific B-cell subsets is not fully understood. We examined the dynamics and immunopathological roles of these subsets before and after treatment.

Methods

Peripheral blood from 40 MS patients was collected at baseline and at 6, 12, 24, 36, and 48 months post-cladribine initiation. High-dimensional flow cytometry quantified and phenotyped seven B-cell subsets, and cytokine production was assessed ex vivo following CpG/PMA/Ionomycin stimulation.

Results

Cladribine rapidly and profoundly depleted memory B cells, particularly MZ-like and classical subsets. MZ-like cells reconstituted slowly, remaining <15% of baseline at 36 months. In contrast, transitional and naïve B cells expanded, shifting the repertoire toward a more "youthful" profile. Ex vivo, MZ-like B cells were highly polyfunctional and expressed CD1c/CD1d, indicating non-classical antigen presentation. DN2 cells showed elevated CD80/CD86 and HLA-DR expression.

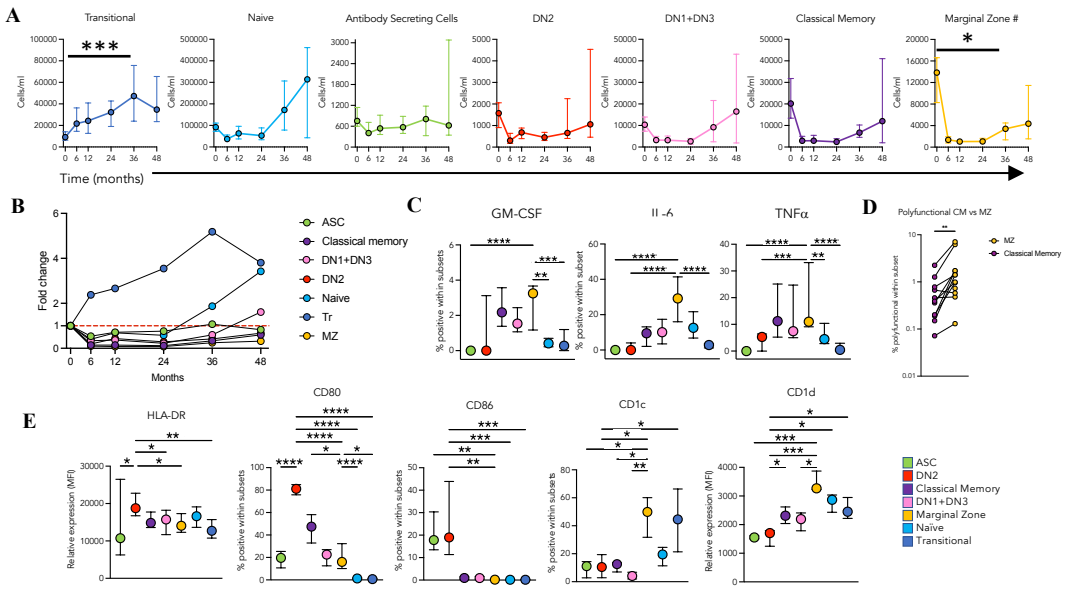

Figure 1: (A) Longitudinal changes in absolute counts of specific B cell subsets. *P < 0.05; **P < 0.01 (Kruskal-Wallis test with Dunn's post hoc correction); **(B)** Fold change in B cell subsets from baseline to 48 months, highlighting major shifts in classical memory and marginal zone B cells; **(C)** Cytokine production by manually gated B cell subsets. Friedman Test with FDR correction for multiple comparisons was performed to compare marker expression on different B cell subsets. * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001; **** P ≤ 0.0001; **(D)** Quantification of polyfunctional cells within CM and MZ subsets, demonstrating significantly higher frequencies in MZ B cells by Wilcoxon matched-pairs signed rank test (** P ≤ 0.01); **(E)** Expression of antigen-presenting molecules (HLA-DR, CD1c, CD1d) and co-stimulatory markers (CD80, CD86) was assessed by flow cytometry in peripheral B cell subsets from healthy donors. For markers expressed by the majority of cells within each subset (e.g., HLA molecules, CD1d), data are shown as median fluorescence intensity (MFI) to reflect quantitative differences in expression. For markers with more restricted or binary expression patterns (e.g., CD80), the percentage of positive cells is shown. Friedman Test with FDR correction for multiple comparisons was performed to compare marker expression on different B cell subsets. * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001; **** P ≤ 0.0001. Circles indicates medians while whiskers show Interquartile Ranges.

Conclusions

Cladribine induces lasting immune resetting by selectively depleting proinflammatory MZ-like memory B cells. Their cytokine profile and antigen-presenting ability suggest a key role in MS inflammation, and their sustained loss may underpin cladribine's long-term efficacy.