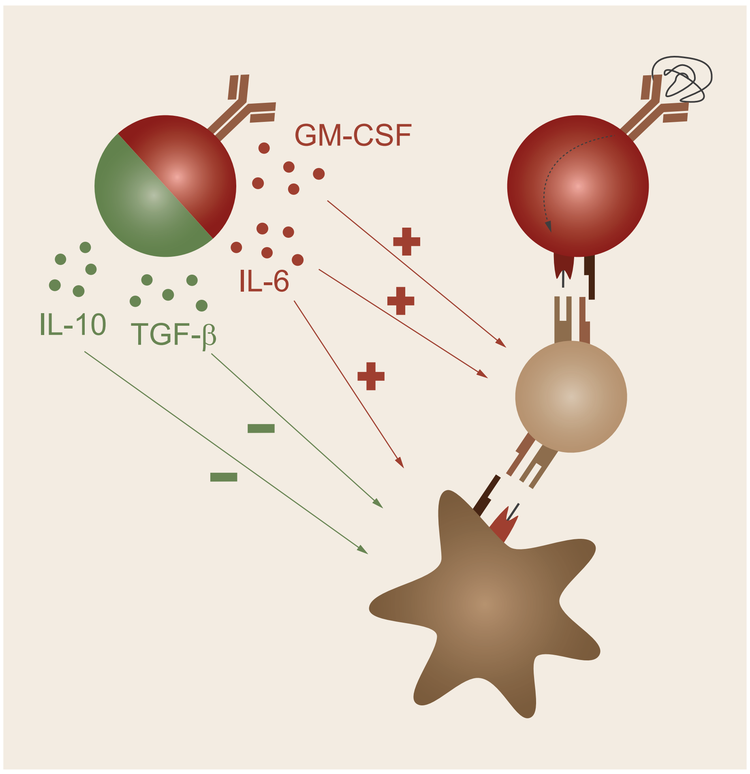
In multiple sclerosis patients, B cells repopulate immature yet more activated upon anti-CD20 antibody therapy

Nitzan Nissimov[1], Zivar Hajiyeva[2], Wolfgang Brück[1], Silke Häusser-Kinzel[1] and Martin S. Weber[1,2]

[1]Institute of Neuropathology, University Medical Center, Göttingen, Germany
[2]Department of Neurology, University Medical Center, Göttingen, Germany

Background & Objective



In multiple sclerosis (MS), B cells are implicated in promoting inflammation through antigen presentation and secretion of pro-inflammatory cytokines. Thus, the therapeutic removal of B cells via anti-CD20 antibodies efficiently reduces annual relapse rates and the development of new and enlargement of existing central nervous system lesions. According to the current guidelines, most patients receive anti-CD20 antibodies every 6 months to avoid B cell repopulation. However, the therapeutic need of a permanent absence of B cells is not well substantiated, as phenotype and function of recurring B cells are not yet thoroughly examined in MS. Hence, we characterized the phenotype of repopulating B cells in MS patients after anti-CD20 antibody infusion.

Materials & Methods

- we isolated human peripheral blood mononuclear cells by Ficoll density gradient centrifugation from whole blood taken by phlebotomy
- for flow cytometry analysis of intracellular cytokines, cells were stimulated for 22 hours with CpG, followed by four additional hours with ionomycin, PMA and golgi plug; all other markers were assessed without stimulation
- prior to antibody staining, cells were incubated with Fc receptor blocking solution to avoid unspecific antibody binding and stained with viability dye for live cell/dead cell discrimination

Results

Table 1: Patient characteristics.

Number of patients (samples before/after/at reappearance	15 (15/14/11)
Sex (w/m)	8/7
Age at start of study (years; mean ± SD)	35.73 ± 8.91
EDSS score at start of the study (mean ± SD)	2.9 ± 2.03
Time since multiple sclerosis diagnosis (years; mean ± SD)	10.76 ± 6.31
Time since initial anti-CD20 treatment (years; mean ± SD	2.68 ± 0.658

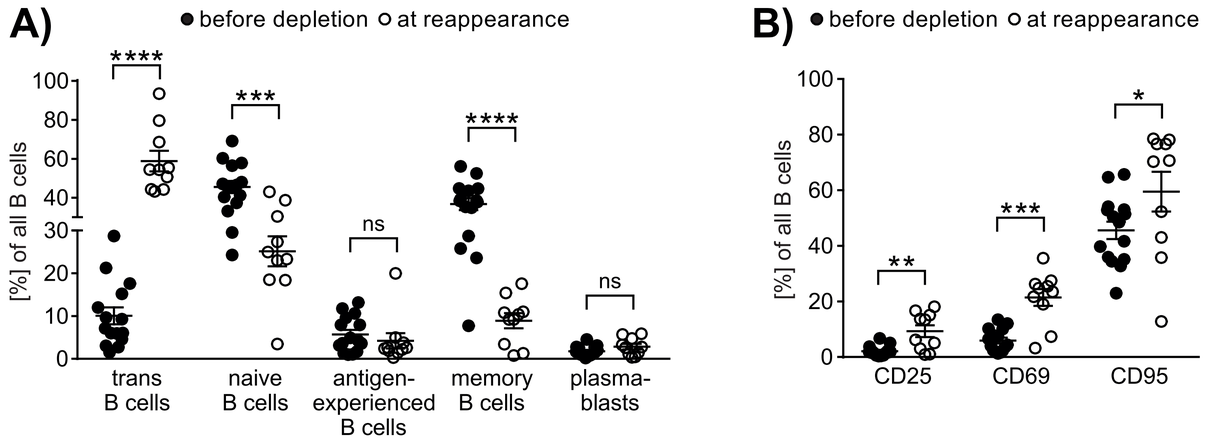


Figure 2: Compared to baseline, recurring B cells are less mature, yet with an enhanced expression of activation marker. We collected PBMC of MS patients before anti-CD20 antibody treatment was initiated (= before depletion) and eight to 24 months thereafter (= at reappearance). **A)** Frequency of transitional ($CD24^{high}$ $CD38^{high}$), mature naïve ($CD27^{-}$ $CD38^{+}$), antigen-experienced ($CD27^{+}$ $CD38^{+}$) and memory ($CD27^{var}$ $CD38^{-}$) B cells as well as plasmablasts ($CD20^{-}$ $CD27^{+}$ $CD38^{+}$) pre-gated on $CD19^{+}$ B cells. **B)** Frequency of CD25, CD69, and CD95 expressing $CD19^{+}$ B cells; * = p < 0.05; ** = p < 0.01; *** p = < 0.001; **** p = < 0.0001; Wilcoxon test/ paired t test.

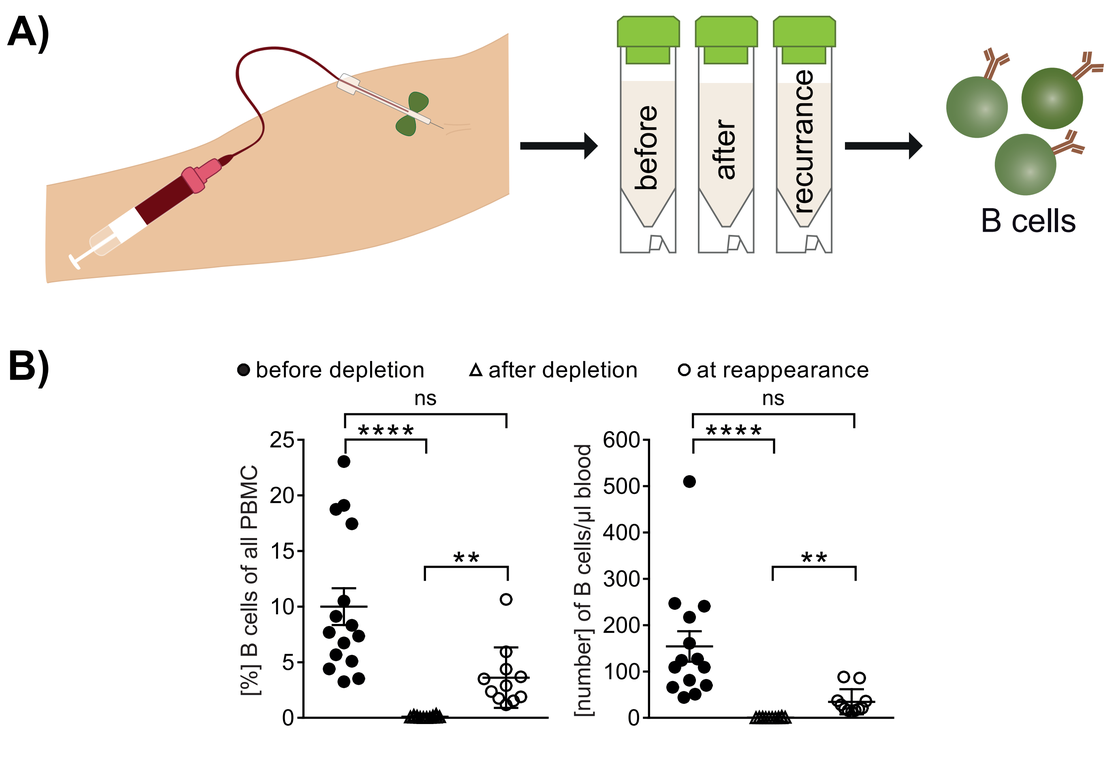


Figure 1: B cells recur after complete depletion mediated by anti-CD20 antibodies. We collected peripheral blood mononuclear cells (PBMC) of MS patients repetitively treated with anti-CD20 antibodies before treatment was initiated (=before depletion), one to five months after initial treatment (=after depletion) and eight to 24 months after initial treatment (=at reappearance). **A)** Schematic illustration of the experimental setup. **B)** Frequency and total number of $CD19^{+}$ B cells at the investigated time points; ** = p < 0.01; **** p = < 0.0001; Kruskal-Wallis test.

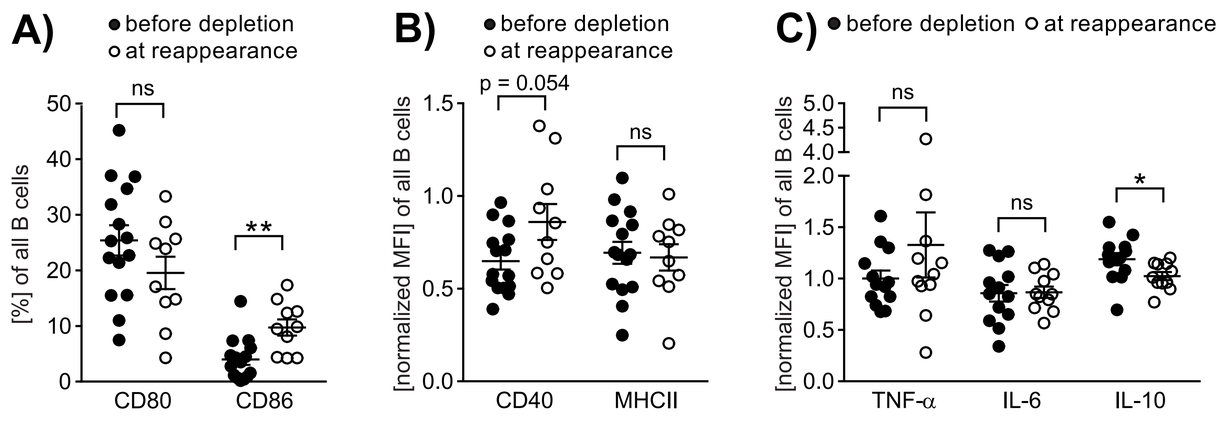


Figure 3: Recurring B cells express high levels of co-stimulatory molecules. We collected PBMC of MS patients before anti-CD20 antibody treatment was initiated (= before depletion) and eight to 24 months thereafter (= at reappearance). **A)** Frequency of CD80 and CD86 expressing $CD19^{+}$ B cells. **B)** Expression of CD40 and MHC class II (MHCII) on $CD19^{+}$ B cells shown as normalized mean fluorescence intensity (MFI). **C)** Expression of tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6 and IL-10 in $CD19^{+}$ B cells depicted as normalized MFI; normalization: patient/control PBMC; * = p < 0.05; ** = p < 0.01; Wilcoxon test/ paired t test.

Conclusion

Our findings implicate that after their anti-CD20 mediated removal, B cells recur less mature, yet more activated in the blood of patients with MS. Whether these immunological changes are associated with changes in the phenotype of other immune cells and have functional and/or clinical implications, remain to be investigated though.

Disclosure

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