Increased frequencies of IgD$^-$CD27$^-$ double negative (DN) B cells with a pro-inflammatory phenotype and function in MS patients

Short title: Age-associated IgD$^-$CD27$^-$ DN B cells in MS

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Immune aging occurs in the elderly and in autoimmune diseases. IgD$^-$CD27$^-$ double negative (DN) B cells have been described as age-associated B cells. This study aimed to investigate the prevalence and functional characteristics of DN B cells in MS patients.

Peripheral blood frequencies of DN, IgD$^-$CD27$^+$ class-switched memory (CSM) and IgD$^+$CD27$^-$ naive B cells and their expression of costimulatory and antigen presentation molecules and chemokine receptors were determined in healthy controls (HC) and MS patients using flow cytometry. DN B cells were also measured in paired blood and cerebrospinal fluid of MS patients. Cytokine production was analysed following *ex vivo* B cell stimulation. CD27$^-$ or CD27$^+$ B cell migration was studied using an *in vitro* chemotaxis assay. The T-box transcription factor T-bet, that has been described in a pathological age-associated B cell subset, was measured in DN B cells.

Peripheral blood DN B cells were significantly elevated in MS patients younger than 60 years compared with age-matched HC (p=0.004), and were further increased in MS cerebrospinal fluid (p=0.03). Expression of MHC class II and costimulatory molecules CD80/CD86 indicated their potential to induce T cell responses. Chemokine receptors CXCR3 and CXCR5 were expressed on DN B cells at similar levels compared with naive or CSM B cells, respectively. DN B cells produced the pro-inflammatory cytokines lymphotoxin-α, tumor necrosis factor-α and granzyme B and demonstrated high migration towards the pro-inflammatory chemokines CXCL10 (CXCR3 ligand) and CXCL13 (CXCR5 ligand). T-bet expression was found in 22[3.3, 52.0]% of DN B cells from MS patients.

Thus, DN B cells are abnormally elevated in MS patients and could migrate into the central nervous system where they could contribute to inflammation by induction of T cell responses and pro-inflammatory cytokine production. Further research on DN B cells could lead to novel targets for more specific MS therapy.