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IgD-CD27- double negative (DN) B cells in multiple sclerosis: a non-exhausted, pro-inflammatory subset with DN1 predominance

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Introduction: Pro-inflammatory immunoglobulin (Ig) D$^-$CD27$^-$ double negative (DN) B cells are abnormally elevated in the peripheral blood (PB) and cerebrospinal fluid (CSF) of MS patients. In aging, DN B cells were described as senescent/exhausted memory cells. Recently, three DN subsets have been described: DN1 (CD11c$^-$CD21$^+$CXCR5$^+$T-bet$^-$), DN2 (CD11c$^+$CD21$^-$CXCR5$^-$T-bet$^+$), and DN3 (CD11c$^-$CD21$^-$CXCR5$^-$).

Objectives/Aims: Here, we studied the subset distribution, activation potential and migration capacity of DN B cells in MS patients.

Methods: The frequency of DN subsets (DN1-3) and expression of T-bet, CD80, chemokine receptors (CXCR3/CXCR5) and adhesion molecules (LFA-1/VLA-4/ALCAM) was measured in paired PB and CSF of MS patients (n=4) and PB of MS patients (n=47/53, respectively) and healthy controls (HC, n=48/25, respectively) using flow cytometry. B cells of MS patients (n=6) and HC (n=6) were stimulated in vitro (CD40ligand+interleukin(IL)-4/CpG2006 +IL-2/CpG2006+IL-21+interferon(IFN)-γ) to evaluate their activation (CD80/CD86) and proliferation (CFSE) potential. B cell receptor (BCR) signaling (SYK/ERK/PLCy2) was analyzed in DN subsets from HC (n=6) and MS patients (n=1) by phosphoflow. Migration of MS B cells towards the pro-inflammatory chemokines CXCL10 and CXCL13 was studied using a chemotaxis assay (n=7) and a blood-brain barrier (BBB) model (n=6).

Results: DN1 was identified as the major DN subset, both in PB (72.2±12.1%) of MS patients and HC (74.7±11.1%), and in CSF (56.0±9.4%) of MS patients. This was confirmed by the expression of T-bet, characteristic of DN2 cells, in only 22.4% of MS DN B cells. Moreover, DN3 cells tended to be increased in MS CSF (35.3±6.5%) compared to PB (10.4±2.0%). MS DN B cells exhibited an activated and migratory phenotype with increased CXCR3+ and CD80+ frequencies and decreased CXCR5+ cells in CSF compared to PB. Following in vitro stimulation, MS DN B cells proliferated and upregulated CD80 and CD86 expression, suggesting that they are not senescent/exhausted cells. Furthermore, DN1 cells showed BCR responsiveness indicated by ERK, SYK and PLCy2 phosphorylation. DN B cell migration over the BBB was demonstrated in vitro and by expression of pro-inflammatory chemokine receptors and adhesion molecules.

Conclusion: DN B cells in MS are predominantly DN1 cells and have pro-inflammatory phenotypic, activation and migration characteristics emphasizing a potential contribution to MS pathology.

Disclosure of interest: L. Beckers, G. Montes Diaz, H. Coenen, P. Baeten, B. Broux, LM. Villar, B. Van Wijmeersch, V. Popescu, O. Gerlach, S. Knippenberg, R. Hupperts, V. Somers, J. Fraussen: nothing to disclose

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