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

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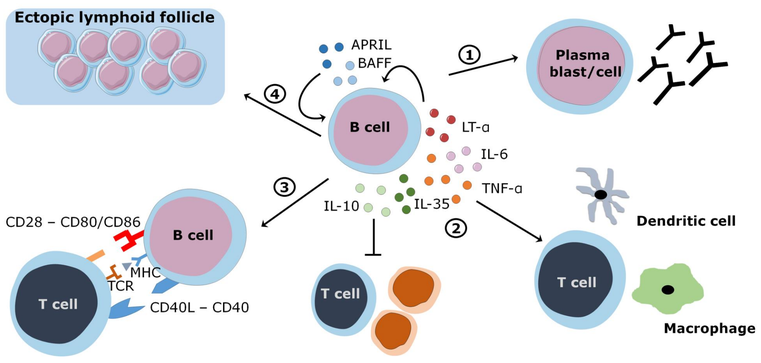
Introduction

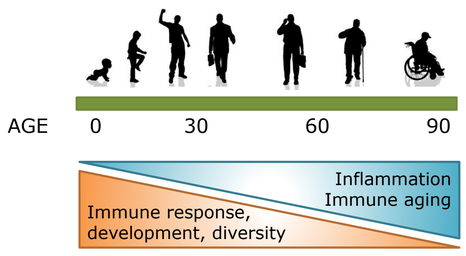
B cells are important players in multiple sclerosis (MS) pathogenesis:



1) Production of (auto)antibodies
2) Cytokine production
3) Antigen presentation & costimulation
4) Formation of ectopic lymphoid follicles

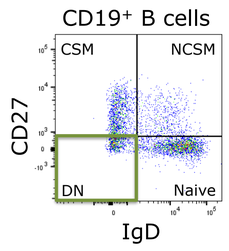
Immune aging

Changes in the immune system when people grow older:



➡ Present in a proportion of MS patients

Age-associated IgD$^-$CD27$^-$ double negative (DN) B cells



- Migration to inflammatory sites
- Autoantibody production
- Described in autoimmune diseases: systemic lupus erythematosus (SLE), rheumatoid arthritis (RA)

CSM: class-switched memory
NCSM: non class-switched memory
DN: double negative

Aim of the study

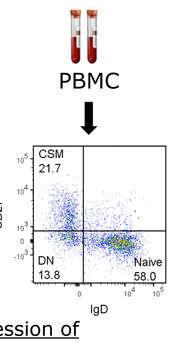
To investigate the prevalence and functional characteristics of DN B cells in MS patients

Patient sampling and methods

Study population

	n	Age[a]	% F	MS type CIS	RR	SP	PP	EDSS[b]	Previous treatment
Frequency of DN B cells									
HC	42	41.8±12.1	69.1	NA				NA	NA
MS	88	41.9±11.5	71.6	0	63	14	11	3.4	UT: 77; TRT: 11
Expression of costimulatory and antigen presentation molecules									
HC	31	31±12.5	61.0	NA				NA	NA
MS	47	48±13.3	70.0	5	30	6	6	3.2	UT: 47
Chemokine receptor expression									
HC	25	42.0±10.6	72.0	NA				NA	NA
MS	49	44.5±10.3	73.5	0	31	12	6	3.7	UT: 41; TRT: 8
T-bet expression									
HC	24	41.3±10.4	70.8	NA				NA	NA
MS	47	44.5±10.4	74.5	0	30	11	6	3.7	UT: 39; TRT: 8

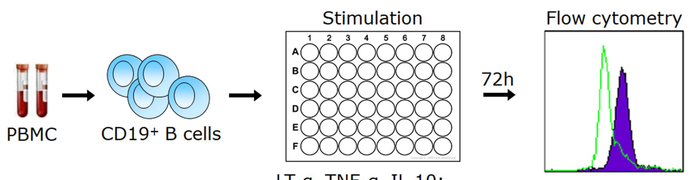
[a] mean±SD; [b] mean; Abbreviations: F, female; CIS, clinically isolated syndrome; RR, relapsing-remitting MS; SP, secondary progressive MS; PP, primary progressive MS; EDSS, expanded disability status scale; NA, not applicable; UT, untreated; TRT, treatment included first-line therapies: interferon-β, glatiramer acetate, teriflunomide.



Expression of
- Costimulatory molecules: CD80, CD86
- Antigen presentation molecules HLA-DR/DP/DQ
- Chemokine receptors: CXCR3, CXCR5
- Transcription factor: T-bet

HLA: human leukocyte antigen; CXCR: C-X-C motif chemokine receptor; T-bet: T-box expressed in T cells

Cytokine production after *in vitro* B cell stimulation



LT-α, TNF-α, IL-10:
- CD40 ligand + IL-4

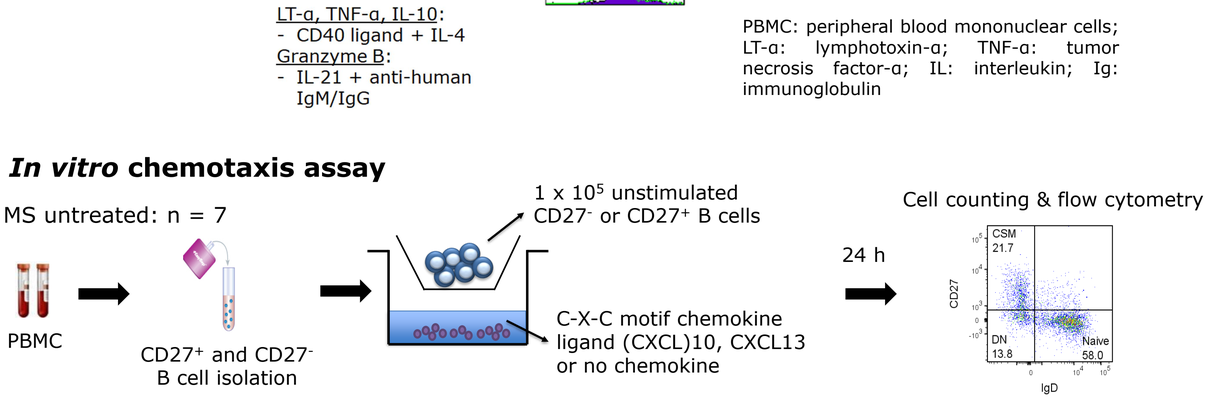
Granzyme B:
- IL-21 + anti-human IgM/IgG

MS untreated: n = 7
MS treated: n = 6
HC: n = 10 (with similar age/gender)

PBMC: peripheral blood mononuclear cells; LT-α: lymphotoxin-α; TNF-α: tumor necrosis factor-α; IL: interleukin; Ig: immunoglobulin

In vitro chemotaxis assay

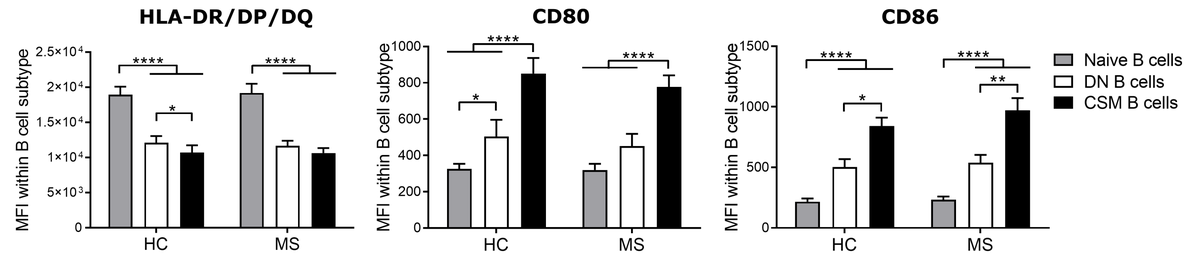
MS untreated: n = 7



Chemotactic index (CI) = $\frac{\#\text{ migrated subtype cells with chemokine} - \#\text{ migrated subtype cells without chemokine}}{\text{start \# of subtype cells in upper chamber}}$

DN B cells express functional molecules

Antigen presentation and costimulatory molecules

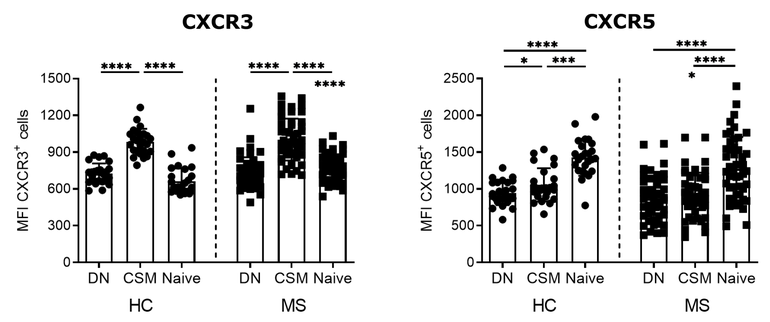


Expression levels (mean fluorescence intensity, MFI) of HLA-DR/DP/DQ, CD80 and CD86 for naive, DN and CSM B cells from HC (n = 31) and MS patients (n = 47). Mean + SD is depicted. * p<0.05; ** p<0.01; **** p<0.0001

➡ DN B cells showed similar or increased HLA-DR/DP/DQ expression as CSM B cells

➡ DN B cells showed CD80/CD86 expression in between that of naive and CSM B cells

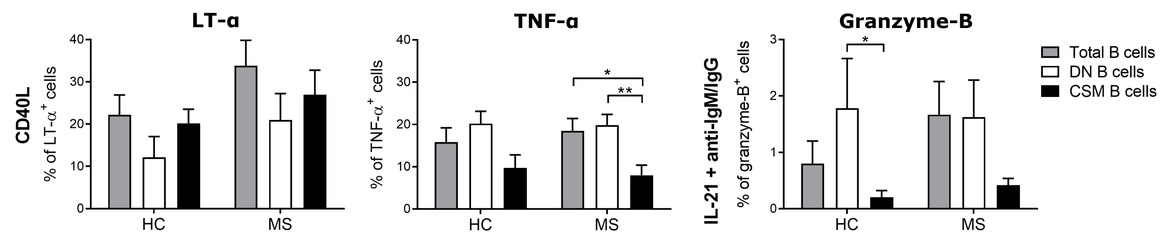
Pro-inflammatory chemokine receptors



Expression levels (mean fluorescence intensity, MFI) of C-X-C motif chemokine receptor (CXCR)3 and CXCR5 for naive, DN and CSM B cells from HC (n = 25) and MS patients (n = 49). Mean (bars) + SD is depicted. * p<0.05; **** p<0.0001

➡ DN B cells showed CXCR3 and CXCR5 expression similar to naive and CSM B cells, respectively

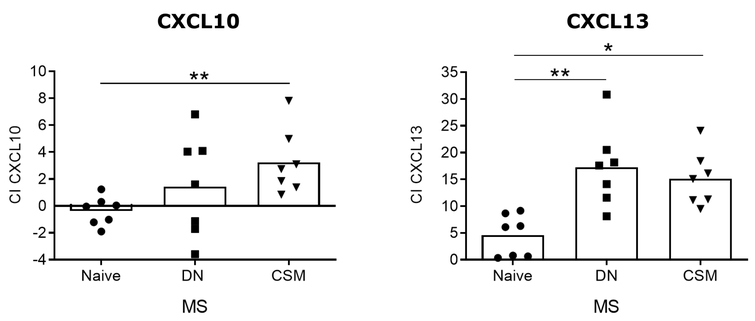
DN B cells produce pro-inflammatory cytokines *in vitro*



The percentage of LT-α$^+$ and TNF-α$^+$ total, DN and CSM B cells after B cell stimulation using CD40 ligand for HC (n = 10) and MS patients (n = 13). Granzyme-B was measured after B cell stimulation with IL-21 + anti-human IgM/IgG. Mean + SD is depicted. * p<0.05; ** p<0.01; ****

➡ DN B cells showed a similar frequency of LT-α$^+$ cells and a higher frequency of TNF-α$^+$ and granzyme-B$^+$ cells compared with the CSM B cell population

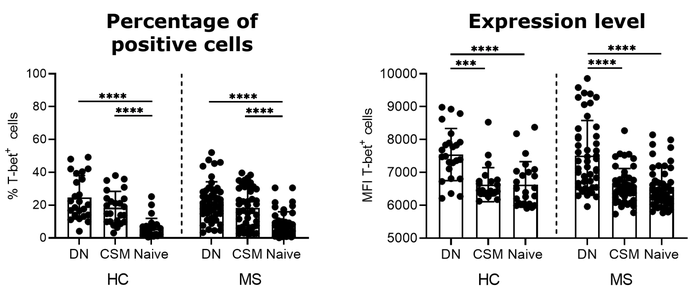
DN B cells migrate towards pro-inflammatory cytokines *in vitro*



Chemotactic index (CI) for naive, DN and CSM B cells of MS patients (n = 7) in the presence of CXCL10 or CXCL13. Mean (bars) + SD is depicted. * p<0.05; ** p<0.01

➡ MS DN B cells showed high migration capacity towards CXCL10 (CXCR3 ligand) and CXCL13 (CXCR5 ligand) that was similar to that of CSM B cells

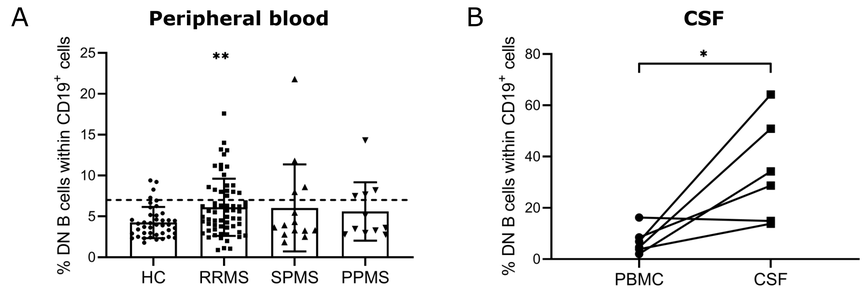
A proportion of DN B cells express the transcription factor T-bet



Percentage and MFI of T-bet$^+$ DN, CSM and naive B cells from HC (n = 24) and MS patients (n = 47). Mean (bars) + SD is depicted. *** p<0.001; **** p<0.0001

➡ DN B cells showed highest T-bet expression compared with naive and CSM B cells, with about 21.6 % of DN B cells being T-bet$^+$

DN B cell frequency is elevated in MS peripheral blood and CSF



(A) The percentage of DN B cells in the peripheral blood of HC (n = 42), RRMS (n = 63), SPMS (n = 14) and PPMS (n = 11) patients younger than 60 years. Mean (bars) ± SD is depicted. Black dashed line represents the cutoff for an increased frequency of DN B cells. (B) The percentage of DN B cells for paired PBMC and CSF cells from 6 MS patients. * p<0.05; ** p<0.01

Conclusions

- DN B cells are abnormally **elevated** in the peripheral blood and CSF of MS patients
- DN B cells could **migrate into the central nervous system** via chemokines involved in MS pathology
- DN B cells have **pro-inflammatory functional characteristics**
- DN B cells show highest expression of **T-bet**, that has been described in another pathological age-associated B cell subset

➡ **Potential importance of DN B cells in MS pathology**

➡ **Could lead to novel targets for more specific MS therapy**

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