Characterisation of the immune system after ocrelizumab treatment in multiple sclerosis

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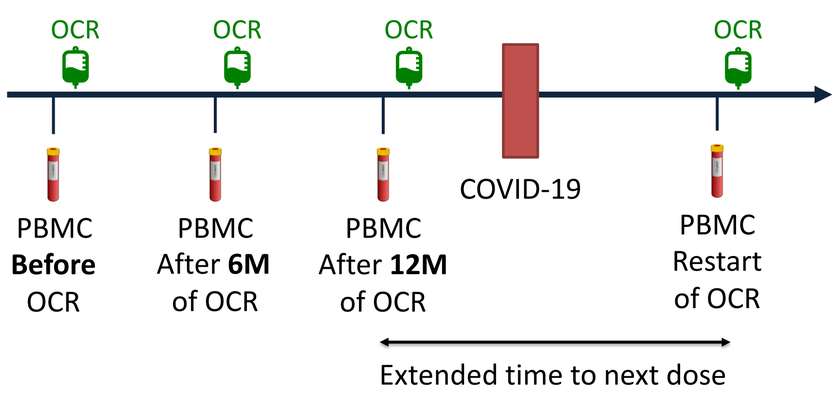
Introduction

B cells are important contributors to the chronic inflammatory processes in multiple sclerosis (MS) by producing **autoantibodies**, inducing pro-inflammatory **T cell responses** and secreting **pro- and anti-inflammatory cytokines**. B cell depletion by the humanized anti-CD20 monoclonal antibody **ocrelizumab (OCR)** is a highly effective treatment for **relapsing-remitting (RR)MS** and was the first immunosuppressive therapy that was approved for early **primary-progressive (PP)MS**. However, detailed information about the effect of OCR on B cell subsets and other immune cells in MS is still lacking.

AIM: To investigate the phenotypic changes of the immune system after OCR treatment in both RRMS and PPMS patients over time

Material and methods



➔ B cells, T cells, monocytes, dentritic cells and natural killer cells were analysed using flow cytometry

Preliminary analysis was performed only on samples for which the 3 time points were available

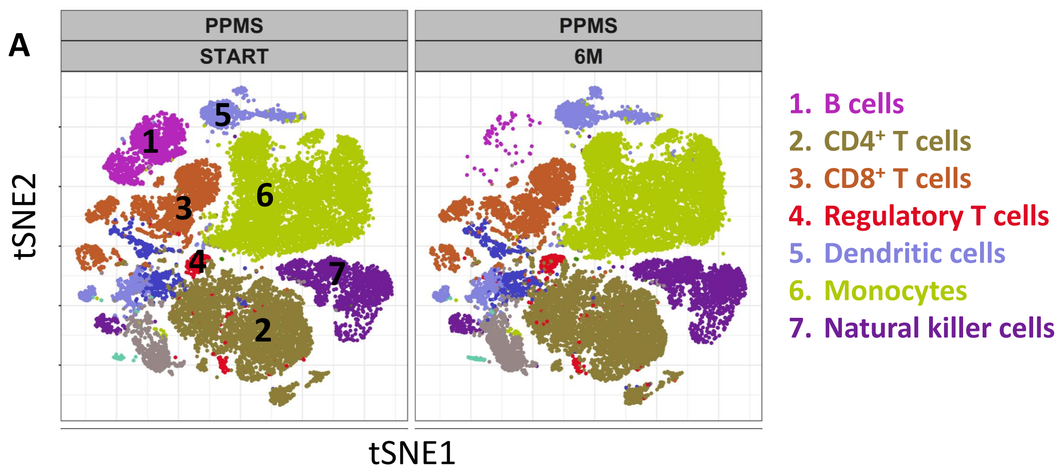
Donors	N	3 time points available	Age[a]	Gender, %F	EDSS[b]	Disease duration[c]
HC	20	N.A.	48.0 ± 11.2	50	N.A.	N.A.
RRMS	18	10	42.0 ± 10.5	61	3	9.6
PPMS	22	9	48.2 ± 8.7	41	5	5.2

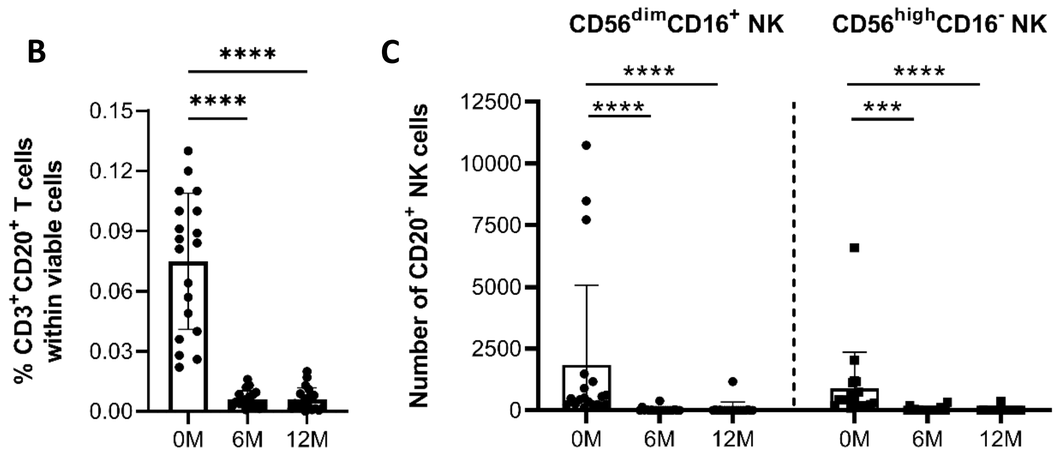
Donors	Time to next dose	Age[a]	Gender	EDSS[b]	Disease duration[c]
RRMSext1	8 months	42	F	2	10
RRMSext2	9 months	34	F	2.5	8

[a] In years, mean ± SD; [b] mean; [c] In years; F: female; EDSS: expanded disability status scale; N.A.: not applicable; HC: healthy control, M: month, PBMC: peripheral blood mononuclear cells.

Results

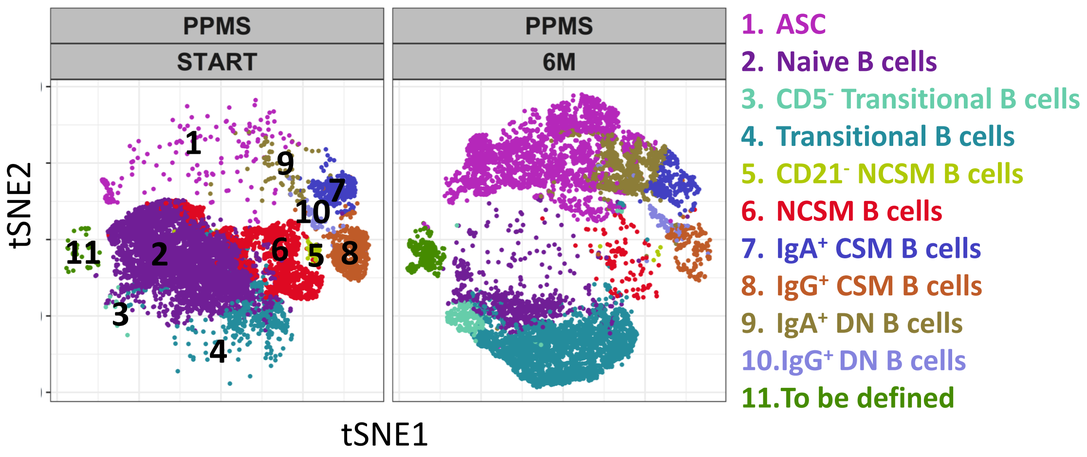
OCR mainly affects CD20⁺ immune cell subsets





(A) tSNE map showing the major immune cell subsets within the viable cell population of PPMS patients (n = 9) before and after 6M of OCR treatment. Viable cells from each sample were down-sampled to 5,000 events. Manually determined gates are plotted on the tSNE maps. (B) Percentage CD3⁺CD20⁺ T cells were gated within the viable cells of all MS patients (n = 19) before, after 6 and 12M of OCR. (C) Number of CD20⁺ NK cells were gated within the CD20⁺ cells of all MS patients (n = 19) before, after 6 and 12M of OCR. Mean levels + SD are depicted. *** p < 0.001, **** p < 0.0001.

OCR induces a shift in the distribution of the B cell population



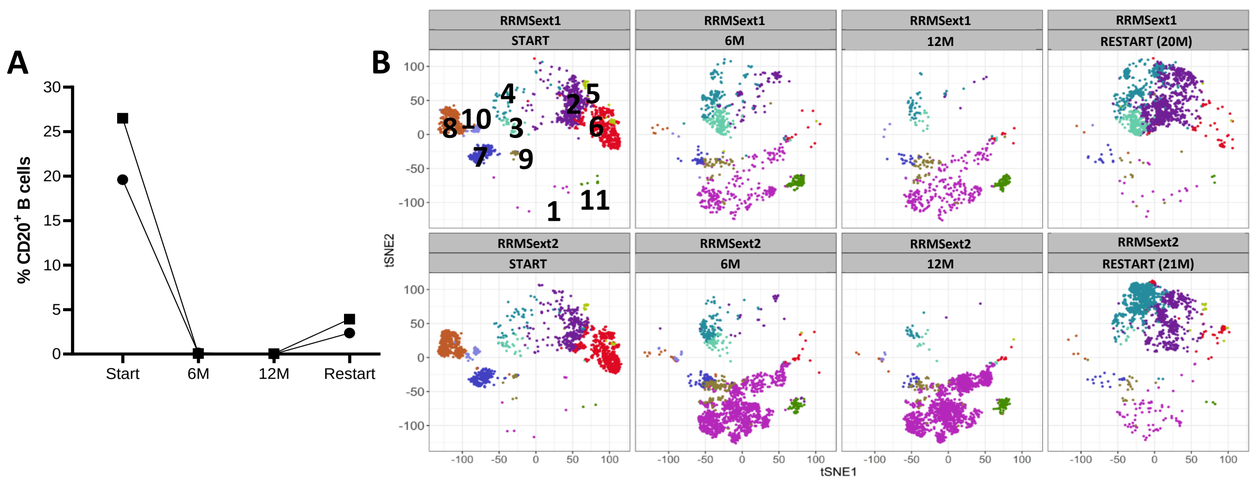
tSNE map showing the major B cell subsets within the lymphocyte gate of all MS patients (n = 19) before and after 6M of OCR treatment. Lymphocytes from each sample were down-sampled to 1,000 events. Manually determined gates are plotted on the tSNE maps. ASC: antibody-secreting cell, NCSM: non class-switched memory, CSM: class-switched memory, DN: double negative

➔ Significantly decreased % of naive and IgG⁺ CSM B cells; increased % of ASC, transitional and IgA⁺ DN B cells after OCR treatment

➔ No significant difference between RRMS and PPMS patients in the distribution of the immune cells subsets (data not shown)

➔ No significant difference between 6 and 12 months of OCR in both RRMS and PPMS patients (data not shown)

Increasing time between 2 OCR doses results in repopulation of early B cell subsets



(A) Percentage CD20⁺ B cells of RRMS patients (n = 2) before, 6M, 12M and 20/21M after OCR treatment.(B) tSNE map showing the major B cell subsets within the lymphocyte gate. Lymphocytes from each sample were down-sampled to 2,000 events. Manually determined gates are plotted on the tSNE maps.

Conclusion

Besides depletion of CD20⁺ B, T and NK cells, OCR treatment induced changes in the distribution of B cell subsets in both RRMS and PPMS patients

➔ Understanding the effect of OCR on innate and adaptive immune cell subsets will contribute to unravelling their role in MS pathology

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