CD4⁺ T cells in the blood of MS patients respond to predicted epitopes from B cell receptors found in spinal fluid

Rune A. Høglund[1,2], A. Lossius[1,2,3], E. J. Homan[4], R.Bremel[4] and T. Holmøy[1,2]

[1]Department of Neurology, Akershus University Hospital, Lørenskog, Norway, [2]Institute of Clinical Medicine, University of Oslo, Oslo, Norway, [3]Department of Immunology and Transfusion Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway, [4] ioGenetics LLC, Madison, WI, USA

Introduction

The pathogenic role of B cells in multiple sclerosis (MS) is not known. We have previously demonstrated that B cells from cerebrospinal fluid (CSF) of MS patients can activate T cells through specific recognition of antigenic determinants (idiotopes) from their own B cells receptors (BCRs). The aim of this study was to evaluate these findings in MS patients using *in silico* prediction models to accurately and quickly identify immunogenic idiotopes of immunoglobulin heavy-chain variable (IGHV) gene repertoires.

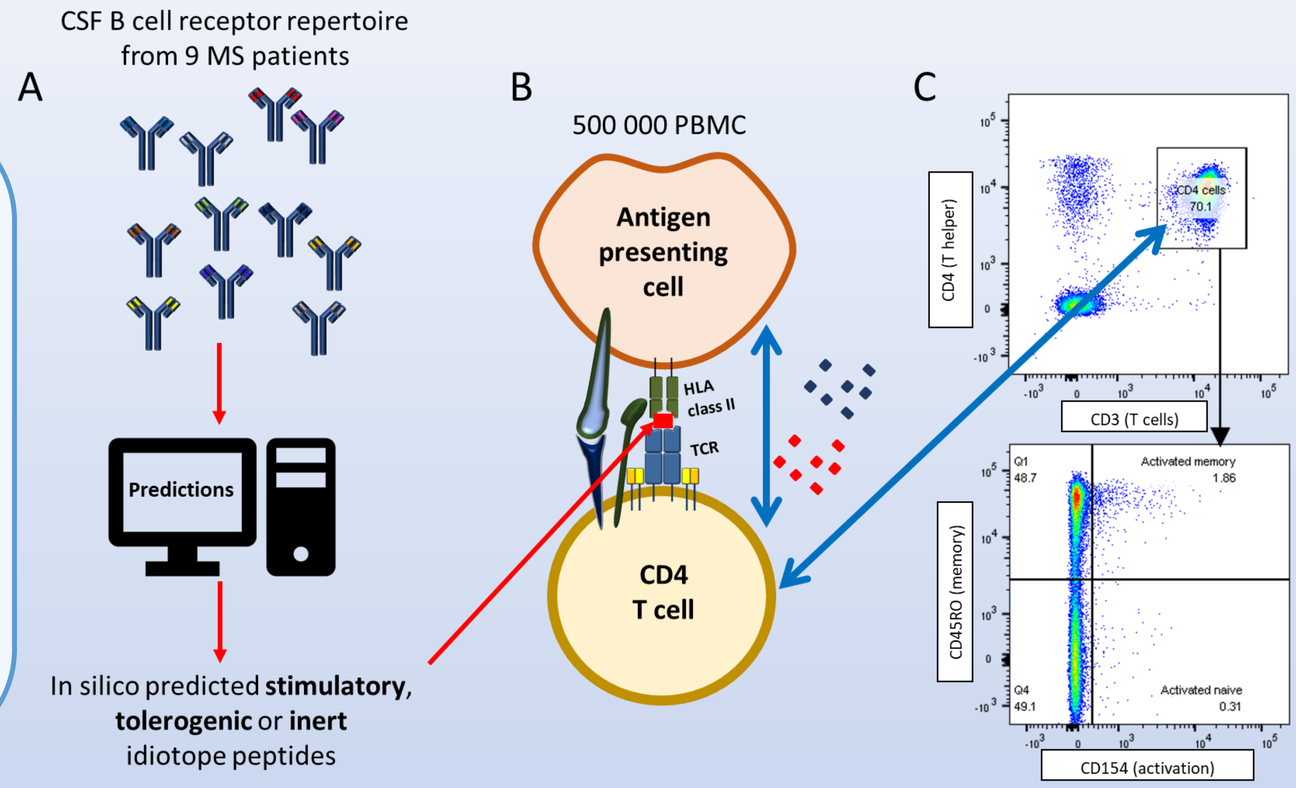

Figure 1. A) IGHV amino acid sequences (1079 [SD=1213]) from MS patients were run through predictive models to identify likely antigenic or inert peptides. B) PBMC were stimulated with idiotope peptides. C) Activated CD4⁺CD45RO⁺ memory T cells were be detected by surface expression of CD154.

Materials and methods

Translated CSF IGHV repertoires were run through bioinformatic predictions *in silico* to predict HLA-DR affinity and endosomal processing, and calculate T cell exposed motif frequency. Predictions and transcript frequencies guided selection of idiotope-peptides from nine MS patients. PBMC from these patients were stimulated *in vitro* with the idiotope peptides in presence of anti-CD40 for 12 hours. Flow cytometry was utilized to identify activated cells using relevant surface makers (Table 1 and Figure 1). Unstimulated cells or insulin peptides were negative controls, and CD3/CD28 beads or EBNA-1 peptides were positive controls

Results

In all nine MS patients, we found blood memory CD4⁺ T cells were activated by predicted idiotope-peptides (Figure 2). Responses were mainly towards peptides affiliated with the CDR3 region, and no robust responses were seen towards peptides with low predicted HLA-DR affinity. Activated memory CD4⁺ T cells also expressed the chemokine receptor CCR6, affiliated with a Th17 phenotype and allowing passage into the central nervous system (Figure 3).

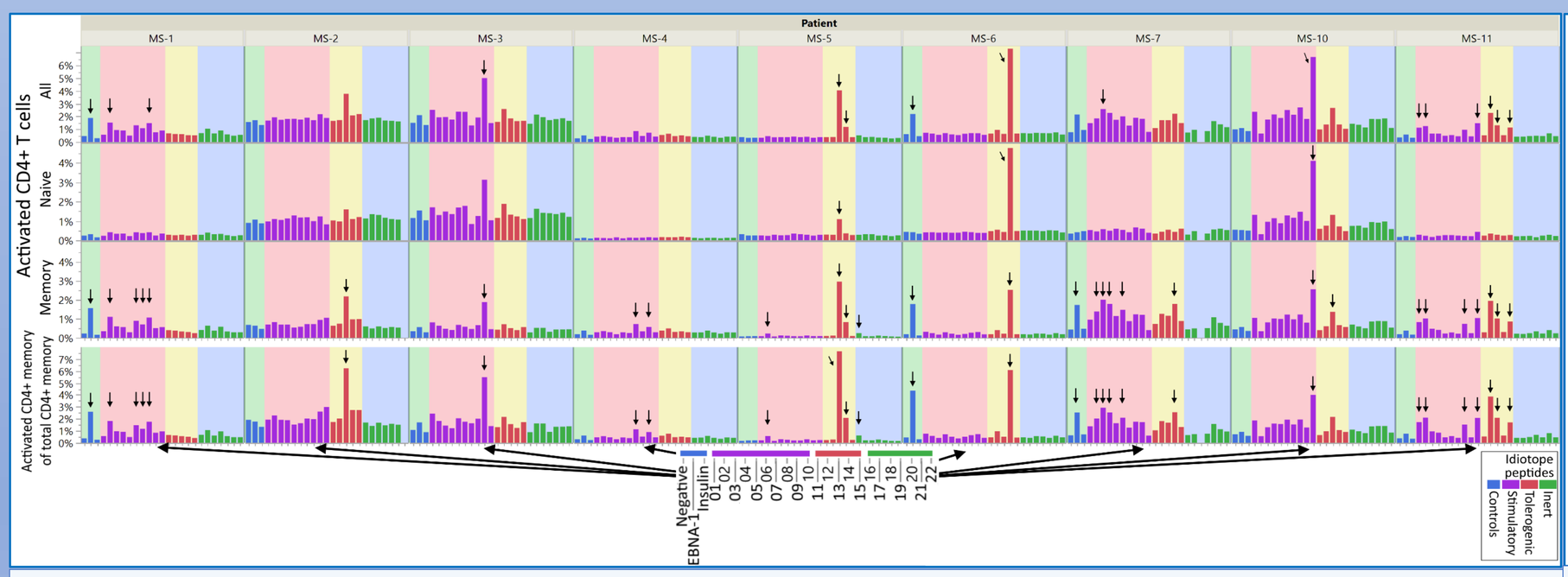

Figure 2. CD3⁺CD4⁺CD8⁻ T cells were assayed for the activation marker CD154. Activated cells among all CD4⁺ cells, CD45RO⁺ memory- or CD45RO⁻ naïve cells are presented as proportions of CD4⁺ cells (upper three panels) or proportion of memory cells (lower panel). Responses were deemed positive (arrows) if the proportion of CD154⁺ cells were 3x higher than in unstimulated (negative) wells.

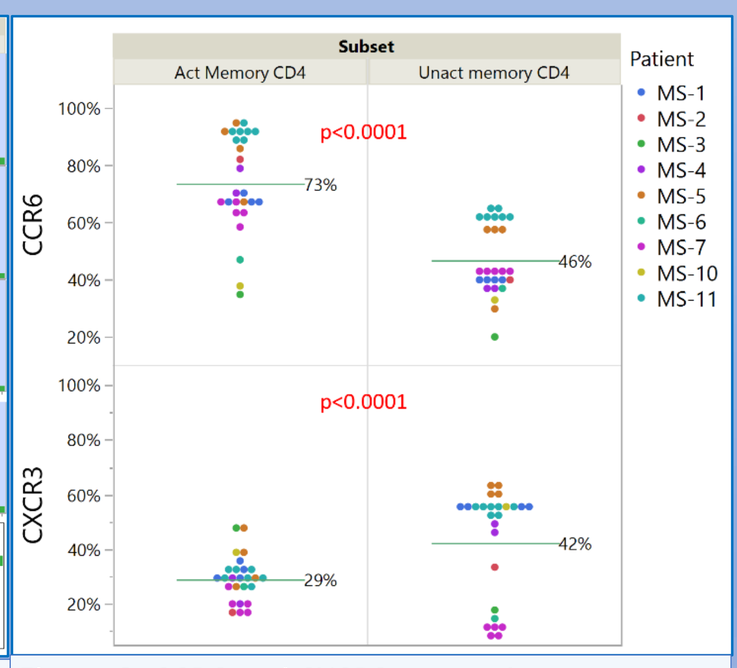

Figure 3. CCR6 and CXCR3 expression among activated (CD154⁺) or un-activated (CD154⁻) memory (CD45RO⁺) CD4⁺ T cells responding to predicted idiotope peptides.
P-values are results of full factorial mixed model.

Table 1: Surface markers and fluorochromes used in experiment

Laser	Fluorochromes	Marker
407 nm (Violet)	BV421	CD154 (activation)
488 nm (Blue)	PE	CXCR3
	PE-Cy7	CD45RO (memory)
	PerCP-Cy5.5	CD4
	FITC	CD3
633 nm (Red)	APC	CCR6
	APC-H7	CD14 CD8
	LIVE/DEAD™ Fixable Near-IR	Viability (dump channel)

Conclusions

This *in vitro* study suggests that MS patients have a memory T cell repertoire capable of recognizing frequent BCRs found in endogenous CSF, and that these T cells express chemokine receptors allowing them to reach the B cells presenting these peptides. It further indicates that antigenic properties of BCR idiotopes can be predicted *in silico* using HLA affinity and endosomal processing predictions.

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UiO : Institute of Clinical Medicine University of Oslo

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