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

Short title: Idiotope-specific CD4⁺ T cells in MS patients

Rune A. Høglund[1,2], Andreas Lossius[1,3], E. Jane Homan[4], Robert Bremel[4] and Trygve 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.

Methods*:* CSF IGHV repertoires from MS patients were sequenced and translated. *In silico* predicted HLA-DR affinity and endosomal processing, as well as transcript frequency, guided selection of idiotope-peptides from nine MS patients. PBMC from these patients were stimulated with the selected peptides in presence of anti-CD40 for 12 hours. T cells were then labeled for activation status with anti-CD154 antibodies and phenotyped with anti-CD3, -CD4, -CD45RO, -CXCR3 and -CCR6, and anti-CD14 and -CD8 for dump channel. Unstimulated cells or insulin peptides were negative controls, and 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. 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.

Conclusion: 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.

Disclosure: RB and EJH hold equity in ioGenetics LLCs, the company responsible for designing the bioinformatics models used in this project. All other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.