Identification of the immunodominant T cell epitopes of AQP4 & MOG in patients with NMOSD

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Introduction

T cells, especially CD4⁺ T cells, are key players in the pathogenesis of autoimmune diseases and mediate cellular and humoral immune responses.[1] Autoantibodies targeting the aquaporin 4 (AQP4) water channel protein and the myelin oligodendrocyte glycoprotein (MOG) are associated with a broad spectrum of human CNS demyelinating diseases including neuromyelitis optica spectrum disorders (NMOSD) and acute disseminated encephalomyelitis (ADEM). [2] Whereas there is some information on the role of AQP4-specific T cells[3], little is known about MOG-specific T cells in these diseases. We therefore aimed to identify the immunodominant human T cell epitopes of AQP4 and MOG in patients with NMOSD.

Aims of the study

1. T cell epitope mapping to identify the immunodominant T cell epitopes of human AQP4 & MOG using the CFSE proliferation assay
2. Functional phenotyping of proliferated CD4⁺ T cells
   a. Cytokine secretion, particularly GM-CSF, IFN-ɣ, IL-4, IL-6, IL-17A in the supernatants using commercial ELISA kits
   b. IFN-ɣ, IL-4, IL-6 & IL-17A production using flow cytometry-based intracellular staining

Methods

Participants of the Study

Ten AQP4-antibody and eight MOG-antibody positive NMOSD patients, one paediatric MOG-antibody positive ADEM patient and ten healthy controls (HC) were included in this study.

Methods

We performed a T cell epitope mapping using the **CFSE proliferation assay (Figure 2)**. Peripheral blood mononuclear cells (PBMCs) were stimulated with a library of eight AQP4 and nine MOG peptides **(Figure 1).** After eleven days, the proliferation of PBMCs in response to single peptides via the dilution of the CFSE-staining was analysed by **flow cytometry**. To gain more information about the functional phenotype of the proliferated T cells, the **cytokine secretion**, particularly granulocyte macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-ɣ, interleukin (IL)-4, IL-6 and IL-17A, in the supernatants of this assay was examined using **ELISA.** For investigating the differentiation of T cells into distinct CD4⁺ T helper cell subsets, particularly Th1, Th2 and Th17 cells producing pro-inflammatory IFN-ɣ, IL-4, IL-6 and IL-17A, respectively (**Figure 4**), a **flow cytometry-based intracellular staining** of these cytokines was performed**.**

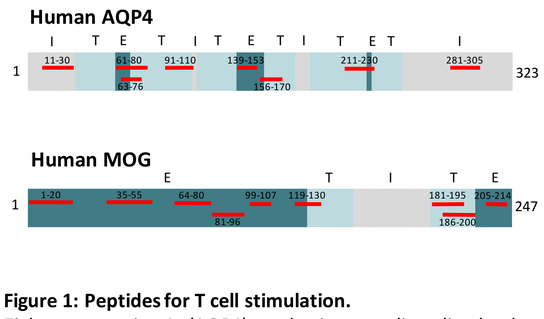

Figure 1: Peptides for T cell stimulation.
Eight aquaporin 4 (AQP4) and nine myelin oligodendrocyte glycoprotein (MOG) peptides corresponding to intracellular (I), extracellular (E) and transmembrane (T) domains of human AQP4 and MOG, respectively, were chosen based on on their encephalitogenicity in animals and/or immunodominance in humans.

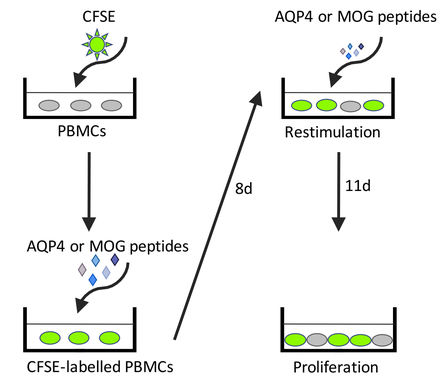

Figure 2: CFSE proliferation assay
CFSE labelled PBMCs were stimulated with AQP4 and MOG peptides and proliferation in response to single peptides via the dilution of the CFSE-staining was analysed by flow cytometry.

Results 1: T cell proliferation

Specific T cell proliferation in response to AQP4 peptides was found in AQP4 and MOG antibody positive NMOSD patients when compared to healthy controls (HC). T cell response to MOG peptides, preferably to peptides corresponding to extracellular regions like the immunodominant N-terminal Ig V-like domain, was found in NMOSD patients as well as in HC.

A		AQP4 antibody NMOSD	MOG antibody NMOSD	HC	p-value
Number of cases		9	5	9	
Female : male		9 : 0	4 : 1	7 : 2	
Age (years)		51.6 (19.9-77.2)[2]	45.5 (20.0-53.1)[2]	27.7 (21.7-48.7)[2]	
	Tetanus toxoid	9 (100%)	5 (100%)	9 (100%)	0.999 [1]
	AQP4 p11-30	4 (44%)	2 (40%)	0 (0%)	0.072 [1]
	AQP4 p61-80	3 (33%)	1 (20%)	0 (0%)	0.173 [1]
	AQP4 p63-76	2 (22%)	2 (40%)	0 (0%)	0.145 [1]
CFSE proliferation	AQP4 p91-110	3 (33%)	2 (40%)	1 (11%)	0.402 [1]
CDI ≥ 2	AQP4 p139-153	5 (56%) *	1 (20%)	0 (0%)	0.026 [1]
	AQP4 p156-170	4 (44%)	0 (0%)	0 (0%)	0.023 [1]
	AQP4 p211-230	3 (33%)	1 (20%)	0 (0%)	0.173 [1]
	AQP4 p281-305	4 (44%)	2 (40%)	0 (0%)	0.072 [1]
	AQP4 any peptide	8 (89%) **	4 (80%) *	1 (11%)	0.002 [1]

B		AQP4 antibody NMOSD	MOG antibody NMOSD	HC	p-value
Number of cases		5	8	10	
Female : male		5 : 0	4 : 4	7 : 3	
Age (years)		51.6 (43.0-73.7)[2]	34.8 (14.3-53.1)[2]	28.4 (21.7-48.7)[2]	
	Tetanus toxin	5 (100%)	8 (100%)	10 (100%)	0.999 [1]
	MOG p1-20	1 (20%)	4 (50%)	1 (10%)	0.145 [1]
	MOG p35-55	1 (20%)	3 (38%)	1 (10%)	0.104 [1]
	MOG p64-80	2 (40%)	2 (25%)	3 (30%)	0.122 [1]
	MOG p81-96	1 (20%)	2 (25%)	2 (20%)	0.962 [1]
CFSE proliferation	MOG p99-107	1 (20%)	0 (0%)	4 (40%)	0.123 [1]
CDI ≥ 2	MOG p119-130	0 (0%)	0 (0%)	0 (0%)	0.999 [1]
	MOG p181-195	0 (0%)	1 (13%)	0 (0%)	0.400 [1]
	MOG p186-200	1 (20%)	0 (0%)	0 (0%)	0.152 [1]
	MOG p205-214	2 (40%)	2 (25%)	1 (10%)	0.346 [1]
	MOG any peptide	4 (80%)	3 (38%)	7 (70%)	0.228 [1]

Table 1: CD4⁺ T cell proliferation in response to AQP4 and MOG peptides in AQP and MOG antibody positive NMOSD patients and healthy controls (HC).
Significance of group differences for proliferation to AQP4 (**A**) and MOG peptides (**B**) was analyzed using [1] Chi-square exact test. [2] Data are shown as median (range).
* significant difference to HC group <0.05, ** significant difference to HC group <0.01, p-values corrected for multiple comparisons.

Results 1: T cell proliferation

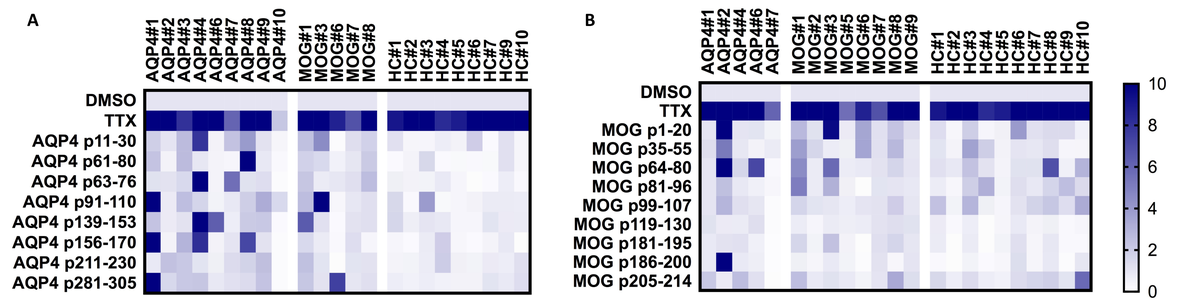

Figure 3: Heatmaps of CD4⁺ T cell proliferation in response to AQP4 and MOG peptides in AQP4 and MOG antibody positive NMOSD patients and healthy controls (HC).
Columns are individual samples with patient or HC ID and rows are different AQP4 (**A**) and MOG peptides (**B**) as well as the negative control DMSO and the positive control tetanus toxoid (TTX). Values range from white (0) to blue (10). Cell division index (CDI) was calculated as follows: (CD4⁺CFSE⁻ cells stimulated with either AQP4 or MOG peptides or TTX (%)) / (vehicle treated CD4⁺CFSE⁻ cells (%)); Cut off: CDI ≥2

Results 2a: Functional phenotyping - ELISA analysis

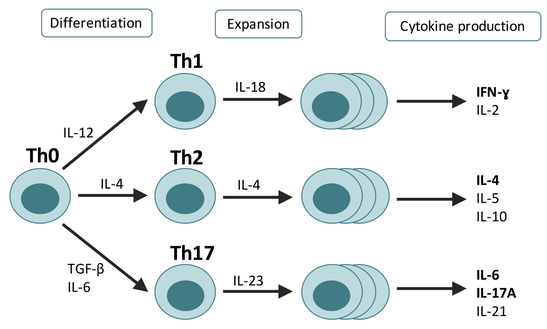

Figure 4: Functional phenotype of autoreactive T cells.
Cytokine secretion, particularly GM-CSF, IFN-ɣ, IL-4, IL-6, IL-17A in response to respective peptides was examined using commercial ELISA kits and cytokine production, particularly IFN-ɣ, IL-4, IL-6, IL-17A was determined using a flow cytometry-based intracellular staining.

ELISA analysis of cytokines secreted in the supernatants after challenging with AQP4 and MOG peptides was either not detectable (IL-4, IL-17A; not shown) or not specific (GM-CSF, IFN- ɣ, IL-6) and therefore give no specific information about the functional phenotype of autoreactive T cells.

GM-CSF	All AQP4 peptides	All MOG peptides
AQP4 IgG⁺	5/7	4/4
MOG IgG⁺	2/2	2/4
HC	4/8	6/10
p-value	ns	ns

IFN-ɣ	All AQP4 peptides	All MOG peptides
AQP4 IgG⁺	5/7	4/4
MOG IgG⁺	2/2	4/4
HC	6/8	8/10
p-value	ns	ns

IL-6	All AQP4 peptides	All MOG peptides
AQP4 IgG⁺	4/7	3/4
MOG IgG⁺	1/2	3/3
HC	3/8	5/10
p-value	ns	ns

Results 2b: Functional phenotyping – Flow cytometry

We identified pro-inflammatory cytokine production (IFN-ɣ, IL-17A) of proliferated CD4⁺ T helper cell subsets (Th1, Th17) using a flow cytometry-based intracellular staining.

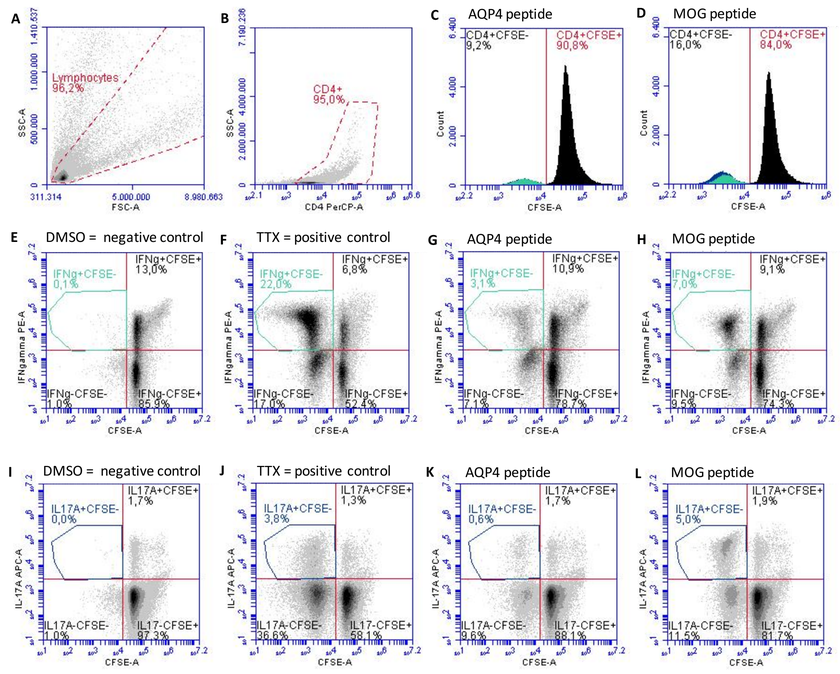

Figure 5: Gating strategy for the identification of proliferated CD4⁺ T helper cell subsets.
(A) Gating of lymphocytes according to empirical values of size and granularity (B) followed by gating of CD4, which is a marker for T helper cells. (C,D) represent the dilution of the CFSE-staining due to proliferation of CD4⁺ T cells in response to an AQP4 or a MOG peptide, respectively.
The second and third rows show representative scatterplots of IFN-ɣ (E-H) and IL-17A (I-L) production of proliferated CD4⁺CFSE⁻ T cells in response to either the vehicle control DMSO (E,I), or to the positive control tetanus toxoid (TTX) (F,J), respectively. (G,K) represent IFN-ɣ and IL-17A production of a patient in response to an AQP4 and (H,L) to a MOG peptide, respectively.

Conclusion

- Our study indicates a specific T cell response to AQP4, but not to MOG, in AQP4 and MOG antibody positive NMOSD patients .
- In contrast, cytokine secretion in the supernatants after challenging with AQP4 and MOG peptides give no specific information about the functional phenotype of autoreactive CD4⁺ T cells.
- We identified pro-inflammatory cytokine production in proliferated CD4⁺ T helper cell subsets.
- Our results could be helpful for the development of new individualised immune tolerance therapies.

Literature

[1] Hohlfeld *et al.*, 2015,
[2] Wingerchuk et al., 2015, Reindl et al., 2013
[3] Kampylafka *et al.,* 2011; Matsuya *et al.,* 2011; Arellano *et al.*, 2012; Varrin-Doyer *et al.,* 2012

Support

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