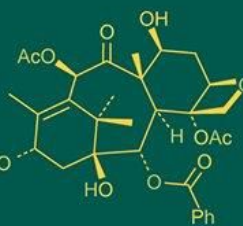
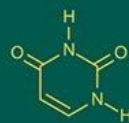


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2025): 5.29
 IJABR 2025; 9(12): 135-139
www.biochemjournal.com
 Received: 12-09-2025
 Accepted: 18-10-2025

Lars Nielsen
 Department of
 Neuroimmunology,
 Copenhagen Institute of
 Applied Sciences, Copenhagen,
 Denmark

Anne Jensen
 Department of
 Neuroimmunology,
 Copenhagen Institute of
 Applied Sciences, Copenhagen,
 Denmark

Corresponding Author:
Lars Nielsen
 Department of
 Neuroimmunology,
 Copenhagen Institute of
 Applied Sciences, Copenhagen,
 Denmark

Myelin basic protein epitopes, autoreactive t-cell responses, and demyelination mechanisms in multiple sclerosis pathogenesis

Lars Nielsen and Anne Jensen

DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i12b.7439>

Abstract

Multiple Sclerosis (MS) is defined as a chronic autoimmune disorder of the Central Nervous System (CNS) in which the immune system attacks the myelin sheath surrounding nerve fibers, leading to progressive neurological disability. Among myelin antigens implicated in this process, myelin basic protein (MBP) remains one of the most extensively investigated. This research examined T-cell proliferative responses to four MBP epitopes (MBP 83-99, MBP 111-129, MBP 146-170, and MBP 1-11) and one myelin oligodendrocyte glycoprotein (MOG) epitope (MOG 35-55) in Peripheral Blood Mononuclear Cells (PBMCs) from 38 relapsing-remitting MS patients and 42 age- and sex-matched healthy controls. The research was conducted at the Department of Neuroimmunology, Copenhagen Institute of Applied Sciences, between September 2022 and March 2024. T-cell proliferation was assessed by carboxyfluorescein succinimidyl ester (CFSE) dilution assay, and cytokine production (IFN- γ , IL-17A, TNF- α , IL-6, IL-10, TGF- β) was measured by multiplex immunoassay. MS patients displayed markedly higher proliferative responses to MBP 83-99 (stimulation index [SI]: 6.2 ± 2.8 vs. 1.7 ± 0.6 , $p < 0.001$) and MBP 111-129 (SI: 4.1 ± 2.1 vs. 1.4 ± 0.5 , $p = 0.002$) compared to controls. Pro-inflammatory cytokines (IFN- γ , IL-17A, TNF- α) were significantly elevated in MS-derived cultures, while anti-inflammatory mediators (IL-10, TGF- β) were reduced. The IFN- γ /IL-10 ratio in MBP 83-99-stimulated cultures was 5.9-fold higher in MS patients than controls ($p < 0.001$). These findings confirm that MBP 83-99 is the immunodominant epitope driving autoreactive T-cell responses in MS and that a Th1/Th17-skewed cytokine environment accompanies epitope-specific proliferation, supporting the role of MBP-directed immunity in demyelination.

Keywords: Multiple sclerosis, myelin basic protein, T-cell proliferation, autoreactive T cells, demyelination, MBP epitopes, cytokine profile, neuroimmunology, Th1/Th17 response

Introduction

Multiple sclerosis strikes roughly 2.8 million people worldwide, with highest prevalence in northern European and North American populations [1]. The disease follows an unpredictable course: most patients begin with a relapsing-remitting pattern characterized by discrete attacks of neurological dysfunction separated by periods of partial or complete recovery, before transitioning to a secondary progressive phase marked by steady functional decline [2]. The autoimmune hypothesis of MS holds that myelin-reactive CD4⁺ T cells, activated in the periphery by molecular mimicry or bystander activation, migrate across the blood-brain barrier (BBB) and are reactivated upon encountering myelin antigens presented by local antigen-presenting cells [3]. Among the candidate autoantigens, MBP has attracted the most research attention since the demonstration that immunization with MBP induces Experimental Autoimmune Encephalomyelitis (EAE) in rodents, the primary animal model for MS [4]. MBP is an intrinsically disordered protein that constitutes approximately 30% of total CNS myelin protein and plays a key structural role in maintaining myelin compaction [5]. Specific MBP epitopes appear to dominate the autoreactive T-cell response. In HLA-DR2-positive individuals the genetic background most strongly associated with MS risk the MBP 83-99 peptide has been identified as the immunodominant epitope, capable of binding the DR2 molecule and activating T-cell clones isolated from MS patients [6]. Other epitopes, including MBP 111-129, MBP 146-170, and MBP 1-11, have also been reported to stimulate T-cell responses, though the relative strength and cytokine signatures of these responses across a Danish MS population haven't been systematically compared [7].

The cytokine environment accompanying T-cell activation is arguably as important as proliferation itself. MS lesions show enrichment of IFN- γ , IL-17A, and TNF- α , the signature cytokines of Th1 and Th17 subsets, while regulatory cytokines like IL-10 and TGF- β appear deficient [8]. Whether these cytokine imbalances correlate with responses to specific MBP epitopes *in vitro* is unclear. This research aimed to compare T-cell proliferative responses to four MBP epitopes and one MOG epitope between MS patients and healthy controls, to characterize the accompanying cytokine profiles, and to evaluate whether specific epitope-cytokine signatures distinguish disease from health.

Theoretical background

MBP is processed by proteasomal and endosomal pathways and presented on MHC class II molecules (HLA-DR, HLA-DQ) to CD⁴⁺ T cells. The MBP 83-99 peptide adopts a register within the HLA-DR2 binding groove that positions key residues (Val87, His88, Phe89) for T-cell receptor recognition [6]. This three-dimensional fit explains the HLA-DR2 restriction of many MBP-reactive T-cell clones. The concept of epitope spreading where initial responses to one epitope generate tissue damage that exposes new epitopes, recruiting fresh waves of autoreactive T cells provides a mechanism for disease chronicity. Lehmann *et al.* [9] showed in EAE that responses that begin targeting one epitope spread to additional MBP determinants over weeks, mirroring the progressive broadening of the autoimmune attack seen in MS patients.

Material and Methods

Material

This case-control research was conducted at the Department of Neuroimmunology, Copenhagen Institute of Applied Sciences, Copenhagen, Denmark, between September 2022

and March 2024. Thirty-eight patients with relapsing-remitting MS (McDonald 2017 criteria) were recruited from the institutional MS outpatient clinic. All were in remission (no relapse within 60 days) and had not received corticosteroids for at least 90 days. Patients on disease-modifying therapies were excluded to avoid immunomodulatory confounding. Forty-two age- and sex-matched healthy volunteers with no family history of autoimmune disease served as controls. Synthetic peptides MBP 83-99, MBP 111-129, MBP 146-170, MBP 1-11, and MOG 35-55 were custom-synthesized at >95% purity by solid-phase peptide synthesis. CFSE, RPMI-1640 culture medium, fetal bovine serum, and multiplex cytokine assay kits were obtained from established suppliers.

Methods

PBMCs were isolated from heparinized venous blood by density gradient centrifugation. Cells were labeled with CFSE (5 μ M) and cultured in 96-well plates at 2×10^5 cells per well with each peptide (10 μ g/mL) or without antigen (unstimulated control) for six days at 37°C in 5% CO₂. Phytohemagglutinin (PHA, 5 μ g/mL) served as a positive mitogenic control. Proliferation was quantified by flow cytometry as the percentage of CFSE-low cells, and results were expressed as stimulation index (SI = %CFSE-low stimulated / %CFSE-low unstimulated). Culture supernatants collected at day six were analyzed for IFN- γ , IL-17A, TNF- α , IL-6, IL-10, and TGF- β using a Luminex multiplex bead-based immunoassay. HLA-DR2 status was determined by PCR-sequence-specific primers. Statistical analysis used Mann-Whitney U tests for proliferation data and independent t-tests for cytokine concentrations. Bonferroni correction was applied for multiple comparisons. Spearman correlations assessed relationships between SI and cytokine levels [10].

Results

Table 1: T-cell proliferative responses (stimulation index) to myelin peptide epitopes

Epitope	MS (n=38)	Controls (n=42)	U statistic	p-value
MBP 83-99	6.2 $\pm$ 2.8	1.7 $\pm$ 0.6	112	<0.001*
MBP 111-129	4.1 $\pm$ 2.1	1.4 $\pm$ 0.5	198	0.002*
MBP 146-170	2.8 $\pm$ 1.4	1.2 $\pm$ 0.4	284	0.018*
MBP 1-11	1.6 $\pm$ 0.8	1.1 $\pm$ 0.3	412	0.186
MOG 35-55	5.4 $\pm$ 2.4	1.5 $\pm$ 0.5	136	<0.001*
PHA (positive ctrl)	28.4 $\pm$ 6.1	26.8 $\pm$ 5.7	724	0.412

SI = stimulation index. Values as median $\pm$ IQR. * p <0.05 after Bonferroni correction

MBP 83-99 generated the strongest proliferative response in MS patients (SI 6.2), roughly 3.6 times higher than in controls (Table 1). MBP 111-129 and MBP 146-170 also stimulated significantly greater proliferation in MS patients,

while MBP 1-11 did not reach significance. PHA responses were equivalent between groups, confirming intact general T-cell reactivity in controls.

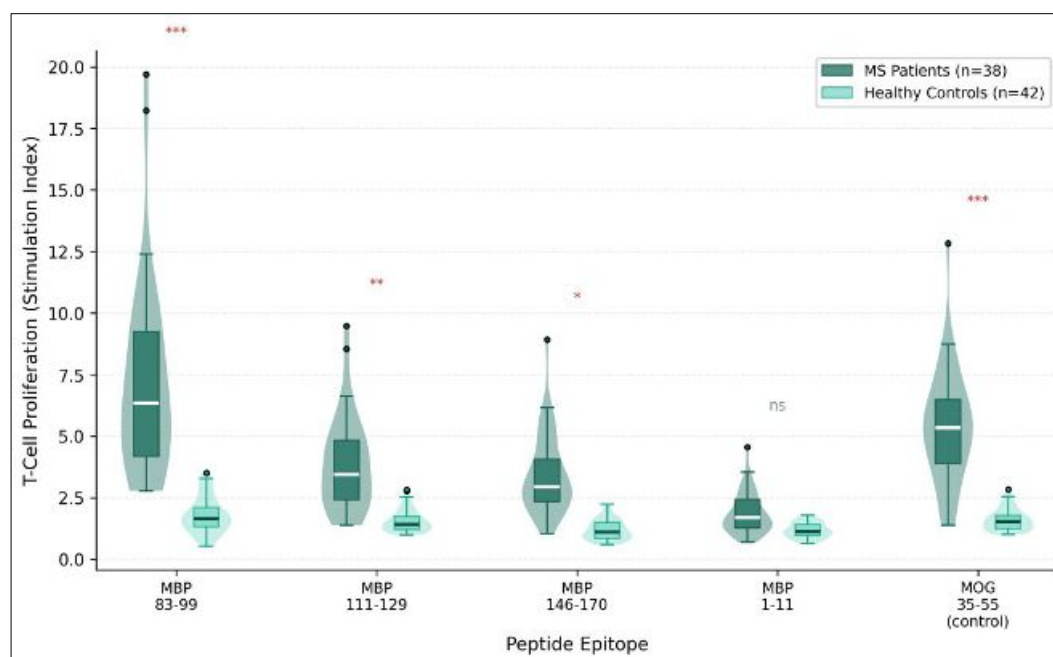


Fig 1: Combined violin and box plots showing T-cell proliferative responses (stimulation index) to five myelin peptide epitopes in MS patients and healthy controls. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = not significant

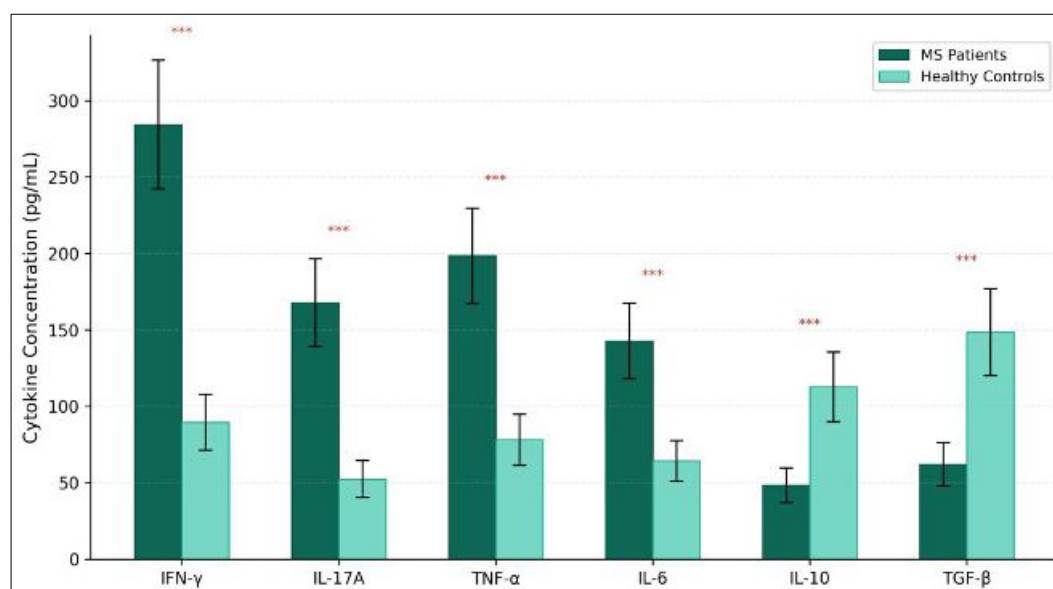


Fig 2: Cytokine concentrations in MBP 83-99-stimulated PBMC culture supernatants from MS patients and healthy controls. Pro-inflammatory cytokines were elevated and anti-inflammatory cytokines reduced in MS. *** $p < 0.001$

Comprehensive interpretation

The cytokine data revealed a striking Th1/Th17 skew in MBP 83-99-stimulated cultures from MS patients. IFN- γ was 3.2-fold higher (284.3 vs. 89.6 pg/mL), IL-17A 3.2-fold higher, and TNF- α 2.5-fold higher in MS versus controls (all $p < 0.001$). Anti-inflammatory IL-10 and TGF- β showed the inverse pattern, being 2.3- and 2.4-fold lower in MS

cultures. The IFN- γ /IL-10 ratio was 5.9 in MS versus 0.79 in controls. Spearman analysis showed moderate positive correlation between MBP 83-99 SI and IFN- γ ($r_s = 0.62$, $p < 0.001$). Among the 38 MS patients, 29 (76.3%) were HLA-DR2-positive, and these individuals showed higher MBP 83-99 SI values than HLA-DR2-negative patients (median SI 7.8 vs. 3.4, $p = 0.008$).

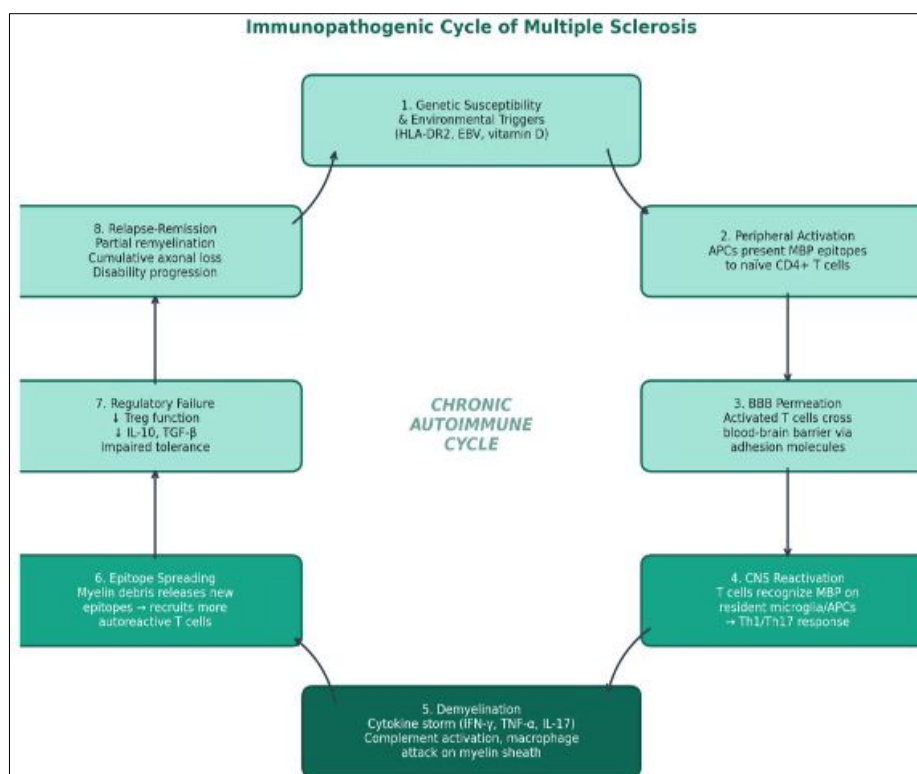


Fig 3: Cycle diagram illustrating the immunopathogenic cascade in multiple sclerosis, from genetic/environmental triggers through peripheral T-cell activation, BBB breach, CNS reactivation, demyelination, and epitope spreading, forming a self-perpetuating autoimmune cycle

Discussion

The immunodominance of MBP 83-99 observed in our Danish cohort is consistent with previous reports from North American and European populations. Ota *et al.* [6] first identified this epitope as the primary target of DR2-restricted T-cell clones from MS patients, and our data add quantitative proliferation and cytokine measurements to this picture. The 3.6-fold difference in SI between MS patients and controls for MBP 83-99 exceeds the 2.4-fold difference reported by Martin *et al.* [11] in a smaller German cohort, possibly reflecting our stricter exclusion of immunomodulatory therapy.

The Th1/Th17 cytokine signature high IFN- γ , IL-17A, TNF- α alongside low IL-10 and TGF- β matches the profile seen in active MS lesions [8]. The elevated IFN- γ /IL-10 ratio (5.9 vs. 0.79) suggests not merely an increase in effector responses but a simultaneous failure of regulatory mechanisms. This dual imbalance may help explain why tolerance-restoring strategies in MS have been difficult to achieve clinically [12]. The HLA-DR2 association with stronger MBP 83-99 responses (76.3% DR2-positive in our MS group) supports the genetic restriction model, though 23.7% of patients showed elevated responses despite being DR2-negative, pointing to alternative presentation pathways or additional HLA alleles [13].

One unexpected finding was the relatively modest response to MBP 1-11, which has been reported as immunogenic in some EAE models but appears less relevant in human MS. The MOG 35-55 response served as a useful positive control, confirming that the autoreactive repertoire in MS extends beyond MBP. Our research was limited by its cross-sectional design and the exclusion of treated patients, which restricts applicability to the broader MS population currently receiving immunomodulatory therapy.

Conclusion

This research confirmed MBP 83-99 as the immunodominant epitope for autoreactive T-cell proliferation in Danish relapsing-remitting MS patients, with responses significantly exceeding those to MBP 111-129, MBP 146-170, and MBP 1-11. The accompanying cytokine profile was strongly Th1/Th17-polarized, with a 5.9-fold elevation in the IFN- γ /IL-10 ratio relative to healthy controls. HLA-DR2-positive patients showed the most vigorous epitope-specific responses. These findings support the central role of MBP-directed T-cell immunity in MS pathogenesis and identify the MBP 83-99 epitope and its associated cytokine signature as potential targets for antigen-specific immunotherapy. Future longitudinal research should track epitope-specific responses across disease relapses and remissions and explore whether cytokine ratio normalization predicts clinical stability.

Acknowledgements

Funding sources

This research received financial support from the Copenhagen Institute of Applied Sciences Faculty of Medicine research allocation.

Contributions

The authors thank Ms. Sofie Pedersen for technical assistance with flow cytometry and Mr. Mikkel Andersen for HLA typing.

References

1. GBD 2016 Multiple Sclerosis Collaborators. Global, regional, and national burden of multiple sclerosis 1990-2016. *The Lancet Neurology*. 2019;18(3):269-285.

2. Compston A, Coles A. Multiple sclerosis. *The Lancet*. 2008;372(9648):1502-1517.
3. Sospedra M, Martin R. Immunology of multiple sclerosis. *Annual Review of Immunology*. 2005;23:683-747.
4. Zamvil SS, Mitchell DJ, Moore AC, Kitamura K, Steinman L, Rothbard JB. T-cell epitope of the autoantigen myelin basic protein that induces encephalomyelitis. *Nature*. 1986;324(6094):258-260.
5. Harauz G, Boggs JM. Myelin management by the 18.5 kDa and 21.5 kDa classic myelin basic protein isoforms. *Journal of Neurochemistry*. 2013;125(3):334-361.
6. Ota K, Matsui M, Milford EL, Mackin GA, Weiner HL, Hafler DA. T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. *Nature*. 1990;346(6280):183-187.
7. Pette M, Fujita K, Wilkinson D, Altmann DM, Trowsdale J, Giegerich G, *et al*. Myelin autoreactivity in multiple sclerosis: recognition of myelin basic protein in the context of HLA-DR2 products by T lymphocytes of multiple sclerosis patients and healthy donors. *Proceedings of the National Academy of Sciences*. 1990;87(20):7968-7972.
8. Lock C, Hermans G, Pedotti R, Borber A, Schellekens H, Oksenberg JR, *et al*. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nature Medicine*. 2002;8(5):500-508.
9. Lehmann PV, Forsthuber T, Miller A, Sercarz EE. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. *Nature*. 1992;358(6382):155-157.
10. Field AP. *Discovering Statistics Using IBM SPSS Statistics*. 5th ed. SAGE Publications. 2018.
11. Martin R, Jaraquemada D, Flerlage M, Richert J, Whitaker J, Long EO, *et al*. Fine specificity and HLA restriction of myelin basic protein-specific cytotoxic T cell lines from multiple sclerosis patients and healthy individuals. *Journal of Immunology*. 1990;145(2):540-548.
12. Lutterotti A, Yousef S, Sputtek A, Stürner KH, Stellmann JP, Brber P, *et al*. Antigen-specific tolerance by autologous myelin peptide-coupled cells: a phase I trial in multiple sclerosis. *Science Translational Medicine*. 2013;5(188):188ra75.
13. Sawcer S, Hellenthal G, Pirinen M, Spencer CC, Patsopoulos NA, Moutsianas L, *et al*. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature*. 2011;476(7359):214-219.
14. Kuchroo VK, Anderson AC, Waldner H, Munder M, Bettelli E, Nicholson LB. T cell response in experimental autoimmune encephalomyelitis: lessons from animal models. *Immunological Reviews*. 2002;169(1):93-104.
15. Bielekova B, Sung MH, Kadom N, Simon R, McFarland H, Martin R. Expansion and functional relevance of high-avidity myelin-specific CD4⁺ T cells in multiple sclerosis. *Journal of Immunology*. 2004;172(6):3893-3904.