

T cell receptor altered peptide ligands of Myelin Basic Protein and Proteolipid Protein (Linear and Cyclic) can modulate immune responses in SJL/J mice: Bioactive peptides for Multiple Sclerosis

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Introduction

Multiple Sclerosis (MS) is commonly occurring chronic, inflammatory and disabling disorder of the central nervous system (CNS). It affects 0.05-0.15% of Caucasians with the onset of the disease in young adulthood and women with the disease outnumber men two to one [1]. CD4⁺ type 1 T helper cells are widely considered to play a pivotal pathological role in mediating an autoimmune attack against components of myelin sheath. Recent studies have challenged the idea that more cells, such as CD8⁺ T cells, macrophages and complement are also responsible for axonal damage and neurodegeneration [2-5]. Myelin Basic Protein (MBP), Proteolipid Protein (PLP) and Myelin Oligodendrocyte Glycoprotein (MOG), have been demonstrated to be encephalitogenic in humans and rodents [5].

Experimental Autoimmune/Allergic Encephalomyelitis (EAE), an animal model for MS, is an inflammatory disease of the central nervous, with variable demyelination (6). It is induced by immunization of susceptible animals with components of the myelin sheath and complete Freund's adjuvant (CFA), and is mainly a CD4⁺ T cell transmitted disease [6]. Although EAE can be elicited in most rodents and in some monkeys, susceptibility, disease course and severity is strain independent.

A modern approach towards the therapeutic management of MS involves the design and use of peptide analogues (Altered Peptide Ligands, APLs) of disease-associated myelin epitopes to induce peripheral T-cell tolerance. Studies have shown that T cell responses in patients are associated with recognition of the 81-105 region of MBP (QDENPVVHFFKNIVTPRTPPPSQGK) (1), with the MBP₈₃₋₉₉ (ENPVVHFFKNIVTPRTP) peptide epitope displaying the strongest binding to HLA-DR2 (2, 3). The binding of MBP₈₃₋₉₉ to HLA-DR2 is via the hydrophobic V⁸⁷ and F⁹⁰ residues, while, H⁸⁸, F⁸⁹, and K⁹¹ are TCR contact residues (Fig. 1) [7-9].

The SJL/J mouse does not express the H2-E- α chain, thus, H2-A^s (I-A^s) is the only functional MHC class II molecule in the SJL/J mouse. In the SJL/J mouse strain residues from the encephalitogenic epitope MBP₈₁₋₁₀₀ have been shown to bind with high affinity. Thus, several overlapping 11-mer of the MBP₈₁₋₁₀₁ epitope were tested in order to determine their binding capacity to H2-A^s. In fact the minimum epitope required for binding was MBP₈₃₋₉₉ and followed by the shorter MBP₈₇₋₉₇ [10]. Moreover, L-Ala scans were performed with single mutated

derivatives, and was shown that these analogues still preserved the capacity to bind to H2-A^s. In addition, the MBP₈₃₋₉₉ peptide may act as a lead peptide for the design of altered peptide ligands and peptide analogues, which could be used to alter T cell responses in the SJL/J mouse model and may aid in new therapeutic approaches in autoimmune diseases.

Moreover, PLP₁₃₉₋₁₅₁ peptide (HSLGKWLGHDPDKF) has been found to be encephalitogenic in SJL/J mice and direct analysis of specific TCR contact residues is not available, however structure activity studies suggest that W¹⁴⁴ and H¹⁴⁷ are the major TCR contact residues (Fig. 1) [11].

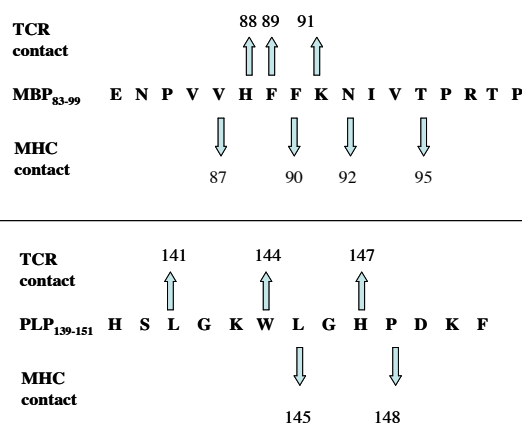


Fig. 1 MHC and TCR contact residues for MBP₈₃₋₉₉ and PLP₁₃₉₋₁₅₁ peptides.

It was previously demonstrated that the linear [R⁹¹, A⁹⁶] MBP₈₇₋₉₉, mutated peptide inhibits EAE. We designed and synthesized cyclic analogue containing the central region of MBP₈₇₋₉₉ residues and the cyclization was achieved between 90-96 aminoacids [12-13]. Although, the C-terminus was replaced with aminocaproic acid resulting in more constrained moiety, this cyclic analogue could not inhibit EAE, compared to linear [R⁹¹, A⁹⁶] MBP₈₇₋₉₉. Moreover, NMR studies based on linear MBP₈₇₋₉₉ and [R⁹¹, A⁹⁶] MBP₈₇₋₉₉ analogues revealed a head-to-tail intramolecular proximity between distant aminoacids, indicating a near cyclic conformation for the linear analogues [14]. Therefore, cyclic analogues were designed

and synthesized by cyclized free amino group of side-chain lysine with C-terminal carboxy group and between the N- and C-termini [15]. Both of the cyclic analogues were able to inhibit EAE similarly. Moreover, cyclo(91-99)[Ala⁹⁶]MBP₈₇₋₉₉ and cyclo(87-99)[Arg⁹¹,Ala⁹⁶]MBP₈₇₋₉₉ were able to inhibit EAE *in vivo*, suppressed proliferation of a human CD4 T cell clone *in vitro* and increased the Th2/Th1 cytokine ratio of human PBMC from MS patients *in vitro* [14]. Furthermore, cyclic analogues were found to bind to HLA-DR4 and were resistant in enzymatic degradation, using lysosomal enzymes and Cathepsin B, D, H, compared to their linear analogues [14].

Since the turn of the century, cyclic peptides have been used for the treatment of several diseases, such as infectious diseases, autoimmunity and cancer. They have been also used as synthetic immunogens [16], transmembrane ion channels [17], antigens for Herpes Simplex Virus [18], potential immunotherapeutic vaccines for diabetes or insulinitis in NOD mice (19) and EAE for the treatment of MS [12, 13], as inhibitors against α -amylase (20) and as protein stabilizers.

Cyclization, apart from enhancing the biological stability of peptides, can stabilize the conformation suitable for enhanced binding to receptor and improved biological activity. In addition, cyclization is an important step towards the design and synthesis of more efficient potential drugs, due to their increased chemical and enzymatic stability, receptor selectivity and improved pharmacodynamic properties, and therefore, help the design of non-peptide mimetics which are considered the new generation of drugs [reviewed in 21].

Based on previous findings, we designed and synthesized a number of linear and cyclic potential APLs by mutating principal TCR contact residues with several substitutions on MBP₈₇₋₉₉, MBP₈₃₋₉₉, and PLP₁₃₉₋₁₅₁ epitopes using Fmoc/tBu methodology (Fig. 2), in order to test immune responses, antibody responses and EAE efficacy in SJL/J mice.

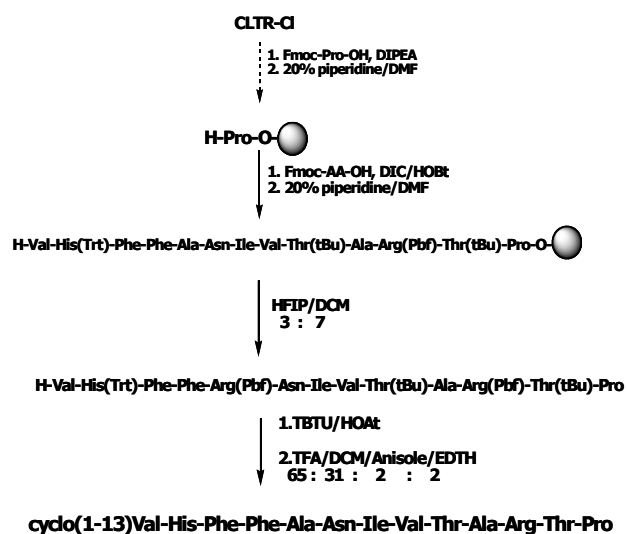


Fig. 2. Synthetic procedure for cyclic analogues using MBP₈₇₋₉₉ peptide as a representative example.

Results and Discussion

MS is a chronic, demyelinating disease of the CNS and its pathology remains unclear. There is increasing evidence that T cell responses to candidate myelin antigens, such as MBP, PLP, MOG play an important role in the pathogenesis of the disease. Another major key player in the induction of MS is the secretion of inflammatory cytokines. IFN- γ is a major inflammatory cytokine which stimulate macrophages and complement to attack and demyelinate the myelin sheath. Therefore, our aim was to test all our agonist and mutated peptides (linear and cyclic) with critical TCR mutations, in order to examine if they could decrease the production of inflammatory cytokines such as IFN- γ and increase the production of IL-4 or IL-10. Overall, we aimed to divert the immune response from Th1 to Th2.

Thus, peptides were either, (i) emulsified in equal volume of CFA and phosphate buffered saline (PBS) and injected once or (ii) conjugated to a novel carrier and injected twice on days 0 and 14. Female SJL/J mice were injected intradermally (id) or subcutaneously (sc) at the base of tail. 14 days after the last immunization T cells were isolated from spleen and examined for their cytokine production profile Th1: IFN- γ , or Th2: IL-4 and IL-10 were measured using a capture ELISpot method. Prior and two weeks after the last immunization sera was collected from the mice and antibody responses were measured by ELISA.

MBP₈₇₋₉₉ peptides (linear and cyclic) induced high levels of both IFN- γ and IL-4 for some of the mutant peptides, when emulsified in CFA or conjugated to the carrier. Also, moderate antibodies were generated when MBP₈₇₋₉₉ analogues were emulsified in CFA, and higher levels when were conjugated to the carrier. However, when analogues of the longer epitope MBP₈₃₋₉₉ were tested in SJL/J the cytokine profile was clearer. We noted that the use of CFA for immunization induced high levels of IFN- γ and moderate levels of IL-4 when the MBP₈₃₋₉₉ mutated peptides were used. Interestingly, high levels of IL-4 and IL-10 were secreted by T cells, when mice were immunized with novel carrier conjugated to mutated peptides, and marginal levels of IFN- γ . Antibody responses were also measured and their cross reaction to MBP protein assessed. Thus, the longer epitope 83-99 with specific mutations at critical TCR contact residues was more efficient compared to shorter epitope at inducing appropriate immune responses.

PLP₁₃₉₋₁₅₁ agonist peptides induced high levels of IFN- γ , while APL induced lower IFN- γ and higher IL-4. The proliferation T cell assays *in vitro* confirmed the results for IFN- γ and IL-4 cytokine profile. Antibody responses were generated to the agonist and weak to APL peptides. In addition, these mice were challenged with PLP₁₃₉₋₁₅₁ in CFA and two consecutive doses of Bordetella Pertussis to induce EAE. Conjugates showed protection or inhibition against EAE development.

ELISpot, ELISA, T cell proliferation, EAE and antagonism experiments have determined the effectiveness of all analogues in these studies.

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