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Suppression of MOG- and PLP-induced experimental autoimmune encephalomyelitis using a novel multivalent bifunctional peptide inhibitor

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ABSTRACT

Previously, bifunctional peptide inhibitors (BPI) with a single antigenic peptide have been shown to suppress experimental autoimmune encephalomyelitis (EAE) in an antigen-specific manner. In this study, a multivalent BPI ($MVB_{MOG/PLP}$) with two antigenic peptides derived from myelin oligodendrocyte glycoprotein ($MOG_{38–50}$) and myelin proteolipid protein ($PLP_{139–151}$) was evaluated in suppressing $MOG_{38–50}$- and $PLP_{139–151}$-induced EAE. $MVB_{MOG/PLP}$ significantly suppressed both models of EAE even when there was some evidence of epitope spreading in the $MOG_{38–50}$-induced EAE model. In addition, $MVB_{MOG/PLP}$ was found to be more effective than PLP-BPI and MOG-BPI in suppressing $MOG_{38–50}$-induced EAE. Thus, the development of MVB molecules with broader antigenic targets can lead to suppression of epitope spreading in EAE.

1. Introduction

Multiple sclerosis (MS) is an immune-mediated neurodegenerative disease of the central nervous system (CNS). The pathogenesis of MS has not been fully elucidated; however, it is categorized as a $CD4^+$ T cell-mediated autoimmune disease (Zhang et al., 1994; Bielekova et al., 1999). It is thought that there is a breakdown in the recognition of self versus non-self antigens, and that the immune system starts recognizing protein components of the myelin sheath as antigens. Major immunodominant proteins of the myelin sheath are myelin proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), and myelin basic protein (MBP). During disease, T cells can recognize epitopes of these proteins and initiate an inflammatory immune response toward them, leading to tissue damage. The debris from the broken down tissue leads to epitope spreading, thus resulting in new tissue components becoming antigenic (Vanderlugt and Miller, 1996).

An animal model known as experimental autoimmune encephalomyelitis (EAE) is often used to study MS. This model mimics some of the pathological features of MS such as CNS inflammation, lesion formation, blood–brain barrier (BBB) breakdown, and the presence of myelin-specific $CD4^+$ T cells (Voskuhl et al., 1993; Lassmann, 2001). In addition, the animal model can be used to study the epitope spreading process (Tuohy and Kinkel, 2000). Unlike MS, however, EAE has an initiating antigen that can be controlled by injection of the encephalitogenic peptide in the presence of complete Freund's adjuvant (CFA). This is a powerful tool because therapies can be developed to specifically suppress the immune response to these antigens. Antigen-specific immunotherapy has become widely investigated recently with the aim of inducing tolerance to specific antigens; therefore, it attenuates the inflammatory response. Previously in our laboratory, bifunctional peptide inhibitors (BPI) composed of antigenic peptides conjugated to adhesion peptides have been developed and have successfully suppressed EAE (Kobayashi et al., 2007; Kobayashi et al., 2008; Ridwan et al., 2010; Zhao et al., 2010; Manikwar et al., 2012). BPI molecules contain a specific antigen (i.e., PLP) and have been shown to suppress EAE induced by a specific antigen (i.e., PLP). Therefore, they will not be useful for suppressing EAE generated by a different antigen (i.e., MOG or MBP). In addition, antigen-specific modulation may not solve the problem of epitope spreading when the disease is in its late stage. Therefore, a new kind of BPI molecule known as multivalent BPI (MVB) has been designed with more than one antigen. The goal is that the MVB molecule will modulate the immune response to suppress the disease regardless of the inciting antigen, thus solving the problem of epitope spreading and making this strategy more applicable for translation into a MS therapy.

In EAE and MS, the activation of inflammatory $CD4^+$ T cells is mediated by two signals that are delivered from antigen-presenting cells (APC) to T cells (Grakoui et al., 1999; Tseng and Dustin, 2002). The first signal (Signal 1) is the antigen presentation by the major histocompatibility complex class-II (MHC-II) molecule, which is recognized by the T-cell receptor (TCR). The second signal (Signal 2) is made up of costimulatory and adhesion molecules on both APC and T cells. After the molecular pair interactions form both signals, signal

Abbreviations: EAE, experimental autoimmune encephalomyelitis; BPI, bifunctional peptide inhibitor; PLP, proteolipid protein; MOG, myelin oligodendrocyte glycoprotein; MVB, multivalent BPI.

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translocation occurs to form the immunological synapse that leads to activation of a sub-population of antigen-specific T cells (Lee et al., 2002; van der Merwe, 2002). The hypothesis is that BPI molecules are designed to simultaneously target Signal 1 and adhesion molecules on the surface of APC to hinder the formation of the immunological synapse, which will prevent the activation of the inflammatory T cells that specifically recognize the antigenic portion of the BPI molecule. MVB molecules are composed of more than one antigenic peptide, and can therefore bind to different MHC-II molecules on the same or different APC. Thus, the inflammatory response toward more than one antigen is prevented.

In this study, MVB comprised of MOG_{38-50} and $PLP_{139-151}$ ($MVB_{MOG/PLP}$) was developed and evaluated for suppressing two different antigen-induced EAE models. As controls, MOG-BPI and PLP-BPI were evaluated for cross-reactivity in MOG-induced and PLP-induced EAE. In this case, the efficacy of MOG-BPI was evaluated in PLP-induced EAE, and the efficacy of PLP-BPI was evaluated in MOG-induced EAE. Finally, some mechanistic aspects of MVB were elucidated by determining the cytokines produced by splenocytes of the EAE animals upon treatment.

2. Materials and methods

2.1. Mice

All protocols for experiments involving SJL/J ($H\text{-}2^s$) (Charles River, Wilmington, MA) and C57BL/6 (Jackson Laboratory, Bar Harbor, ME) were approved by the University's Institutional Animal Care and Use Committee. The mice were housed under specific pathogen-free conditions at a facility at the University of Kansas, which is approved by the Association for Assessment and Accreditation of Laboratory Animal Care.

2.2. Peptide synthesis

Peptides used in this study are listed in Table 1. 9-Fluorenylmethyloxy-carbonyl-protected amino acid chemistry was used to synthesize all peptides, utilizing an appropriate PEG-PS™ resin (Applied Biosystems, Foster City, CA) in an automated peptide synthesis system (Pioneer™:PerSeptive Biosystems, Framingham, MA). The peptides were cleaved from the resin, and removal of the protecting groups from the side-chain was accomplished with 90% TFA with 10% scavenger reagents (1,2-ethane dithiol (3%), anisole (2%), and thioanisole (5%)). The crude products were purified by reversed-phase HPLC using a semi-preparative C18 column with a gradient of solvent A (95%/5% = H_2O (0.1% TFA)/acetonitrile) and solvent B (100% acetonitrile). Analytical HPLC with a C18 column was used to determine the purity of each peptide. The identity of each synthesized peptide was confirmed by electrospray ionization mass spectrometry.

Table 1
List of peptides used in the present study.

Peptide	Sequence
$PLP_{139-151}$ (PLP)	HSLGKWLGHPDKF
MOG_{38-50} (MOG)	GWYRSPFSRVVHL
PLP-BPI	Ac-HSLGKWLGHPDKF-$(AcpGAcpGAcp)_2$-ITDGEATDSG-NH_2
MOG-BPI	Ac-GWYRSPFSRVVHL-XGX-ITDGEATDSG-NH_2
$MVB_{MOG/PLP}$	Ac-GWYRSPFSRVVHL-XGX-ITDGEATDSG-XGX-HSLGKWLGHPDKF-NH_2

Acp in the linker represents ε-aminocaproic acid. Ac—represents the acetyl-capped N-terminus of the peptide. $-NH_2$ represents the amide-capped C-terminus of the peptide. X represents polyethyleneglycol-3.

2.3. Induction of EAE and clinical evaluation

For the PLP-induced EAE, SJL/J female mice (5–7 weeks old) were immunized subcutaneously (s.c.) with 200 μg of $PLP_{139-151}$ peptide in a 0.2 ml emulsion comprised of equal volumes of phosphate-buffered saline (PBS) solution and CFA containing killed mycobacterium tuberculosis strain H37RA at a final concentration of 4 mg/ml (Difco, Detroit, MI). The PLP/CFA emulsion was administered to regions above the shoulder and the flanks (total of 4 sites; 50 μl at each injection site). In addition, 200 ng of pertussis toxin (List Biological Laboratories, Campbell, CA) was injected intraperitoneally (i.p.) on the day of immunization (day 0) and 48 h post-immunization.

For the MOG-induced EAE, C57BL/6 mice (4–6 weeks old) were immunized in a fashion similar to that mentioned above, except that 200 μg of MOG_{38-50} peptide was used and 400 ng/mouse/injection of pertussis toxin was administered on days 0 and 2. The clinical scores that reflect the disease progression were determined by the same observer in a blinded fashion using a scale ranging from 0 to 5 as follows: 0—no clinical symptoms, 1—limp tail or waddling gait with tail tonicity; 2—waddling gait with limp tail (ataxia); 2.5—ataxia with partial paralysis of one limb; 3—full paralysis of one limb; 3.5—full paralysis of one limb with partial paralysis of the second limb; 4—full paralysis of two limbs; 4.5—full paralysis of two limbs with partial paralysis of forelimbs; 5—moribund or dead. Body weight was also measured daily.

2.4. In vivo peptide treatments

2.4.1. Study I—cross reactivity of MOG-BPI and PLP-BPI

This study was performed to study the in vivo cross-reactivity of MOG-BPI and PLP-BPI in suppressing EAE. This was achieved upon induction of the disease with one antigen followed by treating the animals with a BPI molecule containing another antigen. As positive controls, the in vivo efficacies of MOG-BPI and PLP-BPI were evaluated to suppress MOG- and PLP-induced EAE, respectively. Induction of the disease was performed on day 0 as described in Section 2.3. In the MOG-induced EAE, each mouse received s.c. injections of PLP-BPI and MOG-BPI at a concentration of 100 nmol/100 μl/injection (in PBS) on days 4, 7, and 10. The efficacies of both PLP-BPI and MOG-BPI were compared to that of the vehicle (PBS). In the PLP-induced EAE, MOG-BPI was administered s.c. at a concentration of 100 nmol/100 μl/injection (in PBS) on days 4, 7, and 10. The efficacy of each peptide was evaluated by monitoring the clinical score and the change in body weight over a period of 25 days.

2.4.2. Study II—in vivo efficacy of novel $MVB_{MOG/PLP}$ in MOG-induced EAE

The purpose of this study was to evaluate the in vivo efficacy of the novel $MVB_{MOG/PLP}$ in suppressing MOG-induced EAE. Mice were immunized with MOG/CFA on day 0 as described in Section 2.3. The first group of mice received three s.c. injections of $MVB_{MOG/PLP}$ at a concentration of 100 nmol/100 μl (in PBS) on days 4, 7, and 10, and its efficacy was compared to those of the vehicle (100 μl PBS) and positive controls, MOG (100 nmol/100 μl) and MOG-BPI (100 nmol/100 μl). The negative (PBS) control and the positive control were each injected three times on days 4, 7, and 10. The efficacy of each treatment was evaluated using the clinical score and the change in body weight over a period of 25 days.

2.4.3. Study III—in vivo efficacy of novel $MVB_{MOG/PLP}$ in PLP-induced EAE

The efficacy of $MVB_{MOG/PLP}$ was also evaluated in PLP-induced EAE. All mice were immunized with PLP/CFA on day 0 as described in Section 2.3. One group of mice received three s.c. injections of $MVB_{MOG/PLP}$ at a concentration of 100 nmol/100 μl on days 4, 7, and 10; another group received 100 μl of vehicle (PBS) s.c. on the same days. The efficacy of the peptide was evaluated by monitoring the clinical score and change in body weight over a period of 25 days.