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Eluding anaphylaxis allows peptide-specific prevention of the relapsing stage of experimental autoimmune encephalomyelitis ☆

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ABSTRACT

We have used a peptide derived from *Acanthamoeba castellanii* (ACA) to treat the relapsing phase of EAE that develops in SJL mice following immunization with the PLP 139–151 peptide. The native sequence of the ACA 81–95 peptide that shares key residues with the PLP 139–151 peptide is weakly encephalitogenic in SJL mice but is not recognized by antiserum from SJL mice immunized with PLP 139–151. A single amino acid change to the ACA 81–95 peptide sequence significantly enhanced its encephalitogenicity. When administered to SJL mice as a nonlinear peptide octamer, the modified ACA peptide prevented relapsing episodes of EAE in SJL mice previously immunized with the PLP 139–151 encephalitogenic peptide.

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1. Introduction

The inbred SJL mouse strain develops a relapsing/remitting course of EAE (rEAE) following immunization with different myelin-derived proteins, or specific encephalitogenic peptides derived from these proteins (Amor et al., 1994; Bhardwaj et al., 1994; Kaushansky et al., 2007, 2008). Using this model, peptide specific therapies have been developed to successfully prevent and, in some cases, treat established disease (Elliott et al., 1996; Leadbetter et al., 1998; Smith and Miller, 2006). However the concomitant humoral response to the encephalitogen used to induce disease can prompt an anaphylactic response to the therapeutic agent, not only preventing a detailed analysis of the clinical effect that specific peptide therapy might render at the relapse stage of disease, but also perhaps more importantly placing the recipient of the therapeutic agent at significant risk. For example, it has been reported that the humoral response that develops following sensitization to the proteolipid protein (PLP) 139–151 peptide encephalitogen leads to anaphylaxis when soluble PLP 139–151 peptide is subsequently administered to these mice (Pedotti et al., 2001; C.E. Smith et al., 2005). We have also noted the development of anaphylaxis in SJL mice treated with nonlinear peptide octamers of this encephalitogen to modify the course of EAE in mice following immunization with the encephalitogenic PLP 139–151 peptide monomer, thus preventing an analysis of the therapeutic effect of the octameric peptide at the relapsing stage of disease (Wegmann et al., 2008).

In order to assess the treatment effect using soluble peptides in mice following recovery from primary diseases, without the concern for anaphylaxis, we have evaluated the PLP peptide 139–151 molecular mimic derived from the protozoan *Acanthamoeba castellanii* (ACA). This peptide is reported to be encephalitogenic in SJL mice and contains the TCR and MHC contact residues in common with the PLP 139–151 encephalitogenic peptide that has been determined for SJL mice (Massilamany et al., 2010). Other amino acids in the ACA peptide are distinct from the PLP 139–151 peptide sequence providing a natural modification to the PLP 139–151 peptide encephalitogen.

We synthesized the ACA peptide and when used as an immunogen, also found it to be weakly encephalitogenic in SJL mice. We also discovered that the ACA peptide was not recognized by antiserum obtained from SJL mice previously immunized with the PLP 139–151 encephalitogenic peptide. In subsequent studies we substituted amino acids in the native ACA sequence resulting in significant improvement in its encephalitogenicity. Although these modifications promoted ACA encephalitogenicity they did not result in recognition of the altered peptide by antiserum reactive with the PLP 139–151 peptide. Subsequently we evaluated the native ACA and one modified peptide as nonlinear peptide octamers to prevent the relapse phase of EAE in SJL mice immunized with the PLP 139–151 encephalitogenic peptide. We found that although both peptide octamers had some impact on the development of the relapse phase of disease the modified ACA peptide octamer with the greater intrinsic encephalitogenicity was the most effective therapeutic when administered in a soluble form to mice that had

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recovered from their primary course of EAE induced following immunization with the PLP 139–151 encephalitogenic peptide. We further show in this report that this modified ACA octamer prevented the development of rEAE in mice treated with this octamer, and the prevention of the relapsing stage of disease occurred with no signs of anaphylaxis during treatment with the modified ACA peptide-octamer.

2. Materials and methods

2.1. Animals

Female SJL mice (4–6 weeks of age) were obtained from Jackson Laboratories (Bar Harbor, ME) and housed under specific pathogen-free conditions at the Veterans Affairs Medical Center Animal Care Facility (Portland, OR), according to institutional guidelines and were acclimated for at least two weeks prior to experimental use. 5B6 TCR transgenic mice that respond to the PLP 139–151 peptide were obtained from a colony maintained within the animal care facility. All animal studies were reviewed and approved by the IACUC of the VAMC, Portland, OR.

2.2. Antigens

The PLP 139–151 peptide (amino acid 140 C to S substitution, amino acid sequence HSLGKWLGHPDKF) was commercially prepared by Genscript (Piscataway, NJ) and supplied at 90% purity. All MAP peptide octamers were synthesized on a Protein Technologies PS3 peptide synthesizer using standard F-moc chemistry and a double coupling procedure. MAP peptides were prepared by the sequential addition of amino acid residues to an eight-branched MAP resin (#05-24-0151, EMD chemicals San Diego, CA). The MAP peptides were designed to contain the peptide sequence with added lysine spacers when required to enhance solubility (Brokx et al., 2002). The MAP synthesis was started with two glycine residues as spacers for all peptide octamers used in this study. The sequence of the $PLP_{139-151}$MAP peptide with the glycine spacers added is $(HSLGKWLGHPDKFGG)_8$-K_4-K_2-K-beta-A. The sequence of the ACA_{81-95}MAP is $(KRGYFLLKWLGHPNVSKGG)_8$-K_4-K_2-K-beta-A and the modified ACA_{81-95}MAP with a leucine to glycine substitution at position 86 is $(KRGYFLGKWLGHPNVSKGG)_8$-K_4-K_2-K-beta-A. To improve the solubility of all ACA_{81-95}MAP derived peptides, lysines (K) were added to both the N-termini and di-glycine spacer of each octamer branch. Other peptide modifications to the ACA peptide sequence are as indicated in the text. The PLP 141–149 minimal peptide sequence is $(LGKWLGHPDGG)_8$-K_4-K_2-K-beta-A. The sequence of the OSP_{55-71}MAP peptide is $(KKDCVMATGLYHCKPLVDIKGG)_8$-$K_4$-$K_2$-K-beta-A. To improve the solubility of OSP_{55-71}MAP OSP two lysines were added to the N-termini and a single lysine added to the di-glycine spacer of each octamer branch. All peptides were cleaved with a mixture of trifluoroacetic acid (80%), H_2O (8%), 1,2 Ethanedithiol (4%) and Thioanisole (8%) (Sigma, St. Louis, MO). The cleavage reaction was allowed to proceed from 1.5 to 2 h. After cleavage the peptides were purified from the cleavage byproducts by washing 3 times in tert-butyl methyl ether. The peptide pellets are dissolved in a 1:1 mixture of water:acetonitrile, frozen and lyophilized for long-term storage.

The resulting MAP products were assessed for purity by a commercial service (AAA Laboratories, Corvallis, OR). Because of the nature of the MAP octamers, the best indication of a successful synthesis is obtained from an analysis of their complete amino acid composition and then comparing the ratios of amino acid content to the fixed lysine content that constitutes the resin used as the basis of the MAP synthesis. Using the double coupling method for synthesis of the MAP peptides, we found that following cleavage from the resin the determined amino acid content was typically at least 90% of theoretical.

2.3. Induction of active EAE

EAE was induced in 9 to 12 week-old female SJL mice by the subcutaneous injection of encephalitogenic peptide (150–200 μg) emulsified in CFA. The injection volume of 0.2 ml was distributed equally between four sites on the flank. The CFA contained 2 mg/ml *Mycobacterium tuberculosis* strain H37Ra (Difco, Detroit, MI). This immunization protocol was also used to determine the existence of memory responses retained in mice following treatment with MAP peptides.

2.4. Scoring of clinical paralysis

Clinical signs of actively induced EAE in SJL mice typically consist of hind limb weakness with limited forelimb involvement. However, animals that develop the most severe clinical disease signs develop both hind and forelimb paralysis. Degrees of hind limb and forelimb weaknesses were assessed as described below and as previously outlined (Whitham et al., 1991); flaccid tail—clinical severity score of 1; difficulty in righting after being flipped onto their backs—clinical severity score of 2; apparent hind limb weakness and not able to right themselves after being flipped onto their backs—clinical severity score of 3; severe hind limb weakness and walking upright only with difficulty—clinical severity score of 4; severe hind limb weakness and unable to upright—clinical severity score of 5; and hind limb paraplegia with no volitional leg movement—clinical severity score of 6. For some experimental groups the average cumulative disease score was determined. The cumulative disease score is the sum of the daily clinical score for an individual mouse over the observation period. The average cumulative disease score is the group's average daily clinical score summed over the observation period.

2.5. Treatment of actively-induced EAE

MAP peptides were prepared in saline at a concentration of 1.0 mg/ml. Peptide concentrations were adjusted by dilution with sterile saline as required just prior to treatment. Animals received the indicated amount of peptide (i.p.) in a volume of 0.2 ml.

2.6. T cell proliferation assay

Single cell suspensions of spleen cells were prepared from SJL mice previously immunized with PLP139-151/CFA. Cell proliferation responses to MAP peptides were assessed by plating 5×10^5 spleen cells in a 96-well tissue culture plate. The cell mixture was added in a 0.2 ml volume of stimulation media (RPMI 1640-5% fetal calf serum). The ACA derived MAP peptides, PLP 139–151 MAP peptide, or media control was added in a volume of 10 μl/well. Cultures were incubated for 72 h at 37 °C in 7% CO_2. Wells were pulsed for the final 18 h with 0.5 μCi per well [^{3}H]thymidine (Amersham, Arlington Heights, IL). The cells were harvested onto glass fiber filters, and [^{3}H]thymidine uptake was measured using a liquid scintillation counter (1205 Betaplate: Wallac, Turku, Finland). Mean cpm +/1 SD were calculated for quadruplicate wells.

We also used spleen cells from naive 5B6 transgenic mice that respond to the PLP 139–151 peptide. Cell proliferation responses to MAP peptides were assessed by plating 2×10^5 5B6 spleen cells/well in a 96-well tissue culture plate. The cell mixture was added in a 0.2 ml volume of stimulation media. The ACA derived MAP peptides, PLP 139–151 MAP peptide, or media control was added in a volume of 10 μl/well. The 5B6 spleen cells were cultured and harvested as described above.

2.7. Measurement of antibody response

Individual wells of a 96 well ELISA plate (NUNC Maxisorp) were coated with 0.1 ml (0.4 mM) of PLP 139–151 MAP, or ACA MAP derived