Albumin Delivery of Tregitope Peptides for Tolerance Induction in Autoimmunity and Inflammatory Disease

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Abstract

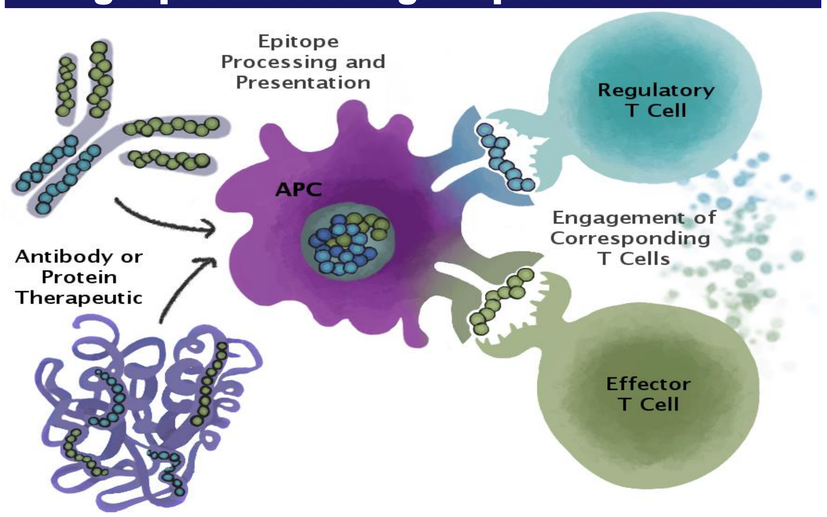
PURPOSE: EpiVax has identified a set of natural T regulatory cell epitopes (Tregitopes), derived from immunoglobulin G (IgG), that bind to multiple MHC class II molecules, activate Tregs and suppress inflammation. Tregitope effects have been validated by independent laboratories across multiple disease models and indicate that Tregitopes have overlapping effects with IVIG therapy. However, as small peptides, effective Tregitope delivery is a significant barrier to their clinical application. Veltis™ is a drug delivery platform from Novozymes Biopharma designed to combine therapeutically active molecules with the long half-life of human serum albumin. We report here the development of a Tregitope-Albumin fusion protein for in vivo animal testing of immunogenicity and inflammatory disease models.

METHODS: EpiVax applied its in silico immunoinformatics tools to develop a polypeptide concatamer of Tregitopes for incorporation into Novozymes' Veltis™ platform. This Tregitope-Albumin fusion product was evaluated for efficacy in a mouse model of OVA immunogenicity where OVA administration elicits a robust, systemic T cell proliferation and cytokine production in which the effects of Tregitope can be efficiently measured. The Tregitope-Albumin fusion was also applied in the prevention or treatment of chronic inflammatory demyelinating polyneuropathy (CIDP) in a spontaneous mouse model.

RESULTS: In the OVA model, mice that received Tregitope-Albumin fusion showed significant reductions in OVA-specific T cell proliferation. Moreover, the Tregitope-mediated modulation T cell responses was achieved with a single dose of Tregitope-Albumin fusion as compared to the daily dosing required for Tregitope peptides. Pilot studies suggest that the Tregitope-Albumin fusion, administered either prophylactically or therapeutically, may ameliorate CIDP disease progression. Immunologic correlates of Tregitope-mediated protection will also be examined.

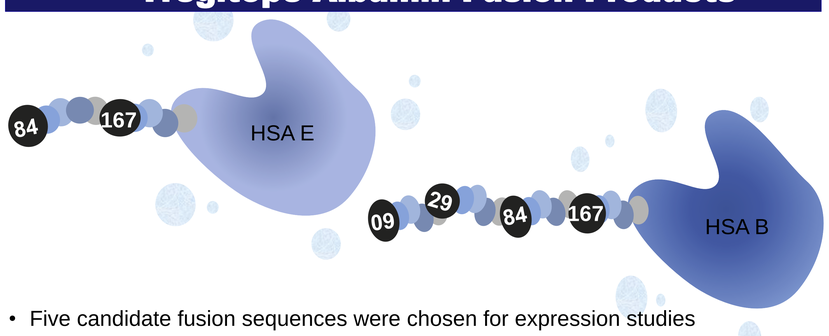
CONCLUSIONS: Tolerance-inducing Tregitopes are relevant in the treatment of autoimmune and inflammatory disorders. Delivered by albumin, they may represent a safer and more effective alternative to IVIG therapy. The Tregitope-Albumin fusion is an important advance in bringing the potential of Tregitope to clinical fruition.

Tregitopes Elicit Antigen Specific Tolerance



- Tregitopes are short, linear peptide sequences that activate regulatory T cells
- They are highly conserved in IgG across species
- Experimental evidence supports a role for Tregitopes in suppressing inflammation
- Co-formulated with, or attached to immunogenic proteins, Tregitopes elicit antigen-specific tolerance

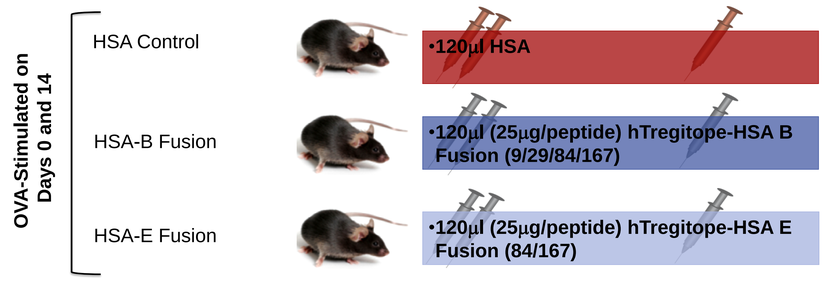
Tregitope-Albumin Fusion Products



- Five candidate fusion sequences were chosen for expression studies
- Based on expression, two were chosen for production: Fusion E and Fusion B
- HSA-Tregitope Fusion E contains human Tregitopes 167 and 84, is very well expressed and produced in large quantities
- HSA-Tregitope Fusion B contains human Tregitopes 84, 167, 09 and 29; although well expressed, Fusion B contains significant breakdown products and is difficult to purify

Study 1: Methods

Preliminary Testing in the OVA Model



- C57BL/6 mice were injected SQ (Figure A) or IP (Figure B) with OVA in CFA on Days 0 and 14. On day 0, mice also received an injection of either vehicle alone (HSA) or the indicated HSA-Tregitope Fusion
- On Day 17 spleens were harvested and splenic leukocytes were isolated, labeled with CFSE and incubated at 37°C for 72 hrs +/- OVA antigen
- Post incubation, cells were stained with CD4 antibodies and acquired on a FACSCalibur for determination of OVA antigen-specific proliferation

Study 1: Results

CD4+ T Cell Proliferation is Reduced in the Presence of Tregitopes

Figure A. Subcutaneous Delivery

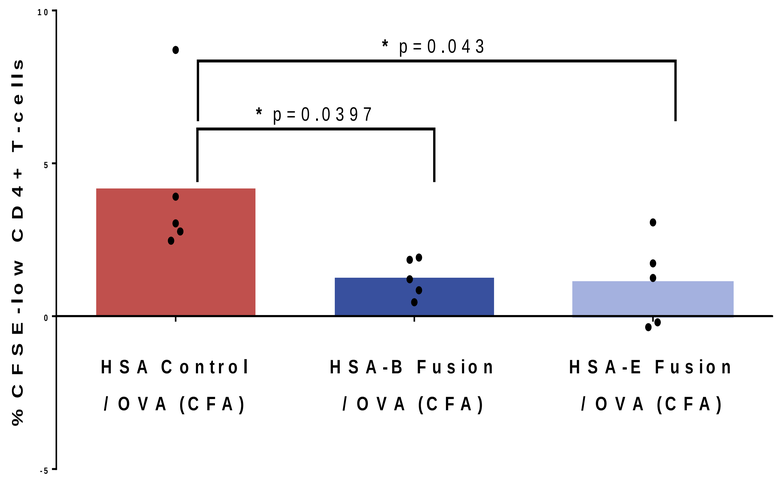
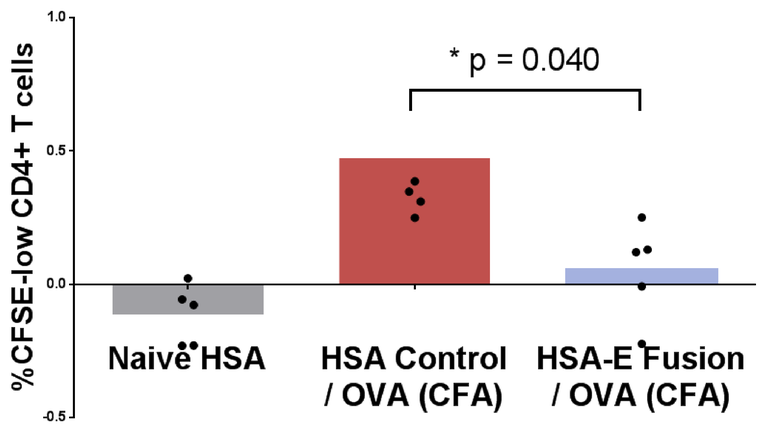


Figure B. Intraperitoneal Delivery



- These studies demonstrate statistically significant reductions in OVA-specific CD4-T Cell proliferation when first antigen exposure is co-administered with fusion
- SQ and IP are both effective routes of administration for achieving this immunomodulatory effect

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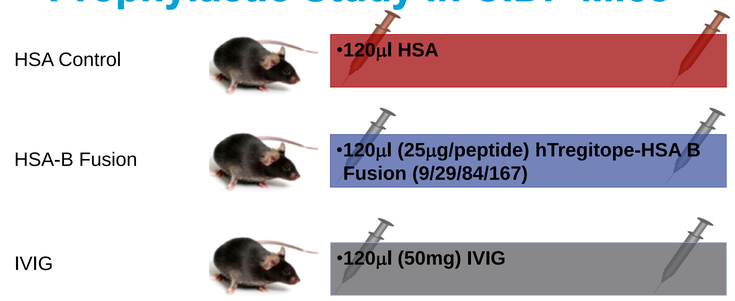
Study 2: Methods

Prophylactic Study in CIDP Mice

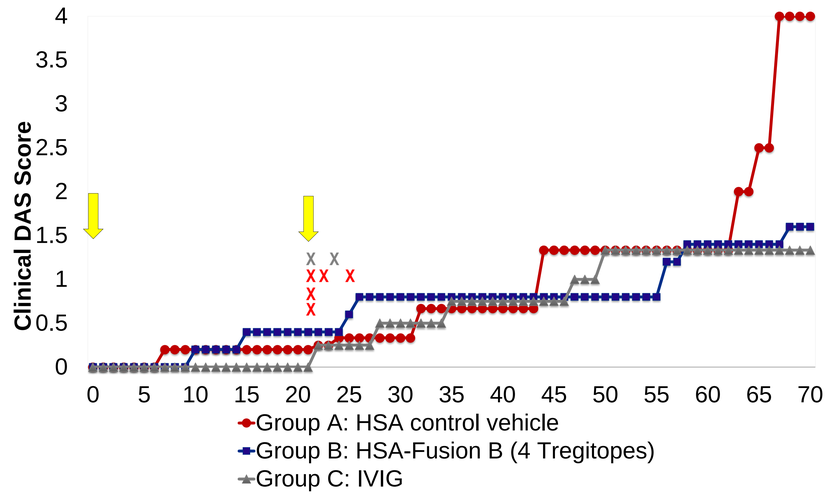


- Asymptomatic CIDP-susceptible mice were enrolled at 16 weeks of age
- Groups were treated with **HSA (n=8)**, **Tregitope-Albumin Fusion B (n=5)**, or **IVIG (n=7)** on days 0 and 21 (yellow arrows on graph below)
- Disease incidence and progression were measured over 10 weeks by digital abduction score (DAS)
- Mouse deaths in HSA and IVIG groups are indicated by "X" (on graph below)

Study 2: Results

CIDP Progression is Reduced in Mice that Receive HSA-Tregitope Fusion

Prophylactic Tregitope-Albumin Fusion Treatment



- CIDP progression was limited to and average DAS score of 1.5 with Tregitopes, compared to 4 in HSA vehicle control group
- **HSA Arm**: 5/8 deaths after second dose
- **HSA-Tregitope Arm**: NO DEATHS after second dose
- **IVIG Arm**: 2/7 deaths after second dose

Conclusions and Next Steps

- ❖ **HSA-Tregitope administration, either SQ or IP, reduces antigen-specific CD4+ T-cell proliferation**
- ❖ **Prophylactic Tregitope administration may mitigate spontaneous CIDP progression in the B7-2-deficient NOD mouse model**
- ❖ **Tregitopes have a defined mechanism of action while IVIG does not**

Next steps:

- ✓ **Refinement of dose and regimen using the OVA Immunogenicity Model**
- ✓ **Follow up studies in the mouse model of CIDP: Applying optimal dose/regimen and extending the observation period**
- ✓ **Application of Tregitope-Albumin Fusions in another chronic autoimmune disease: NOD mouse model of T1D**
- ✓ **In light of the adverse events observed in the CIDP mice (NOD background), a toxicity study is also planned**

For questions regarding immunogenicity prediction services and deimmunization options, please contact: Anthony Marcello at 401-272-2123, ext. 149; or at amarcello@epivax.com