Tregitope Applications to Tolerance Induction in Autoimmune Disease and Therapeutic Protein Immunogenicity

L.P. Cousens[1], A.S. De Groot[1,2], F. Mingozzi[3,4], D. Koeberl[5], W. Elyaman[6], B. Mazer[7], S. Khoury[6], and W. Martin[1]

[1]EpiVax, Inc., Providence, RI USA, [2]University of Rhode Island, Providence, RI USA, [3]Genethon, Evry, FR, [4]University Pierre and Marie Curie, Paris, FR, [5]Duke University, Durham, NC, USA, [6]Brigham and Women's Hospital, Boston, MA USA, [7]McGill University Health Center, Montreal, Canada

www.epivax.com

ABSTRACT

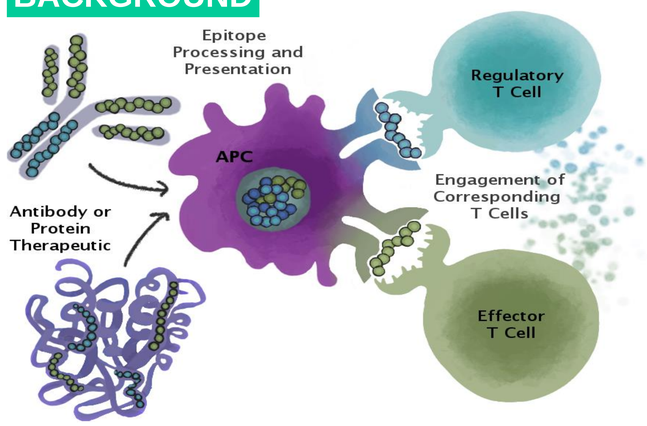
Purpose: Modulation of T cell responses provides new opportunities for the treatment of autoimmune and inflammatory diseases. Tregitopes are regulatory T cell (Treg) epitopes endogenously found in IgG that are presented on MHC II to regulatory T cells (Treg) and provide beneficial immunomodulatory effects, paralleling effects attributed to intravenous immunoglobulin (IVIG) therapy. In this presentation, we will provide evidence that Tregitopes derived from human IgG can reproduce immunomodulatory effects of IVIG *in vitro* and *in vivo* that may bypass the harmful side effects of this current treatment.

Methods: More than six collaborating laboratories have evaluated the mechanisms of action and beneficial effects of Tregitopes in mouse models of MS (EAE), OVA-induced allergic airway disease, enzyme replacement therapy in mice lacking acid alpha-glucosidase (GAA) as a model of Pompe disease, and AAV-mediated gene transfer.

Results: Tregitopes cause CD4+CD25+FoxP3+ Treg to expand and produce IL-10 *in vitro*, and iTreg to be induced *in vivo*. Induction and functions of Tregs have been examined in *in vivo* mouse model systems such as EAE, enzyme replacement therapy for Pompe disease, and AAV-mediated gene transfer. The protective effects of Tregitopes are comparable to IVIG in EAE and allergy models. In GAA-deficient mice, under conditions known to induce anti-drug-antibodies (ADA), Tregitope administration modestly inhibited ADA production, while a significant increase in GAA activity was observed for Tregitope compared to vehicle-treated controls, consistent with a functional improvement where Tregitope inhibited glycogen buildup in muscle. In the allergy model, significant and reproducible expansion of Tregs was observed in conjunction with decreased airway reactivity comparable to, if not greater than, IVIG. We will provide additional unpublished evidence demonstrating the antigen specificity of tolerance induction using Tregitopes in conjunction with target antigens, and discuss the relevance of Tregitopes to the treatment of human immune-mediated diseases.

Conclusions: Tregitopes, by inducing Tregs and tolerance, are promising for the treatment of autoimmune and inflammatory disorders and may be a powerful tool for the design of safer, more effective protein therapeutics, including monoclonal antibodies, novel scaffolds, replacement therapies or biosimilars.

BACKGROUND



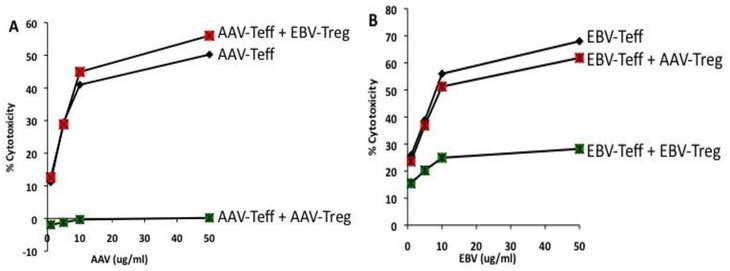
Tregitopes Elicit Antigen Specific Tolerance

- Highly conserved linear peptide sequences derived from autologous proteins such as immunoglobulin.
- Bind multiple MHC class II haplotypes with high affinity.
- Activate Treg cells to dampen immune response to antigens.
- Mechanism of Treg cells may be contact-dependent or mediated by cytokines.

RESULTS

1 Tregitopes suppress antigen-specific CD8+ responses

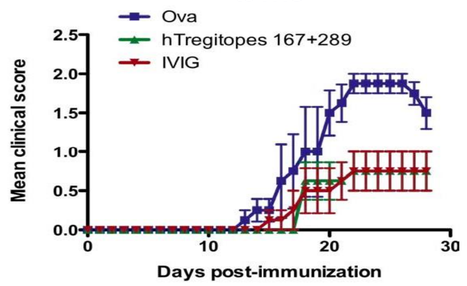
AUTOIMMUNITY



A. AAV-specific-CTLs (AAV-Teff) incubated with PBMC exposed to Tregitope and EBV (EBV-Treg) efficiently kill AAV-peptide loaded targets; AAV-Teff incubated with AAV-Treg do not.

B. Vice versa: EBV-Teff alone or incubated with AAV-Tregs kill EBV-peptide loaded targets; EBV-Teff incubated with EBV-Treg are not.

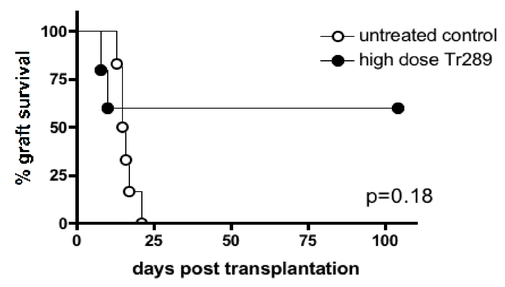
2 Tregitopes suppress EAE comparable to IVIG



- Day 0: EAE induced in C57BL/6 mice with 100 µg MOG35-55/CFA.
- Mice treated every 2nd day with Ova (control), Tregitopes, or IVIG.
- Human Tregitopes 167 + 289 suppress EAE clinical score to the same extent as IVIG.

3 Tregitopes suppress T_H1 type allogeneic responses in vivo

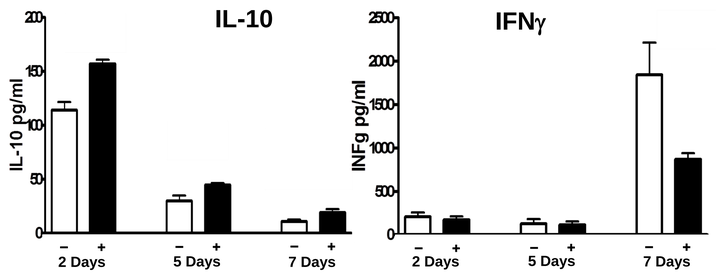
TRANSPLANT



BALB/c heart → **C57BL/6 CD28KO recipients**

- Tregitope 289 (Tr289) administered at day 0 (100 µg/dose) and every other day from days 2-10 (50 µg/dose) enhances the survival of fully allogeneic heart transplants from CD28-KO mice.

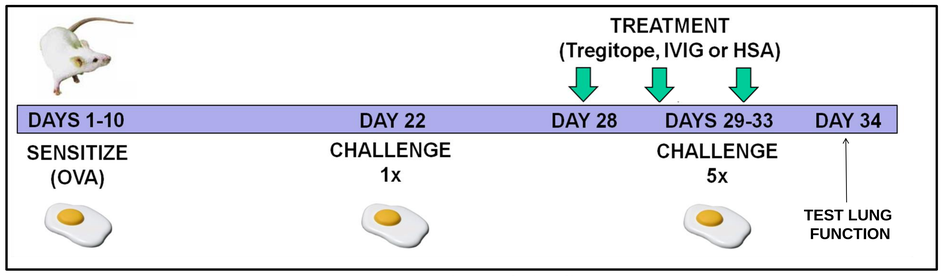
4 Tregitopes suppress T_H1 type allogeneic responses in vitro (human MLR model)



- Human MLR: PBMCs cultured with irradiated stimulator cells with (+) or without (-) 10 µg/ml Tregitope 289. Supernatant cytokines measured by Luminex.
- Addition of Tregitopes modulates human PBMC cytokine production, decreases proliferation, and increases percent of FoxP3+ Treg cells; effects are HLA-restricted (data not shown).

5a Tregitopes mirror effect of IVIG in allergic airway disease model

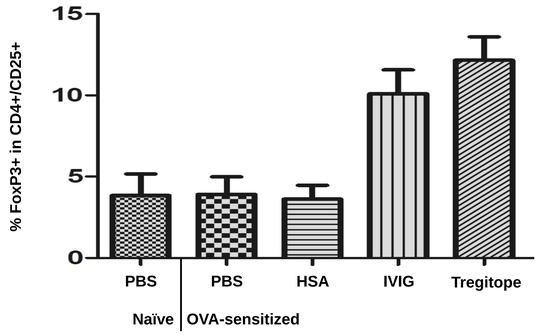
ALLERGY



Experimental Timeline

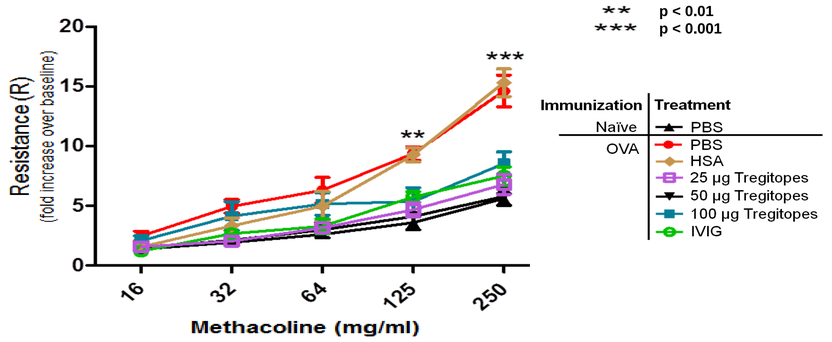
- Tregitopes were dissolved in DMSO and subsequently diluted in PBS.
- Following OVA sensitization, mice were injected with Tregitopes subcutaneously at 25, 50 or 100 µg/dose on days 28, 30 and 32.
- Treg phenotype was assessed by flow cytometry (5b), and treatment effectiveness was quantified by measuring airway resistance (5c) by FlexiVent.

5b Proportions of Tregs in lungs



- After challenge, the number of Tregs in lungs was determined by evaluating $CD4^+CD25^+Foxp3^+$ populations using flow cytometry.
- Treatment with IVIG and Tregitopes at 25 µg per dose led to significantly increased Treg frequency.

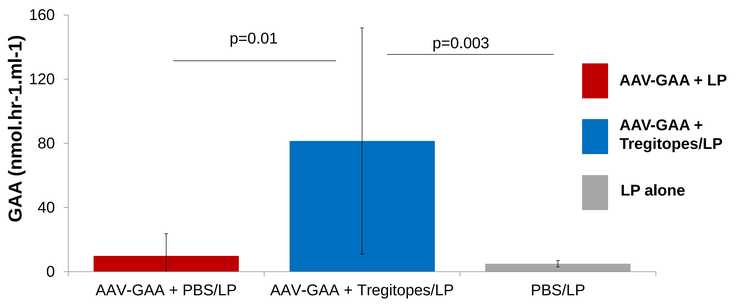
5c Changes in airway responsiveness



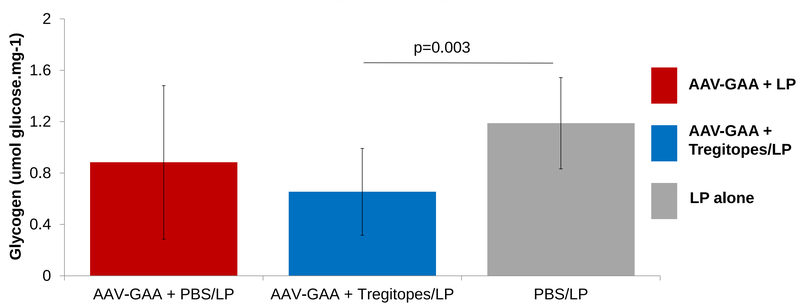
- Tregitopes markedly inhibited pulmonary cellular inflammation and dramatically attenuated airway hyper-responsiveness (AHR), measured using a small-animal ventilator (FlexiVent).
- Tregitope-mediated reduction in airway resistance was statistically significant at 125 mg/ml and 250 mg/ml Methacoline.

6a Tregitopes increase GAA activity in GAA knockout mice

PROTEIN THERAPEUTICS



6b Tregitopes decrease glycogen buildup in GAA knockout mice



- B6129-Gaatm1Rabn/J (GAA Knockout) mice at 12-16 weeks of age were treated with Tregitopes formulated in liposomes +/- GAA (in AAV) vector.
- Serum samples were collected for 12 weeks after GAA administration (24 hours before each Tregitope injection) to monitor ADA responses to the GAA protein.
- At week 18, Tregitope increased GAA activity (6a) and decreased buildup of glycogen (6b) in muscle was observed in Tregitope- compared to vehicle-treated mice.
- Conclusion: The protective effect of Tregitopes may be improved by more effective and longer term delivery relative to the therapeutic GAA protein.

CONCLUSIONS

- **These studies are an exciting first step towards understanding tolerance induction by Tregitopes. The data indicate that Tregitopes induce tolerance by:**
  - ✓ **Activating Treg populations.**
  - ✓ **Shifting cytokine production from inflammatory (IFNγ) to tolerogenic (IL-10).**
  - ✓ **Modulating APC phenotypes.**
- **The studies suggest a parallel mechanism of action for IVIG and Tregitopes.**
- **Tregitope-induced tolerance is antigen-specific; its effects can be long lasting.**
- **Tregitopes may improve therapeutic approaches in autoimmune disease, transplantation, gene transfer and allergy as well as enabling safer, more effective protein therapeutics.**

This work was supported by a Juvenile Diabetes Research Foundation "Industry Grant", a GBS-CIDP Foundation grant, SBIR Phase I/II awards (1/5R43DK081261) and SBIR Phase I award (5R43AI102454-02) from the National Institute of Health.

For more information contact: Anthony Marcello at 401-272-2123, ext. 149; or at amarcello@epivax.com