Antigen-specific tolerance induction by Tregitopes in vivo

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ABSTRACT

Modulation of T cell responses provides new opportunities for the treatment of autoimmune and inflammatory diseases. Tregitopes are regulatory T cell (Treg) epitopes found in IgG that provide beneficial immunomodulatory effects, paralleling effects attributed to intravenous immunoglobulin (IVIG). In this presentation, we will provide evidence that Tregitopes derived from human IgG can reproduce immunomodulatory effects of IVIG in vitro and in vivo. 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, cardiac transplant, diabetes (NOD), and AAV-mediated gene transfer. Tregitopes cause CD4+CD25+Foxp3+ Treg to expand and produce IL-10 in vitro, and Tregitopes to be induced in vivo. Induction and functions of Tregs have been examined in vivo model systems such as D01.10 TCR transgenic mice, transplant of BM12 to C57BL/6, AAV-mediated gene transfer and EAE. Together, the data show that effector T cells, Th17, and Th9 cells are modified in the presence of Tregitopes. In OVA-induced allergic airway disease, we observed significant and reproducible expansion of Tregs 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 We will provide additional unpublished evidence demonstrating the antigen specificity of tolerance induction using Tregitopes in conjunction with target antigens, and discuss the relevance of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. Additionally, Treg epitopes (Tregitopes?) have been identified in other common serum proteins, suggesting that peripheral control of inflammatory signals may in part be due to intrinsic self-protein “off switches”.

RESULTS

1. Tregitope effect is antigen specific

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

2. Tregitopes and IVIG both suppress EAE

• 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 Th1, Th2 type allogeneic responses in vivo

Tregitopes suppress Th1, Th2 type allogeneic responses in vivo (human MLR model)

4. Tregitopes mirror effect of IVIG in allergic airway disease model

Experimental Timeline

- Tregitopes dissolved in DMSO, 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 assessed by flow cytometry (A); treatment effectiveness was quantified by measuring airway resistance (% by FlexiVent).

5. Prophylactic treatment of NOD mice

- At 8 weeks of age, and again at weeks 9, 10, 14 and 15, eight NOD mice/group injected with pre-pro-insulin (PPI) peptides +/− Tregitopes (200 µg/mouse) or a Tatanus Toxin irrelevant peptide control.

- Prophylactic Tregitope treatment blocked onset of diabetes and suppressed the elevation of blood glucose levels.

CONCLUSIONS

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

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