Immunogenic Biologics: Validation of Screening, Deimmunization and Tolerization Approaches

Leslie P. Cousens[1], Leonard Moise[1,2], Frances Terry[1], William Martin[1], and Anne S. De Groot[1, 2]

[1]EpiVax, Inc., Providence, RI

[2]Institute for Immunology and Informatics, University of Rhode Island, Providence, RI.

www.epivax.com

ABSTRACT

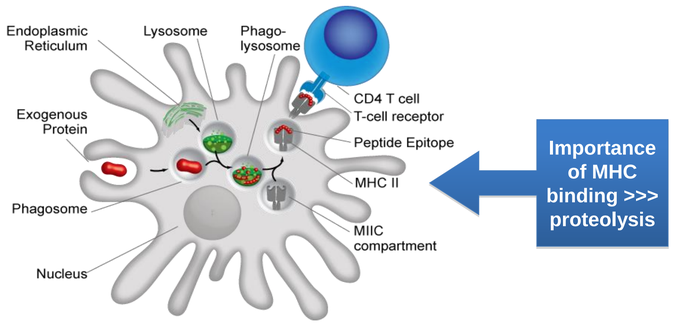
Purpose: Immune responses to protein therapeutics can directly impact drug pharmacology, safety, and efficacy. While many factors contribute to protein immunogenicity, T cell-dependent responses play a critical role. Tools to predict and reduce T cell responses to protein therapeutics present benefits at every stage of drug development.

Methods: We provide evidence for two different approaches to mitigate therapeutic protein immunogenicity: epitope modification and antigen-specific tolerance induction. In vitro and in vivo validation of computational predictions and iterative in silico modification of immunogenic epitopes were experimentally validated for selected modified sequences. Tolerance induction was achieved by co-delivery or by chemical or recombinant linkage of regulatory T cell epitopes (Tregitopes) to therapeutic proteins.

Results: (1) Deimmunization: Rational epitope modification applied to either Factor VIII or botulinum toxin demonstrates reduced immunogenicity when following a systematic process of in silico epitope mapping, in vitro and in vivo validation of computational predictions, iterative in silico modification of immunogenic epitopes and experimental validation of carefully selected modified sequences. (2) Tolerance induction: This approach involves co-delivery, chemical or recombinant linkage of regulatory T cell epitopes (Tregitopes) to therapeutic proteins. We have demonstrated that Tregitope incorporation leads to lower immune responses against target epitopes identified in Factor VIII and botulinum toxin.

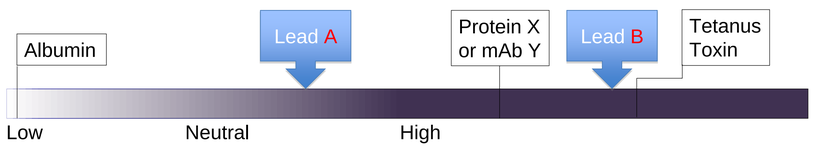
APPROACH 1: DEIMMUNIZATION BY EPITOPE MODIFICATION

T-cell epitopes as predictors of immunogenicity



- Proteins containing many T cell epitopes are predicted to be highly immunogenic, while proteins containing few epitopes are more likely to be less immunogenic.
- Immunoinformatics is a good method for predicting T cell responses and thus immunogenicity.

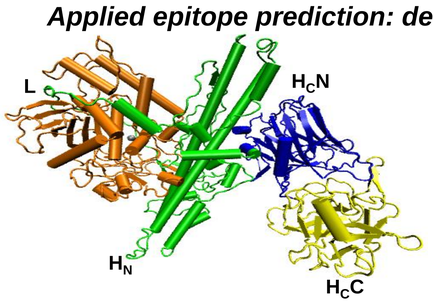
Selecting a biological lead by T cell epitope content



- Predicted epitopes are identified by EpiMatrix (CTL/T helper epitopes) and ClustiMer (promiscuous epitopes), then ranked on an immunogenicity scale by T epitope content.

De Groot, AS & Martin, W. Reducing risk, improving outcomes: bioengineering less immunogenic protein therapeutics. *Clinical Immunol*. (2009) 131(2):189-201.

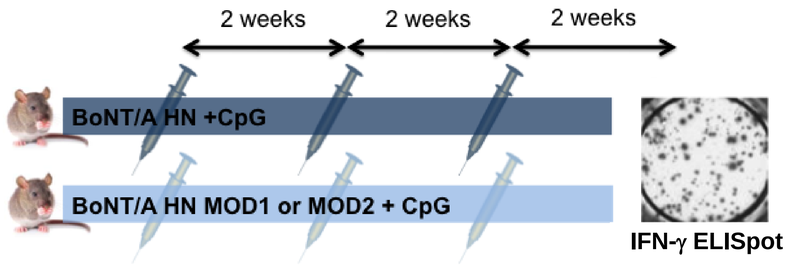
Applied epitope prediction: deimmunization of Botox



Domain	Number of clusters
Heavy chain	15
HN	4
HC	11
H_CN	5
H_CC	6

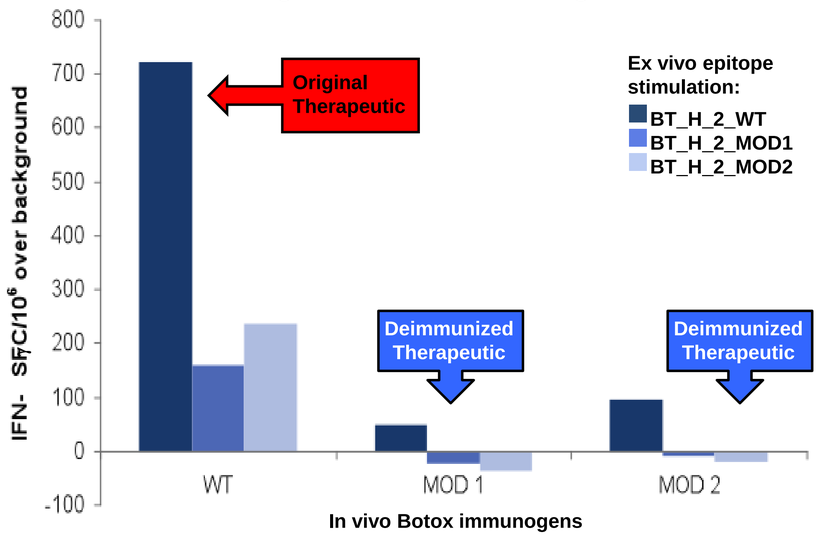
- Botox predicted epitope clusters mapped with EpiMatrix, modified in silico via OptiMatrix pinpoint deimmunization.
- Modified sequences validated in vitro (HLA binding assays, ex vivo immunoassays), in vivo (class II HLA transgenic mice) for immunogenicity.
- *Hypothesis:* Disruption of immunogenic epitopes will destroy HLA binding and thus T cell stimulation.

Validation of Botox deimmunization



- HLA A2/DR1 transgenic mice were immunized subcutaneously with Botox HN (BoNT/A HN) domain wild-type (WT) or modified (MOD) proteins together with CpG.
- Mice were immunized three times, at two-week intervals.

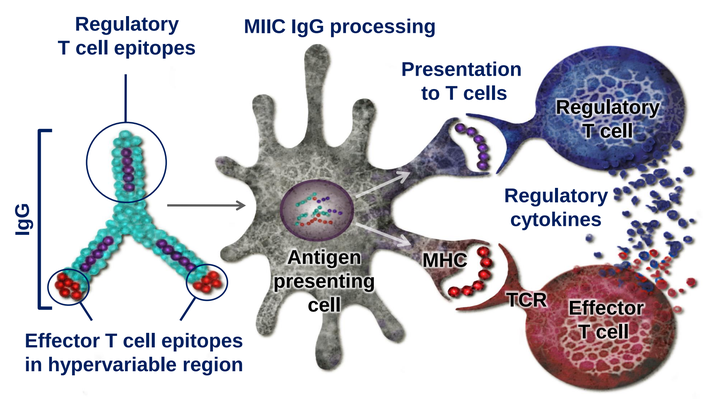
Modified Botox epitopes are less antigenic and less immunogenic



- Splenocytes were harvested and tested for recall responses to wild-type (WT) and deimmunized/modified (MOD) epitopes in an IFN-γ ELISpot assay.
- In mice sensitized to WT Botox, Botox MODs were less antigenic in vitro.
- Botox MODs administered in vivo were less immunogenic.

APPROACH 2: TREGITOPE-MEDIATED TOLERANCE INDUCTION

Balance between effector and regulatory T-cell epitopes



- Multiple mechanisms evolved to maintain central and peripheral self-tolerance. Tregitopes play a critical role.
- Tregitopes: 15-20mer peptides highly conserved in IgG that elicit regulatory T cell responses. T effector epitopes can be balanced by Tregitopes to modify immune responses.
- Tregitopes can mitigate T effector responses, promote tolerance to an immunogenic protein .

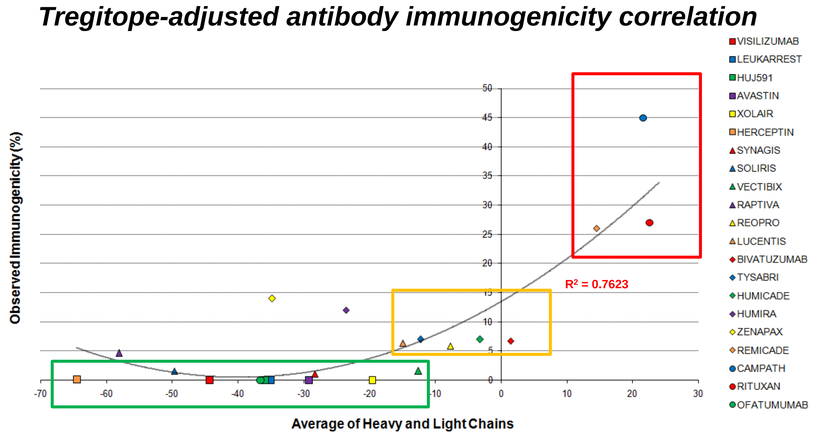
Factoring Tregitopes into protein therapeutics

1 + 1 - Regulatory T cell epitope* = Response

T cell response depends on:
T effector epitope content – Tregitope content + HLA of subject

- *Hypothesis:* Addition of Tregitopes will enhance the natural T-regulatory immune response to a recombinant protein.

Tregitope-adjusted antibody immunogenicity correlation

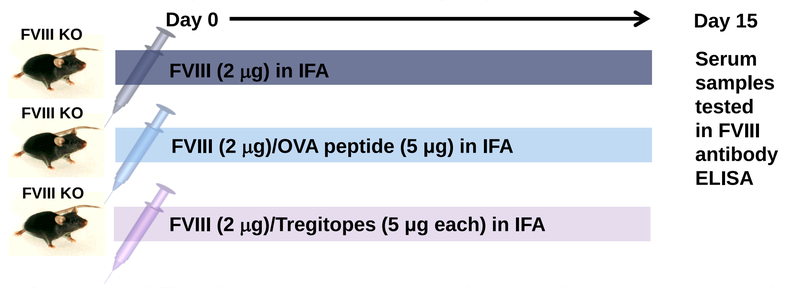


- EpiMatrix predicted immunogenicity of mAb sequences as sum of T effector epitopes, adjusted for Tregitope content*.
- Predicted immunogenicity adjusted for Tregitope content correlates with observed immunogenicity**.
- For protein therapeutics without many natural Tregitopes, co-administered Tregitopes could mitigate T effector responses and reduce immunogenicity.

*Weber, CA et al. T cell epitope: friend or foe? Immunogenicity of biologics in context. *Adv Drug Deliv Rev.* (2009) 61(11):965-76.

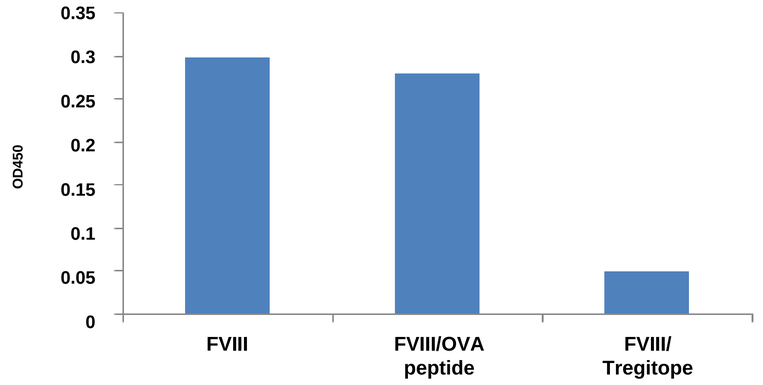
**De Groot, AS & Martin, W. Reducing risk, improving outcomes: bioengineering less immunogenic protein therapeutics. *Clinical Immunol*. (2009) 131(2):189-201.

Preliminary study: in vivo Tregitope FVIII tolerization



- Impact of Tregitopes on therapeutic protein immunogenicity tested by co-administration with FVIII in FVIII knockout (FVIII-KO) mice.
- Three groups (n=5 mice) immunized with: FVIII (2 μg); FVIII (2 μg) with mTregitope167 (5 μg) and mTregitope289 (5 μg); or FVIII (2 μg) with control peptide $OVA_{323-339}$ (5 μg).
- Sacrificed on day 15, sera assayed for anti-FVIII antibodies.

Co-administration of Tregitopes with FVIII tolerizes FVIII-KO mice to the therapeutic protein



- Tregitopes co-administered with FVIII significantly lowered humoral responses (anti-FVIII antibody levels) compared to mice given FVIII +/-OVA peptide.

CONCLUSIONS

- ✓ In silico immunogenicity screening is a useful tool to predict potential clinical immunogenicity, providing opportunities to improve therapeutic design through lead selection, deimmunization, and tolerance induction.
- ✓ Tregitopes are relevant for therapeutic antibody development and inducing nTregs and tolerance, and may potentially make immunogenic therapeutics more tolerable.
- ✓ Deimmunization and Tregitope-induced tolerance may be a powerful way to address immunogenic therapeutic proteins.

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