Regulating Immune Responses to Biologics: Validation of Screening, Deimmunization and Tolerization Approaches

Leslie P. Cousens[1], Leonard Moise[1,2], Frances E. Terry[1], William D. 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 directed against 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 benefits every stage of drug development.

Methods: We provide evidence for two different approaches to mitigate therapeutic protein immunogenicity of botulinum toxin and FVIII as examples: 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 recombinant linkage of regulatory T cell epitopes (Tregitopes) to therapeutic proteins.

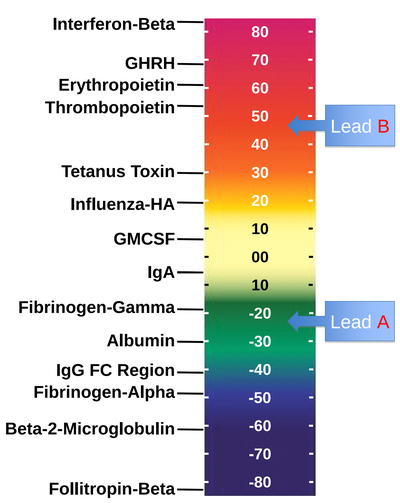
Results: (1) Deimmunization: Rational epitope modification, as applied to either botulinum toxin or Factor VIII, demonstrates reduced immunogenicity when following a systematic process of in silico epitope mapping, in vitro and in vivo validation of computational predictions, iterative in silico modification of immunogenic epitopes and experimental validation of carefully selected modified sequences. (2) Tolerance induction: This approach involves co-delivery 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 botulinum toxin and Factor VIII.

Conclusions: Analysis, prediction, and modification of T cell-mediated immunogenicity of therapeutic proteins expands the available strategies for mitigating therapeutic protein immunogenicity. The deimmunization and tolerization approaches presented here, either individually or combined, will facilitate the design of critical safety attributes early in development and will improve licensed biologics, including the construction of biobetters.

BACKGROUND

T cell epitopes as predictors of immunogenicity

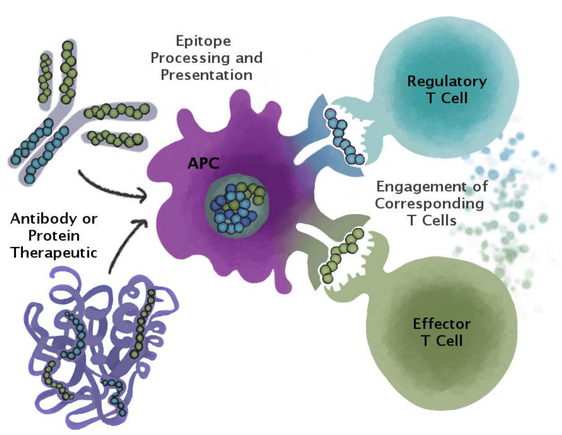
- Proteins containing many T cell epitopes are predicted to be highly immunogenic, while proteins containing few epitopes are likely to be less immunogenic.
- Immunoinformatics is a reliable and robust method for predicting T cell responses and thus immunogenicity.



Selecting a biological lead by T cell epitope content

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

Balance between effector and regulatory T cell epitopes



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

IN SILICO SCREENING TOOLS TO PREDICT IMMUNOGENICITY OF PROTEIN THERAPEUTICS

Protein Therapeutic:

1 + 1 + 1 = response

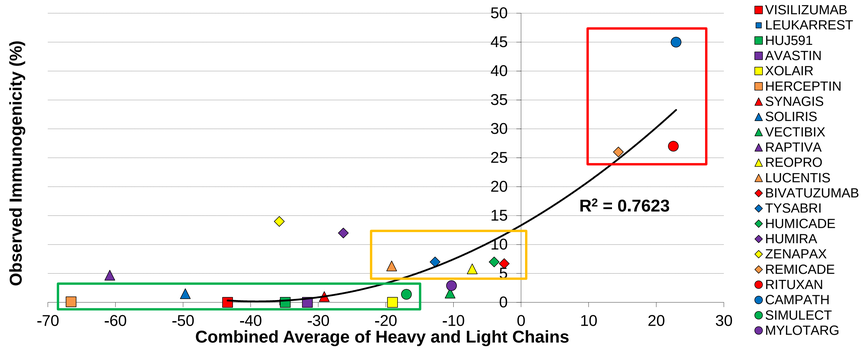
T cell response depends on:
T effector epitope content + HLA of subject

Monoclonal Antibody:

1 + 1 - regulatory T cell epitope = response

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

- EpiMatrix predicted immunogenicity of mAb sequences as the 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.

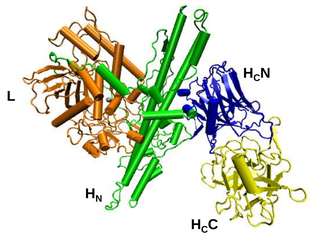


* Weber, CA et al. T cell epitope: friend or foe? Immunogenicity of biologics in context. *Adv Drug Deliv Rev.* (2009) 61(11):965-76.
** De Groot, AS & Martin, W. Reducing risk, improving outcomes: bioengineering less immunogenic protein therapeutics. *Clinical Immunol*. (2009) 131(2):189-201.

APPROACH 1: DEIMMUNIZATION BY T CELL EPITOPE MODIFICATION

Hypothesis: **Modification of immunogenic epitopes will disrupt HLA binding and thus T cell stimulation.**

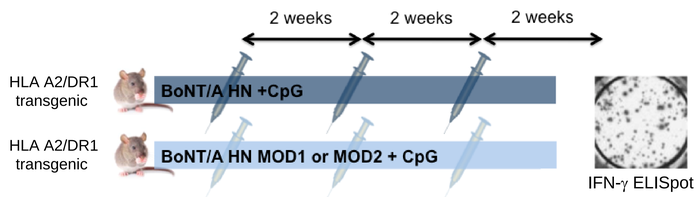
Applied epitope prediction: deimmunization of Botox



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

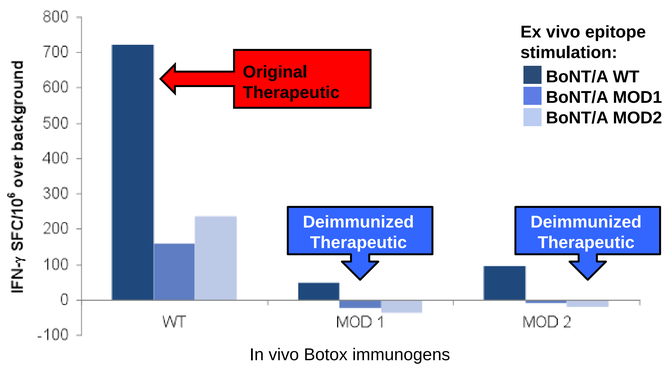
- Predicted epitope clusters in Botox are pinpointed with EpiMatrix and deimmunized in silico via OptiMatrix.
- Modified sequences are produced and validated in vitro (HLA binding assays, ex vivo immunoassays), and in vivo (class II HLA transgenic mice) for immunogenicity.

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 adjuvant.
- Mice were immunized three times, at two-week intervals.

Modified Botox epitopes are less antigenic and less immunogenic than wild-type Botox epitopes

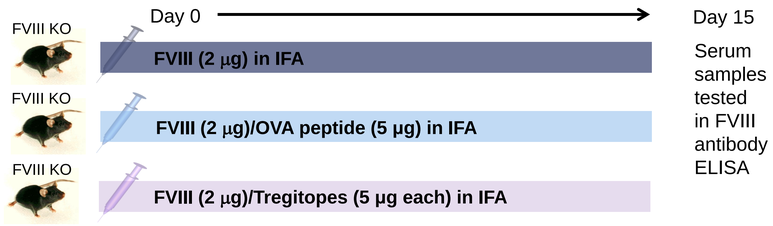


- Splenocytes were harvested and tested for recall responses to wild-type (WT) and deimmunized/modified (MOD) epitopes in an IFN-γ ELISpot assay.
- In mice sensitized to WT Botox, Botox MODs were less antigenic in vitro.
- Botox MODs administered in vivo were less immunogenic.

APPROACH 2: TREGITOPE-MEDIATED TOLERANCE INDUCTION

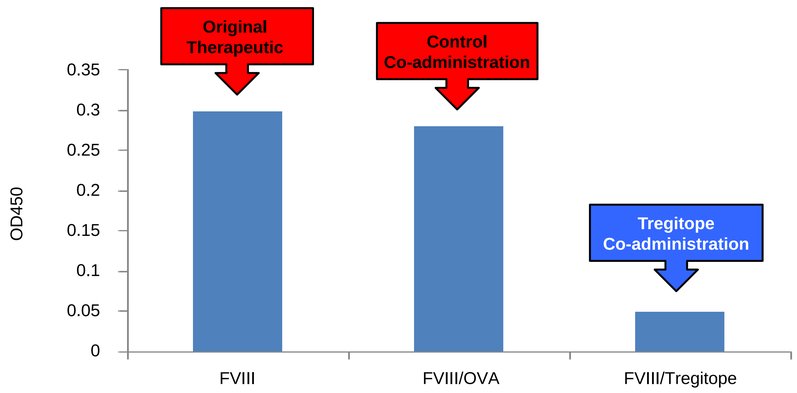
Hypothesis: **Co-delivery of Tregitopes to a protein therapeutic will prevent anti-drug antibody development.**

Preliminary study: in vivo Tregitope FVIII tolerization



- The impact of Tregitopes on therapeutic protein immunogenicity was tested by co-administration with FVIII in FVIII knockout (FVIII-KO) mice.
- Three groups (n=5 mice per group) were 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).
- After 15 days, mice were sacrificed and sera were 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 measured by anti-FVIII antibody levels, as compared to mice who received FVIII +/- OVA control peptide.

CONCLUSIONS

- ✓ **Our in silico immunogenicity screening tools predict clinical immunogenicity potential, providing opportunities to improve therapeutic design through lead selection, deimmunization and tolerance induction.**
- ✓ **Tregitopes are relevant for therapeutic antibody development and for inducing natural Tregs and tolerance, and are a potential tool to make immunogenic therapeutics more tolerable.**
- ✓ **Combining deimmunization and Tregitope-induced tolerance strategies may provide a powerful solution to address protein immunogenicity issues.**

Ongoing support for this work is funded by an SBIR Phase I award (1R43HL114308) from the National Institutes of Health

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