**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.

**APPORCH 1: DEIMUNIZATION BY EPITOE 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 ClustMHR (promiscuous epitopes), then ranked on an immunogenicity scale by T cell epitope content.


**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. Efector epitopes cannot be balanced by Tregitopes to modify immune responses.

- Tregitopes can mitigate T effector responses, promote tolerance to an immunogenic protein.

**Factorizing Tregitopes into protein therapeutics**

- 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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