

Do Tregitopes have the potential to impact the current treatment landscape of autoimmune diseases?

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Anne S De Groot

Author for correspondence:
EpiVax, Inc., 146 Clifford St,
Providence, RI 02903, USA
and
University of Rhode Island,
Institute for Immunology and
Informatics, RI, USA
Tel.: +1 401 272 2123
Fax: +1 401 272 7562
dr.annie.degroot@gmail.com;
annied@epivax.com

Current treatment of autoimmune disease usually involves the use of cytotoxic drugs or biologic agents that interfere with the activity of B cells, T cells or key cytokines, such as TNF, IL-1 and IL-6. On occasion, polyclonal immunoglobulin G (IgG), intravenous (IVIg) is used. The discovery of IgG- (and hence IVIg-) derived T regulatory (Treg) epitopes that trigger the expansion of Tregs *in vitro* and *in vivo* provides a novel explanation for the effect of IVIg. These IgG-derived Treg epitopes (also known as Tregitopes) appear to be effective on their own (*in vivo*, in autoimmune disease models) and when co-administered with a specific autoimmune disease antigen, contribute to antigen-specific tolerance induction. A description of Tregitopes and a brief discussion of their potential applications in autoimmune disease are provided here. Tregitope-based immunotherapy has the potential to modify the current autoimmune pharmacopeia.

Tregitopes are regulatory T-cell epitope sequences contained in IgG that were discovered by the team of De Groot and Martin in the course of searching for T effector (Teff) epitopes in monoclonal antibody (mAb) therapeutics [1,2]. These highly conserved (in IgG), promiscuous Treg epitopes have been shown to trigger the expansion of Tregs *in vitro*. A retrospective review of the T-cell epitope (and Tregitope) content of therapeutic mAbs, published in 2009 [3], revealed a close correlation between the presence of Tregitopes and the absence of HLA-binding Teff epitopes, with the lack of mAb immunogenicity in clinical use.

Taking this observation one step further, the presence of Treg epitopes in IgG might explain why immunoglobulins, which are produced by B cells that undergo somatic hypermutation in the periphery, do not generally elicit the expected immune response against the new 'foreign' hypervariable (complementarity determining regions, or CDR) sequences. Recognition of the importance of Tregitopes in mAb therapeutics led to the incorporation of Tregitope-

specific adjustments in certain immunogenicity predictions, improving the accuracy of the results [4,5].

Tregitopes may also explain, in part, the tolerance-inducing effect of intravenous immunoglobulin (IVIg) therapy [6]. This theory is supported by reports that IVIg-induced expansion of Tregs *in vitro* and *in vivo*, [7–9], and IVIg experts generally agree that Treg epitopes such as Tregitopes may be contributing to the tolerizing effects of IVIg [10,11]. Although it is only approved for use in a handful of diseases [12], IVIg is used off-label for hundreds of conditions [13–19].

Recently published *in vivo* studies of Tregitopes in mouse models of human autoimmune diseases have further validated the Tregitope discovery [20]. Additional publications demonstrate that co-administration of Tregitopes with target antigens *in vivo* and *in vitro* leads to the induction of antigen-specific tolerance [2,21] and suppression of both humoral [22] and cellular immune responses, including antigen-specific CD8⁺ T-cell response [6,23]. 'Control' peptides have been compared *in vitro*

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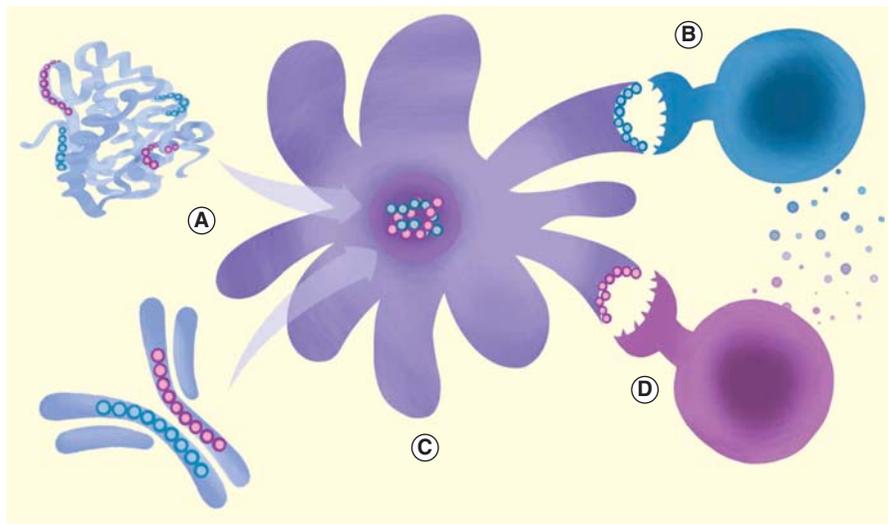


Figure 1. Naturally occurring Tregitopes in monoclonal antibodies and therapeutic proteins. T-cell epitopes (blue = Tregitope; pink = T effector epitope) can be identified within the sequences of antibody molecules or protein therapeutics using immunoinformatics tools, and validated *in vitro*. (A) Antigen-presenting cell presents Tregitopes to CD4⁺/CD25⁺ Tregs via MHC II, causing them to be activated; they may also proliferate. (B) Tregitope-specific regulatory T cells then actively secreted IL-10 and TGF- β , and the phenotype of nearby antigen-presenting cells (C) is either directly or indirectly altered, leading to downregulation of co-stimulatory molecules (i.e., CD80, CD86 and MHC II) and upregulation of tolerogenic factors such as ILT3. (D) Suppression of co-stimulatory molecules may directly inhibit T effector cell activation, while elaboration of cytokines such as IL-10 may indirectly suppress T effector responses. T-cell epitopes and Treg epitopes not drawn to scale in this illustration.

and *in vivo* [24] and have not been shown to have the same effect. In another recent publication, cross-conservation (at the T-cell receptor surface) with other highly conserved T-cell epitopes in autologous proteins is identified as a potential distinguishing feature of Treg epitopes (such as Tregitopes) but not from Teff epitopes [25]. Other groups have reported that specific IgG-derived peptides subsequently identified as Tregitopes [26–28] induce tolerance in animal and human autoimmune disease, providing independent confirmation of Tregitope studies.

The proposed Tregitope mechanism of action is distinct from some IVIg mechanisms of action but similar to others (FIGURE 1). For example, Tregitopes are unlikely to be involved in the induction of tolerance by the formation of immune complexes, blockade of Fc receptors and thereby clearance of anti-self-antibodies; immuno-modulation via anti-idiotypic interactions, inhibition of complement-mediated tissue damage and inhibition of superantigen-mediated T-cell activation; but may be involved in tolerance induction following interaction of sialylated Fc with DC-SIGN, and in direct modulation of cytokine expression (by Tregs); as well as induction of Tregs [13]. IVIg has also recently been shown to be associated with modulation of the regulatory T-cell axis, reduction of IL-17 and enhancement of the suppressive function of Tregs. Many of these effects can be explained by uptake of Tregitope by antigen-presenting cells, processing and presentation of Tregitopes to T cells, which respond by inducing Tregs to be activated and express FoxP3 and CD25. IgG processing

and epitope presentation is likely to occur when the IgG recycling protein, FcRN, is overwhelmed (as it may be when high doses of IgG, as in IVIg, are given).

The discovery of Tregitopes naturally led to the concept of actively integrating Tregitopes into biologics. Tolerization of immune responses to protein therapeutics (by introduction of Tregitopes) could be considered to be an alternative to humanization of mAbs, and it might also be applied to non-mAb biologic products [20]. Tolerization across the breadth of HLA alleles expressed in the general human population would involve inclusion of the complete repertoire of Tregitopes present in each IgG that is developed for clinical applications. Studies that support the effectiveness of this approach have been carried out by De Groot and Cousens *et al.* [6,20,21] and are currently underway in the laboratories of a number of other research groups [22,23].

The origin of Tregitope-specific T cells is as yet undetermined. They may be natural Tregs, induced Tregs or both. Regardless of the origin or mechanism of action of Tregitopes, the potential for these peptides

to alter the course of autoimmune diseases as a replacement for cytotoxic therapy, or as an alternative to biological disease modifiers, is certainly worth consideration. Albumin has been proposed as a carrier for Tregitopes, since albumin itself may contain Tregitopes, and it has been used with a wide range of therapeutic proteins as a stable and non-immunogenic excipient.

Tregitopes may also be useful for their IVIg-like effects in conditions such as allergy, where Treg induction is considered to be important but IVIg was considered to be too dangerous (and expensive) to use as therapy. Tregitopes could be combined with the target of autoimmunity, where the target is known, such as glutamic acid decarboxylase (GAD 65) protein in diabetes. For example, early studies in autoimmune disease models clearly demonstrate that Tregitopes have promise as a standalone treatment for autoimmune disease [6,20]. Where the antigen is unknown, Tregitope immunotherapy might be given during a flare, when autoimmune antigens are being presented to the immune system by activated antigen-presenting cells. Whether used on their own, or co-administered with a specific autoimmune disease antigen, Tregitope-based immunotherapy has the potential to augment future therapeutic options for autoimmune disease.

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