Review

Tregitope update: Mechanism of action parallels IVIg

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Abstract

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In the course of screening immunoglobulin G (IgG) sequences for T cell epitopes, we identified novel Treg epitope peptides, now called Tregitopes, contained in the highly conserved framework regions of Fab and Fc. Tregitopes may provide one explanation for the expansion and stimulation of Treg cells following intravenous immunoglobulin (IVIg) therapy. Their distinguishing characteristics include in silico signatures that suggest high-affinity binding to multiple human HLA class II DR and conservation across IgG isotypes and mammalian species with only minor amino acid modifications. Tregitopes induce expansion of CD4+/CD25hi/FoxP3+ T cells and suppress immune responses to co-incubated antigens in vitro. By comparing the human IgG Tregitopes (hTregitopes 167 and 289, located in the IgG CH1 and CH2 domains) and Fab to murine sequences, we identified class II-restricted murine Tregitope homologs (mTregitopes). In vivo, mTregitopes suppress inflammation and reproducibly induce Tregs to expand. In vitro studies suggest that the Tregitope mechanism of action is to induce Tregs to respond, leading to production of regulatory signals, followed by modulation of dendritic cell phenotype. The identification of Treg epitopes in IgG suggests that additional Tregitopes may also be present in other autologous proteins; methods for identifying and validating such peptides are described here. The discovery of Tregitopes in IgG and other autologous proteins may lead to the development of new insights as to the role of Tregs in autoimmune diseases.

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1. Introduction

IVIg is considered to be a low-risk form of treatment for inflammatory disorders and autoimmune diseases. Although it is only approved for use in a handful of diseases [1], IVIg is also used off-label for more than 100 conditions. Recent reports indicating that polyclonal immunoglobulin therapies induce Treg expansion and IL-10 secretion in vivo in animals and humans [2–4] have improved our understanding of the mechanism of action of IVIg. As a result, research on the effects of IVIg in autoimmune disease models has expanded, and more off-label uses are being reported [5–10]. In addition to its application in autoimmune diseases, IVIg is used as a component of the “Bonn-Malmö protocol” therapy for acquired inhibitors to Factor VIII [11,12] and for suppressing immunorejection after transplantation [13]. Although initially used to protect against infection, IVIg is now used in combination with other medications (Rituximab) for tolerance induction in Pompe disease [14]. The link between IVIg and induction of tolerance in these clinical settings and in autoimmune disease therapy sparked our interest in the potential for parallel mechanisms of action between IVIg and Tregitopes, novel peptides that were previously described by our group.

Tregitope peptides were discovered because they contain in silico signatures that suggested high-affinity binding to multiple human class II Major Histocompatibility Complexes (class II MHC, HLA-DR) [15]. We previously published the sequences of two Tregitopes identified in the constant fragment (Fc) region of immunoglobulin G (IgG) that are highly conserved across IgG Fc sequences. In previous work, we demonstrated that these Fc-derived T regulatory epitopes (Tregitopes) are able to (i) bind to many different HLA with high affinity and (ii) cause expansion of CD4+/CD25+FoxP3+ regulatory T cells (Treg) both in vitro and in vivo. Taking together their location in (and conservation across) IgG, their ability to bind to MHC class II, and their observed stimulation of Treg, we speculated that the purpose of these Tregitopes was to help to suppress human anti-human (anti-idiotypic) immune responses targeting neo-epitopes contained in antibody hyper-variable regions (see Fig. 1). In addition to suppressing immune responses to adjacent complementarity determining region (CDR) epitopes, we reasoned that the peptides might also suppress immune responses to other co-administered antigens in vitro and in vivo. We were able to confirm this hypothesis, and in the original publication, we described Tregitope-mediated suppression of immune responses to allergens such as dust mite lysate and birch pollen in vitro and in vivo. The incubation of human T cells with Tregitope peptides was also associated with activation of Tregs (as measured by expression of FoxP3 and IL-10 up-regulation) and a numerical expansion of Tregs. Induction of natural Tregs in this manner has also increased expression of adaptive Treg cell surface proteins (GITR and CTLA-4) [15]. Elyaman et al. have further explored the induction of antigen-specific adaptive tolerance to the MOG35–55 epitope using human Tregitopes 167 and 289 in vivo [16].

Another validation of the original Tregitope hypothesis emerged from clinical studies of monoclonal antibodies. We analyzed a wide range of commercially available monoclonal antibodies for the presence of Tregitopes. Consistent with the original hypothesis that these epitopes may be associated with regulation of anti-idiotypic responses, we reported that monoclonal antibodies (used in the clinic) that contained more Tregitopes were less immunogenic in human subjects than those that contained few Tregitopes [17].

Moving beyond their natural function and applications to co-administered antigens, we hypothesized that the presence of these epitopes in human IgG might explain at least one mechanism of action of IVIg in humans and animals, and, in part, how IVIg acts to suppress autoimmune diseases [18,19]. In this summary of recent work, we describe the process used to identify additional human Tregitopes and the murine homologs of human Tregitopes. We report the MHC binding affinity of human and murine Tregitopes (hTregitopes and mTregitopes, respectively) to murine MHC and review the mechanism(s) of action (MOA) by which Tregitopes engage Treg populations to induce immune tolerance. The purpose of this article is to highlight our rationale for proposing that the MOA of Tregitopes closely parallels certain aspects of the MOA established for IVIg.

2. Identification and validation of Tregitopes

Human Tregitopes 167 and 289 were originally identified while screening for monoclonal antibody immunogenicity using the EpiMatrix immuninformatics tools developed by Martin and De Groot [15,20,21]. The EpiMatrix tools are designed to predict the probability that peptides will bind to particular MHC class II alleles. This analysis is typically carried out by first parsing the complete amino acid sequence of a protein (such as FVIII, IgG monoclonal antibody sequences) into overlapping 9-mer frames. Then, each frame is evaluated for binding potential to a panel of eight common HLA alleles (DRB1*0101, *0301, *0401, *0701, *0801, *1101, *1301, and *1501) using the EpiMatrix T cell epitope prediction tool [20,21]. The protein sequences are then re-examined with the ClustiMer algorithm [22], which identifies regions of epitope clustering. The best-defined human Tregitopes and a brief description of their MHC-binding characteristics are summarized in Table 1.

2.1. In silico identification of Tregitopes

We have now developed a consistent protocol for in silico identification of candidate Tregitopes. We start by downloading human IgG sequences from Genbank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Downloaded sequences are aligned, and CDR regions are identified through conventional means. We then identify all 9-mer peptides that are conserved in at least 10% of the antibodies in our sample. These relatively conserved peptides are subsequently scored for HLA binding affinity using the EpiMatrix and ClustiMer algorithms, searching for affinity to the eight common HLA alleles mentioned above [20–22]. IgG peptides that are both highly conserved and high-scoring (in terms of both epitope content and strong epitope clusters) are evaluated for their potential to induce Tregs, as described below.

In Table 1, we list the two previously published Tregitopes (hTregitope 167 and 289) and three additional highly conserved (in more than 30% of published IgG sequences) epitopes that we classify as Tregitopes. The five Tregitopes each contain at least one EpiBar, which is defined as a single 9-mer frame that contains the potential to bind to four or more HLA alleles (for a comprehensive discussion of EpiBars and their relevance to T cell response, see reference [22]). These putative Tregitopes, listed in Table 1, are derived from both the Fc region and from the framework regions of variable domains (Fab). Together, they broadly cover heterogeneity in the HLA repertoire of humans, and thus they comprise our list of Tregitopes that may be clinically useful for induction of tolerance.

We do not believe that Tregitopes are conserved IgG. Thus, we have compiled a list of an additional 35 candidate Tregitopes, including sequences from other prevalent autologous proteins such as collagen and albumin, that have also been identified using the method described above (not shown). These include unique peptides as well as several families of frequently occurring variant sequences derived...
from IgG and other proteins. Validation studies of these putative Tregitopes in vitro, ex vivo, and in vivo using reproducible testing procedures are ongoing in the EpiVax laboratory.

2.2. Identification of murine homologs

To support in vivo testing of the Tregitope hypothesis in various murine-based disease models and TCR-transgenic mice (BALB/c, C57BL/6, NOD, DO11.10, OT-II), we defined murine homologs of the human Tregitopes. To accomplish this, we searched murine IgG constant domain sequences and identified regions corresponding to the human Tregitopes described above; see sequences in Table 2 below. Retention of MHC binding affinity was estimated using the EpiMatrix prediction tools designed for C57BL/6 and BALB/c mice (alleles I–Ab and I–Ad, respectively).

As human IVIg has been shown to induce the expansion and activation of Tregs in C57BL/6 mice [18], we also evaluated whether hTregitopes (derived from human IgG) were predicted to bind to murine MHC. As shown in Table 2a, hTregitope 167 has a total of three murine MHC motifs; one high-scoring and one moderate-scoring murine motif for

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than human-origin monoclonals that are hTregitope-replete. The pre-
has been associated with greater clinical immunogenicity in humans
origin monoclonal antibodies that naturally contain murine Tregitopes
human HLA may explain (in part) why administration of murine-
HLA binding motif scores. The relative lack of high-af
HTREG_IGGC-167 scores (22 and 12 for mTregitope 167 and 289, respec-
Tregitopes 167 and 289 also have slightly lower than average human
lower than the comparable hTregitope scores for murine MHC. Murine
is the J peptide identi
2.3. Additional Tregitopes are present in murine IgG
We note that the murine Tregitope homologs included in Table 2b
do not represent all possible murine Tregitopes. An example of a likely
Tregitope (for which no clear human homolog has yet been identi
fl
Tregitope 029 and is also highly conserved across mouse and human
genes. This peptide has been extensively described in publications
by the Mozes group but not previously identified as a Treg epitope,
even though it has been demonstrated to have Treg-inducing effects in
vivo in mice and in humans. In vivo mouse studies determined that
administration of Edratide results in an up-regulation of CD4+CD25
Tregs and increased FoxP3 and TGF-beta RNA expression [24]. The
Edratide peptide has also been evaluated in clinical studies involving
lupus patients; induction of TGF-beta and FoxP3 genes in peripheral
blood mononuclear cells (PBMC) of patients treated with Edratide was
demonstrated [27].

2.4. Tregitope peptide synthesis
Tregitope peptides contain multiple amino acids with hydrophobic
amino acid R groups that contribute to promiscuous MHC binding.
The presence of these R groups also makes Tregitope peptide synthesis
more difficult and reduces their solubility in aqueous solutions.
Tregitope peptides are synthesized using a solid-phase peptide synthesis
strategy employing N-(9-fluorenyl)methoxycarbonyl (Fmoc)
based chemistry to a purity greater than 80% by HPLC [21st Century
Biochemicals, Marlborough, MA]. Peptide sequence and purity are then
re-confirmed by MALDI/TOF mass spectrometry on a QSTAR
XL Pro mass spectrometer and, when appropriate, by HLA binding
affinity as compared to historical reference samples. Tregitope peptides
are initially solvated in an organic solvent such as DMSO, and diluted
stepwise into an aqueous solution of either 50% ethanol and PBS
(Tregitope 167) or sodium bicarbonate (Tregitope 289). Peptides are
either combined or administered individually for in vitro assays or in
vivo delivery. In all cases, following the dilution protocol as outlined
here, the final concentration of organic solvents is equal to or less
than 2% DMSO and 12.5% ethanol. All control peptides are formulated
in a similar manner; alternatively, we use an equivalent volume of
cell-culture medium with DMSO as a vehicle control.

2.5. Validation in HLA binding assays
The competition-based HLA binding assay used to validate the
HLA-binding properties of Tregitopes was adapted from Steere and
Kwok [28]. Standard curve analysis using the four-parameter logisitic
equation is performed in SigmaPlot software. IC50 values are calculated
for each peptide/allele combination and compared to an extensive
internal data set [29-34]. We currently test each putative Tregitope
peptide against a panel of eight HLA alleles representing greater
than 90% of human populations.
As shown in Table 3, even though the Tregitope peptides were
initially identified because of their predicted ‘promiscuous’ HLA binding
affinity, the peptides do not uniformly bind to all of the highly

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of human Tregitopes in IgG.</th>
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<tbody>
<tr>
<td>Tregitope</td>
<td>Human MHC motifs</td>
</tr>
<tr>
<td>---------</td>
<td>------------------</td>
</tr>
<tr>
<td>HTREG_IGGC-167</td>
<td>20</td>
</tr>
<tr>
<td>HTREG_IGGC-289</td>
<td>14</td>
</tr>
<tr>
<td>HTREG_IGGH-009</td>
<td>7</td>
</tr>
<tr>
<td>HTREG_IGGH-029</td>
<td>7</td>
</tr>
<tr>
<td>HTREG_IGGGK-084</td>
<td>7</td>
</tr>
</tbody>
</table>

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I-A<sup>d</sup>, in addition to a high scoring motif for I-A<sup>b</sup>. Human Tregitope 289 has a total of six murine MHC binding motifs, including two high-scoring murine motifs for I-A<sup>b</sup> binding, and three moderate scores for NOD (only the highest scores for each of the MHC alleles is shown in Table 2a). Murine Tregitope 167 has better scores for Balb/C and C57BL/6 MHC than mTregitope 289 (Table 2b); these scores are nonetheless lower than the comparable hTregitope scores for murine MHC. Murine Tregitopes 167 and 289 also have slightly lower than average human HLA Clustimzer scores (22 and 12 for mTregitope 167 and 289, respectively, as compared to 30 and 22 for the corresponding human Tregitopes) and, compared to hTregitopes, lower than average human HLA binding motif scores. The relative lack of high-affinity binding to human HLA may explain (in part) why administration of murine-origin monoclonal antibodies that naturally contain murine Tregitopes has been associated with greater clinical immunogenicity in humans than human-origin monoclonals that are hTregitope-replete. The predicted ability of hTregitopes to bind to murine MHC with high affinity may also explain why human IVIg is able to induce Tregs in mice in vivo.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>a. Human Tregitopes 167 and 289.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tregitope</td>
<td>AA</td>
</tr>
<tr>
<td>---------</td>
<td>----</td>
</tr>
<tr>
<td>HTREG_IGGC-167</td>
<td>26</td>
</tr>
<tr>
<td>HTREG_IGGC-289</td>
<td>21</td>
</tr>
<tr>
<td>2b. Murine Homologs to human Tregitopes</td>
<td></td>
</tr>
<tr>
<td>Tregitope</td>
<td>AA</td>
</tr>
<tr>
<td>---------</td>
<td>----</td>
</tr>
<tr>
<td>MTRREG_IGGC-167</td>
<td>20</td>
</tr>
<tr>
<td>MTRREG_IGGC-289</td>
<td>19</td>
</tr>
</tbody>
</table>

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The mechanism of J peptide action is mediated by Tregs. Thus, it is likely
that other Tregitope-like peptides remain to be discovered in murine IgG.
An additional murine Tregitope has been identified by Mozes et al. in
Israel [2425-26]. Their peptide, known as the ‘hCDR1’ peptide (sequence
GYYWSWIQPQPVKGEEWIG) or ‘Edratide’, is very similar to human
Tregitope 029 and is also highly conserved across mouse and human
genomes. This peptide has been extensively described in publications
by the Mozes group but not previously identified as a Treg epitope,
even though it has been demonstrated to have Treg-inducing effects in
vivo in mice and in humans. In vivo mouse studies determined that
administration of Edratide results in an up-regulation of CD4+CD25
Tregs and increased FoxP3 and TGF-beta RNA expression [24]. The
Edratide peptide has also been evaluated in clinical studies involving
lupus patients; induction of TGF-beta and FoxP3 genes in peripheral
blood mononuclear cells (PBMC) of patients treated with Edratide was
demonstrated [27].
Table 3

The six hTregitopes listed in this table bind to a wide range of human HLA (the HLA molecules tested in vitro are archetypical alleles and represent >60% of human populations). As shown here, Tregitopes are highly promiscuous but not universal binders to all human HLA. We use recombinant HLA molecules supplied by Bill Kwok of Benaroya Institute and his published method for HLA binding. Briefly, in 96 well plates, non-labeled test peptides compete for binding to purified HLA molecules against a labeled standard peptide for 24 h at 37°C. The non-biotinylated test peptides are evaluated over a range of concentrations while the biotinylated reference peptide is held at a fixed concentration (0.1–1 μM depending on the HLA). The peptides bound to class II molecules are then captured on ELISA plates using pan-class II antibodies (1243, anti-HLA-DR alpha chain). The assays are developed by addition of streptavidin–europium and read on a time-resolved fluorescence plate reader. Even though the Tregitope epitopes were identified for their predicted ‘promiscuous’ HLA binding affinity, they were neither predicted to bind to all HLA uniformly, nor are they shown here to bind to all of the highly prevalent human HLA. The moderate HLA-restriction of Tregitopes underscores the importance of performing HLA typing on subjects when looking for a Tregitope effect in vitro and in vivo.

<table>
<thead>
<tr>
<th>HLA Binding IC50s of 6 class II-restricted regulatory T cell epitopes to HLA-DR.</th>
<th>Non-binder</th>
<th>Weak binder</th>
<th>Moderate binder</th>
<th>Strong binder</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTREG_IGGH-009</td>
<td>20.10</td>
<td>0.60</td>
<td>Non-binder</td>
<td>Non-binder</td>
</tr>
<tr>
<td>HTREG_IGGH-029</td>
<td>47.60</td>
<td>Non-binder</td>
<td>Non-binder</td>
<td>Non-binder</td>
</tr>
<tr>
<td>HTREG_IGGK-084</td>
<td>Non-binder</td>
<td>0.60</td>
<td>Non-binder</td>
<td>Non-binder</td>
</tr>
<tr>
<td>HTREG_IGGG-134</td>
<td>Non-binder</td>
<td>1.23</td>
<td>1.31</td>
<td>1.68</td>
</tr>
<tr>
<td>HTREG_IGGC-167</td>
<td>1.30</td>
<td>0.90</td>
<td>23.35</td>
<td>3.88</td>
</tr>
<tr>
<td>HTREG_IGGC-289</td>
<td>78.26</td>
<td>1.30</td>
<td>2.10</td>
<td>1.00</td>
</tr>
</tbody>
</table>

2.6. Validation in murine MHC-binding assays

As shown in Fig. 1a, the human Tregitopes (hTregitope 167 and 289) were able to compete with FITC-GP66 for binding to I-α- and β-chains with bovine thrombin. The binding affinity of the Tregitopes is measured by their ability to inhibit the specific binding of the FITC-labeled GP66 or EoS52 peptides. Unlabeled GP66 or EoS52 (moderately high-affinity peptides for I-αβ and I-αβ, respectively), were compared to the human and murine Tregitope peptides, at equal concentrations.

As shown in Fig. 1a, the human Tregitopes (hTregitope 167 and 289) were able to compete with FITC-GP66 for binding to I-αβ equally well as unlabeled GP66. mTreg167 was also a strong binder, whereas mTregitope 289 bound the least of all four Tregitopes. So as to further explore the MHC-binding capacity of mTregitope 289, a second experiment was performed to compare it with FluO6–318, a negative control peptide. In this experiment, mTregitope 289 was a better competitor than the negative control FluO6–318, although not with affinity equal to the other Tregitopes (data not shown). When tested for binding to I-αβ, all of the Tregitopes bound with less affinity than the unlabeled control, with hTregitopes 167 and 289 showing higher affinity than mTregitope167 and mTregitope 289 binding poorly, if at all.

Although individual MHC-binding affinities varied among the Tregitope peptides, nearly all of the peptides showed dose-dependent inhibition of the FITC-labeled control for both murine MHC molecules. Taken together, these results are consistent with the predictions listed in Table 2a. This may explain the efficacy of human IVIg in ameliorating a large number of experimental models in mice (e.g. murine EAE [18], murine allergic airways disease [35], ITP [36], arthritis [37]).

2.7. Tregitopes are present in the IgG of other species

While we have satisfied our immediate need for murine Tregitope homologs by identifying the two listed in Table 2b, the method described above for identifying murine homologs could be extended to identify Tregitopes in the proteins of other species. Since identifying putative Tregitope epitopes by promiscuity across class II alleles is not necessarily applicable to laboratory animal strains that are highly inbred, we suggest that the most effective method for identifying homologs to Tregitopes found in humans, mice, or other species may be to perform direct sequence comparison together with in vitro and in vivo validation. Using this method, we have identified, but not yet validated, close homologs to human Tregitopes in cynomolgus monkey IgG. Another approach would be to search for sequences that are highly conserved in common autologous proteins and that are predicted to be both highly likely to bind to multiple MHC molecules of that species. Validation of the Tregitopes identified in non-human primate (NHP) is an active area of interest, particularly as Tregitopes may have an influence on NHP immune responses to monoclonal antibodies, and NHP are often used for pre-clinical proof of efficacy and safety-toxicity studies of therapeutic proteins.

3. Mechanism of action parallels with IVIg

3.1. Proposed Tregitope mechanism of action

We have performed extensive studies on the mechanism by which Tregitopes activate Tregs and induce the expansion of CD4+/CD25+ T cells in vivo. Based on these studies (Cousens and DeGroot, submitted for publication), we propose that Tregitopes engage regulatory T cells and that these cells subsequently modulate the phenotype of nearby antigen-presenting cells, as illustrated in Fig. 2. According to this model, Tregitopes are recognized in the context of MHC by circulating Tregs (1). This leads to expansion and activation of the nTregs (2); IL-10 and/or TGF-beta are secreted and the phenotype of antigen-presenting cells is either directly or indirectly altered, leading to down-regulation of co-stimulatory molecules (i.e. CD80, CD86, and MHC II) and up-regulation of tolerogenic factors such as IILT3. Suppression of antigen-presenting cells is either directly or indirectly altered, leading to down-regulation of co-stimulatory molecules (i.e. CD80, CD86, and MHC II) and up-regulation of tolerogenic factors such as IILT3. Suppression
3.2. IVIg mechanisms of action

The effects of IVIg have been attributed to a wide array of mechanisms. These include: the formation of immune complexes [38]; blockade of Fc receptors and thereby clearance of anti-self antibodies [39]; immuno-modulation via anti-idiotypic interactions [40]; inhibition of complement-mediated tissue damage [41]; direct modulation of cytokine expression by leukocytes and endothelial cells; and inhibition of superantigen-mediated T cell activation [42–44]. IVIg has recently been shown to be associated with modulation of the regulatory T cell axis, induction of nTregs [18], reduction of IL-17 [45], and enhancement of the suppressive function of Tregs [46]. IVIg therapy for autoimmune or inflammatory conditions is based on the intravenous administration of four to five times more IgG than for replacement therapy in immune deficiency. As such, IVIg administration can potentially deliver large quantities of Tregitopes.

3.3. Tregitopes are present in IgG Fc and Fab

The part of IgG that mediates the immunosuppressive activities attributed to IVIg has been the subject of some controversy in the literature. Certain studies have linked the anti-inflammatory efficacy of IVIg to the Fc region, mediated through the interaction of immunoglobulin Fc domains with receptors that suppress immune activation (FcRIIB), while others have characterized Fc-independent mechanisms such as epitope masking [47,48]. For example, Bayry et al. have noted that the immunomodulatory effects of IgG are not restricted to the Fc region [49]. A number of studies have shown that the Fab region is capable of inducing suppression equal to the Fc region [see references 50–53]. Still others believe antibodies generate a mix of effector and regulatory influences, depending on their makeup [54]. For IVIg, both may be true. As we have found Tregitopes in both the Fc and Fab regions of IgG, we postulate that the Tregitope contributions to the mechanisms of action of IVIg could be associated with both Fc and Fab segments of IgG. However, their inherent characteristics as epitopes presented by MHC class II would make their efficacy dependent on processing of IgG by APC, independent of FcRIIB and antigen specificity.

3.4. Tregitope activity is independent of sialylation

Tregitopes may explain some of the paradoxical observations for the requirements of Fc-sialylation in the anti-inflammatory activity of IVIg. DC-SIGN is known to traffic sialylated IgG to the antigen processing and presentation pathway [55]. Thus IgG processed by APC can present putative Tregitopes to Treg at the APC surface, leading to Treg activation. The Tregitope peptides studied here were prepared without sialylation; we conclude that sialylation is not required for the induction of Tregs following direct administration of Tregitope peptides [24,25]. However, the enhanced processing of sialylated immunoglobulins when internalized through the DC-SIGN or other lectin receptors may result in the more efficient presentation of Tregitopes by MHC II. Moreover, Tregitopes may serve somewhat specialized roles related to the degree of sialylation of the whole antibody. For example, reduced immune responses to co-delivered antigen, particularly when the Fc domain is sialylated, have been observed during pregnancy and lactation [56,57]. Thus, the maternal-fetal transfer of sialylated IgG bearing Tregitopes and allergens or other antigens may lead to the induction of antigen-specific tolerance in the fetus.

4. Conclusion

The concept that IgG, and particularly the Fc domain, is associated with the potential to induce immunologic tolerance is not new. Scott et al. described the tolerizing effect of Fc-conjugated antigens in multiple models [58,59]. They demonstrated that the tolerogenic effect of engineered antigen-IgG fusion constructs did not require Fc receptor binding [60] and that HLA class II was involved [61]. The possibility that the tolerizing effect of IVIg may also be due to elements that are present in the Fab domain of IgG has been raised by other authors [62]. Clinical proof of the presence of Tregitopes in IgG Fab can be found in Edratide, a well-studied homolog of...
Tregs that are antigen-specific may be that Tregitope-specific Tregitope peptides. Thus the discovery of Tregitopes may lead to development of appropriate targets for a Tregitope drug that contains synthetic Tregitopes have been observed. Traditional safety and toxicity studies are present in many monoclonal antibodies, in anti-thymocyte globulin (ATG), and in polyclonal IVIg, all of which are already in use for the treatment of autoimmune diseases. We have performed more than 20 individual animal studies with Tregitopes, and no toxicities associated with Tregitopes have been observed. Traditional safety and toxicity studies are underway.

Here, we have described methods that led to the discovery of Tregitopes and described several new Tregitopes in IgG identified using a combination of computational and experimental methods. We believe that additional Tregitopes remain to be discovered in other highly prevalent human proteins. We propose that such Tregitopes would also conform to the following characteristics: (i) MHC class II promiscuity, (ii) high sequence conservation, and (iii) in vitro evidence that these peptides can activate FoxP3-expressing Tregs and modify T effector responses. It is important to note that the origin of Tregitope-specific T cells is unknown. They may originate among T cells with moderate TCR affinity that escape deletion in the thymus to circulate in the periphery as “natural” regulatory T cells (nTreg), suppressing immunity against self-antigens. This hypothesis is supported by the expansion of CAR T cells in the thymus of mice treated with Tregitope 167 [63]. Regardless of their origin, Tregitope-specific T cells may act as peripheral immune system regulators. If an immunomodulatory therapy based on Tregitopes can be developed, it may have applications in many clinical settings. If further evidence emerges that the regulatory T cell effects of IVIg can be attributed to Tregitopes, then certain indications for IVIg therapy may be appropriate targets for a Tregitope drug that contains synthetic Tregitope peptides. Thus the discovery of Tregitopes may lead to development of synthetic alternatives to plasma-based IVIg therapy for inflammatory and autoimmune diseases. An advantage to Tregitope therapy may be that Tregitope-specific T cells can be used to expand adaptive Tregs that are antigen-specific. This would represent a major advance in the field of autoimmune disease therapy.

Conflict of Interest

Dr. David W. Scott is a current consultant/collaborator of EpiVax, Inc., the company that discovered Tregitopes and holds the IP. Ryan Tassone and Drs. De Groot and Cousens are employees of EpiVax, Inc. Dr. De Groot owns stocks and options in EpiVax, Inc. The authors recognize that there is a potential conflict of interest that could inappropriately influence, or be perceived to influence, their work; however, they also attest that the results and discussion are free of any influence to the best of their knowledge.

Take-home messages

- Regulatory T cell epitopes (Tregitopes) found in IgG framework regions are highly conserved peptides that suppress immune responses to co-delivered antigens.
- Intravenous immunoglobulin (IVIg) contains Tregitopes, which may explain its immunotherapeutic efficacy, at least in part.
- Human Tregitope homologs are present in murine IgG, and may be present in the IgG of other species.
- Studying Tregitopes may shed light on the mechanism of action of IVIg and similar therapies, and lead to novel treatments for autoimmunity.
- The methods described here may be used to identify Tregitope-like peptides in other autologous proteins.

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