Application of Tregitopes for tolerance induction in Type 1 Diabetes

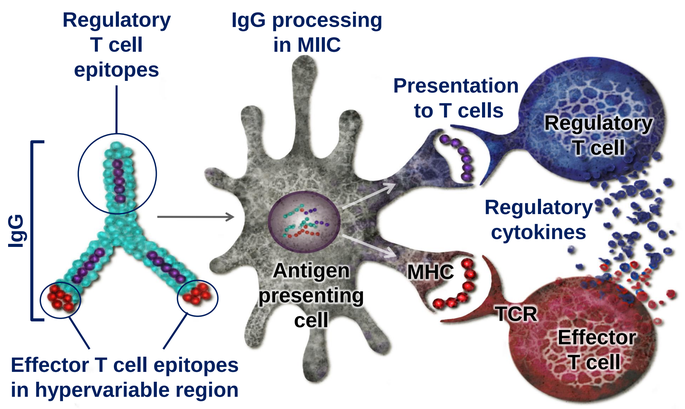
Leslie P. Cousens[1], Yan Su[2], Xin Li[2], Frances Terry[1], Jinhee Lee[3], David W. Scott[2], Anne S. De Groot[1,4]

[1]EpiVax, Inc., Providence, RI; [2]Uniformed Services University of Health Sciences, Bethesda, MD; [3]University of Massachusetts Medical School, Worcester, MA; and [4]Institute for Immunology and Informatics, University of Rhode Island, Providence, RI.

Abstract

HLA class II-restricted regulatory T cell (Treg) epitopes in IgG ("Tregitopes") have been reported to suppress immune responses to co-administered antigens by stimulating expansion of natural Tregs (nTregs). Here we evaluate their impact on human immune responses to islet cell antigens ex vivo and on modulation of Type 1 Diabetes (T1D) in mice in vivo. Co-administration of Tregitopes and T1D antigens delayed development of hyperglycemia and reduced T1D incidence in NOD mice. T1D suppression was observed even following disease onset. To measure the impact of Tregitopes on T cell responses, we evaluated the Tregitope treatment effect in DO11.10 mice. FoxP3 upregulation in KJ1-26-stained OVA-specific CD4+ T cells was seen after Tregitope treatment of DO11.10 mice, along with reduced IFNγ production and T effector responses. Current studies aim to identify a suitable delivery vehicle and dosing regimen for Tregitope therapy. In ex vivo human T cell studies, T1D patient peripheral blood mononuclear cell (PBMC) responses to GAD65 epitopes in the presence and absence of Tregitopes were measured. Immune responses to GAD65 epitopes and Tregitopes in these assays correlated with HLA type. Implementation of these defined regulatory T cell epitopes for therapy of T1D and other autoimmune diseases may lead to a paradigm shift in disease management.

Background

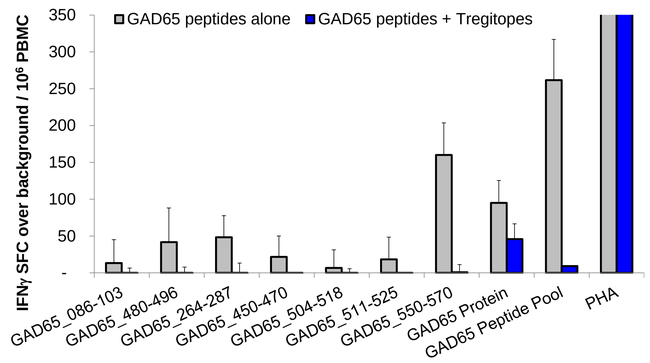


Postulated T regulatory cell epitope (Tregitope) mechanism of action:

- **Tregitopes are highly conserved Treg epitopes found in human and other species' IgG.**
- **Tregitopes are postulated to reduce immunogenicity of neo-epitopes in the hypervariable region of IgG CDR.**
- **Tregitopes also suppress immune response to other co-delivered T cell epitopes.**

Ex Vivo Results

Tregitope effects on human PBMC in IFNγ ELISpots



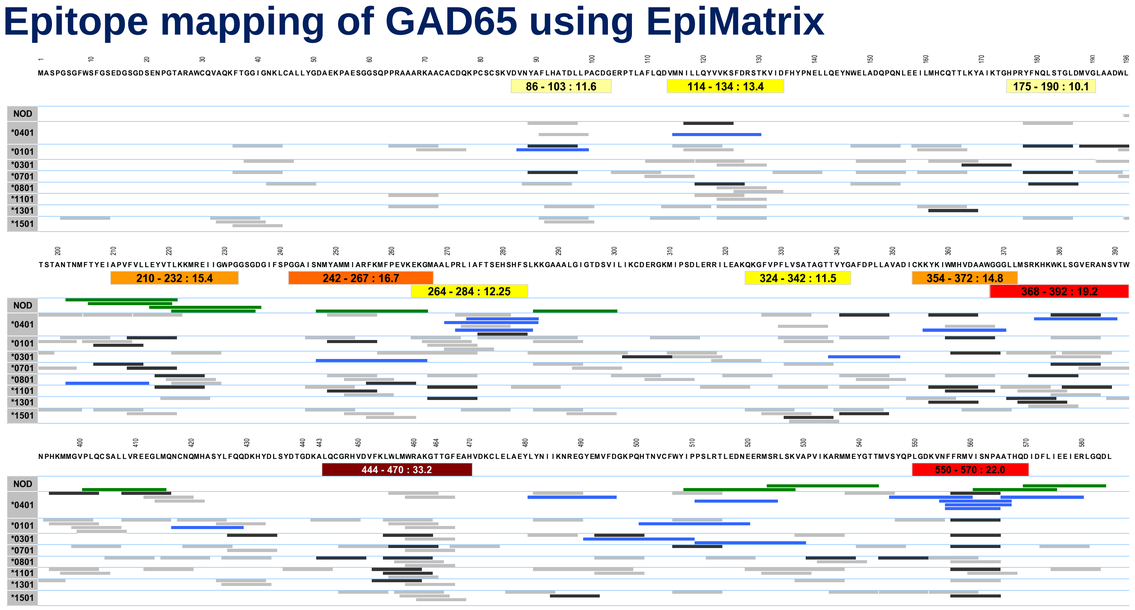
- **PBMC cultured with GAD65 peptide pool plus (blue) or minus (grey) Tregitope peptides.**
- **IFNγ ELISpot responses to GAD65 peptides cultured with recently diagnosed (<6 months) donor PBMC were low or not significant.**
- **Tregitopes suppressed responses to whole protein and to individual and pooled GAD65 peptides.**

Subject ID	# peptides tested in ELISpot	Responses suppressed by Tregitopes	Responses suppressed significantly (p<0.05)
D1100H	9	6 (67%)	2 (22%)
D1101H	14	12 (86%)	4 (29%)
D1104H	14	11 (79%)	3 (21%)
D1105H	11	8 (73%)	4 (36%)
D1106H	12	8 (67%)	2 (17%)
D1107H	7	7 (100%)	3 (43%)
N668C	10	9 (90%)	4 (40%)
N669C	11	10 (91%)	3 (27%)
N670C	11	11 (100%)	4 (36%)
N672C	8	1 (13%)	0 (0%)
N674C	14	9 (64%)	3 (21%)

- **IFNγ ELISpot responses to GAD65 peptides were suppressed by Tregitopes for 78% of peptides tested using diabetic subject (D) PBMC and for 74% of peptides tested with normal controls (N).**
- **Suppression was statistically significant for 26% of assays.**

In Silico Results

Epitope mapping of GAD65 using EpiMatrix



- **EpiMatrix-predicted epitopes (low to high binding potential)**
- **Clusters (low to high predicted immunogenicity)**
- **Published human epitopes**
- **Published NOD epitopes**

- **Predicted, published epitope clustering clearly illustrated for EpiMatrix-predicted epitope clusters 450-470 and 550-570.**

GAD65 450-470 and 550-570 cluster maps

GAD65 Position 450-470

Frame Start	AA Sequence	Frame Stop	DRB1*0101 Z score	DRB1*0301 Z score	DRB1*0401 Z score	DRB1*0701 Z score	DRB1*0801 Z score	DRB1*1101 Z score	DRB1*1301 Z score	DRB1*1501 Z score	Hits
450	VDVFKLWLM	458									0
451	DVFKLWLMW	459									0
452	VFKLWLMWR	460									0
453	FKLWLMWRA	461						2.62	2.61		2
454	KLWLMWRAK	462									0
455	LWLMWRAKG	463	1.82	2.41			2.53	2.83			4
456	WLMWRAKGT	464	2.26		2.01	3.07		1.81			4
457	LMWRAKGTT	465					1.72			1.86	2
458	MWRAKGTTG	466								1.69	1
459	WRAKGTTGF	467	1.67	1.81	2.18	1.71	1.78		1.74		6
460	RAKGTTGFE	468									0
461	AKGTTGFEA	469								1.84	1
462	KGTTGFEAH	470									0
Assessments Performed: 104			Hydrophobicity: 0.03			EpiMatrix Score: 30.4		EpiMatrix Cluster Score (w/o flanks): 33.9			

GAD65 Position 550-570

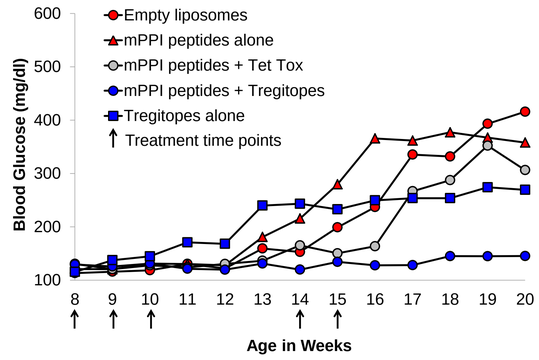
Frame Start	AA Sequence	Frame Stop	DRB1*0101 Z score	DRB1*0301 Z score	DRB1*0401 Z score	DRB1*0701 Z score	DRB1*0801 Z score	DRB1*1101 Z score	DRB1*1301 Z score	DRB1*1501 Z score	Hits
550	LGDKVNFFR	558									0
551	GDKVNFFRM	559									0
552	DKVNFFRMV	560									0
553	KVNFFRMVI	561					1.76			2.24	2
554	VNFFRMVIS	562									0
555	NFFRMVISN	563									0
556	FFRMVISNP	564									0
557	FRMVISNPA	565	2.68	2.32	3.24	2.75	1.91	2.54	1.69	2.97	8
558	RMVISNPAA	566									0
559	MVISNPAAT	567	1.87	1.75							2
560	VISNPAATH	568						1.70			1
561	ISNPAATHQ	569									0
562	SNPAATHQD	570									0
Assessments Performed: 104			Hydrophobicity: -0.23			EpiMatrix Score: 17.87		EpiMatrix Cluster Score (w/o flanks): 22.32			

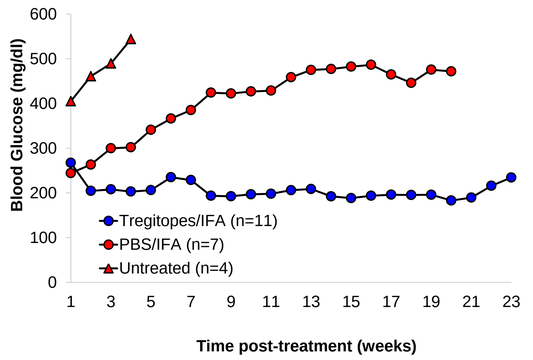
- **Score >1.64 on EpiMatrix 'Z' scale (top 5%) indicates a 'hit', a potential 9-mer epitope.**
- **Score >2.32 (top 1%) indicates extremely likely to bind MHC.**
- **Hits across four or more HLA class II alleles form an EpiBar.**

- **Cluster 450-470 has three EpiBars.**
- **Cluster 550-570 has one EpiBar.**

In Vivo Results

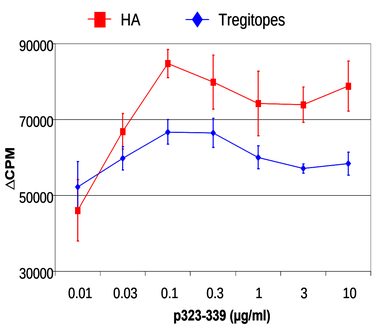
T1D prevention (left) and treatment (right) in NOD mice

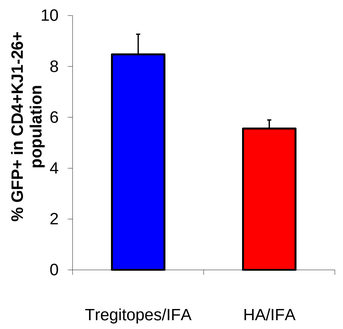




- **Five murine pre-proinsulin (mPPI) peptides and four Tregitopes (mouse 167, 289; human 167, 289) delivered in liposomes completely suppressed T1D.**
- **mTregitopes 167 & 289 (total 20 µg) suppressed disease progression when delivered after T1D onset, demonstrating successful T1D 'cure'.**

Tregitope induction of Ag-specific Treg in DO11.10 mice





- **In DO11.10 FoxP3 transgenic mice, Tregitope therapy suppressed proliferation of OVA-specific T cells as compared to Flu HA control (left).**
- **Tregitope also increased FoxP3+ T cells specific for OVA (KJ126+; right).**

Conclusions

- **T1D prevention: Co-administration of Tregitopes and mPPI peptides prior to T1D onset suppressed diabetes in NOD mice for 15-20 weeks.**
- **T1D treatment: Single dose of mTregitopes 167 & 289 in IFA following T1D onset in NOD mice led to significant reduction in diabetes incidence for >15 weeks.**
- **Co-incubated human Tregitopes with PBMC in vitro suppressed immune responses to epitopes derived from GAD65; suppression correlated with HLA allotype.**
- **Tregitope tolerance induction to islet cell antigens could result in a paradigm shift in disease management and expand immunotherapeutic strategies to treat T1D.**

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