Immunogenicity: It's Personal

Presented by
Annie De Groot
Founder and CSO, EpiVax

Analysis by
Andres Gutierrez
Jacob TIvin
Aimee Mattei
Bill Martin

Collaboration with:
Nanobu Sugiyama Pfizer Japan
Keizo Fujio U tokyo
Sophie Tourdot (Pfizer USA)

In vitro studies by
Sandra Lelias
Brian Roberts
EpiVax's Amazing Lab Team

EpiVax

Outline

EpiVax

- Background
- Individual differences in immunogenicity
- Regional differences in immunogenicity
- HLA (Personal)-restricted evolution of antibody affinity
- Conclusions

Immunogenicity: It's a Problem.
Immunogenicity: It's Personal.

EpiVax

MAbs. 2010 May-Jun;2(3):256-65. doi: 10.4161/mabs.2.3.11641. Epub 2010 May 1.

The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions

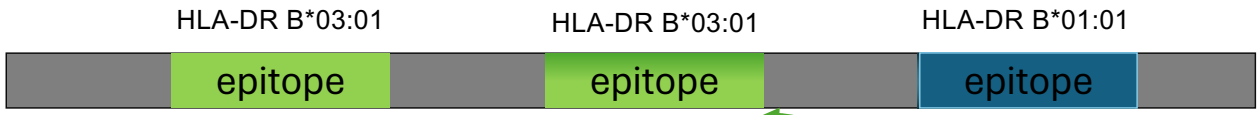
Fiona A Harding [1], Marcia M Stickler, Jennifer Razo, Robert B DuBridge

"Humanization, the replacement of mouse constant regions and V framework regions for human sequences, results in a significantly less immunogenic product. However, some humanized and even fully human sequence-derived antibody molecules still carry immunological risk." (Fiona Harding (PDL/Facet), MAbs, 2010)

Immune Response to Protein Therapeutics
Corr. w/T cell epitopes in biologic sequence & HLA

EpiVax

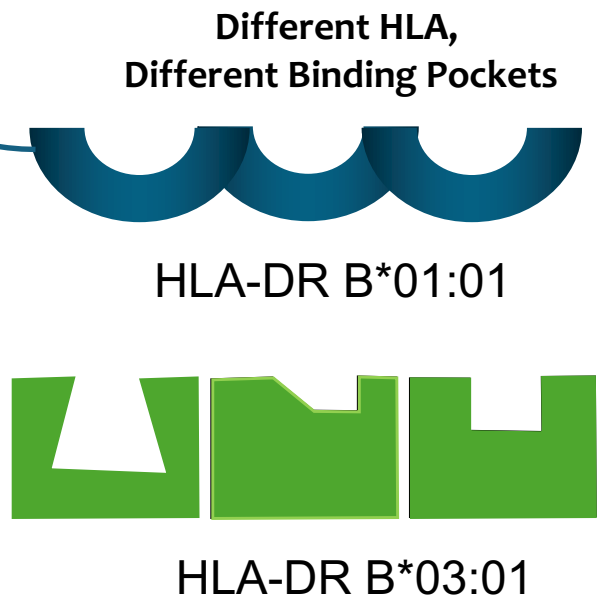
Protein Therapeutic contains sequences that bind to many different HLA:



T cell response depends on:

T cell epitope content + HLA of subject

"Patient Factors" (EIP)



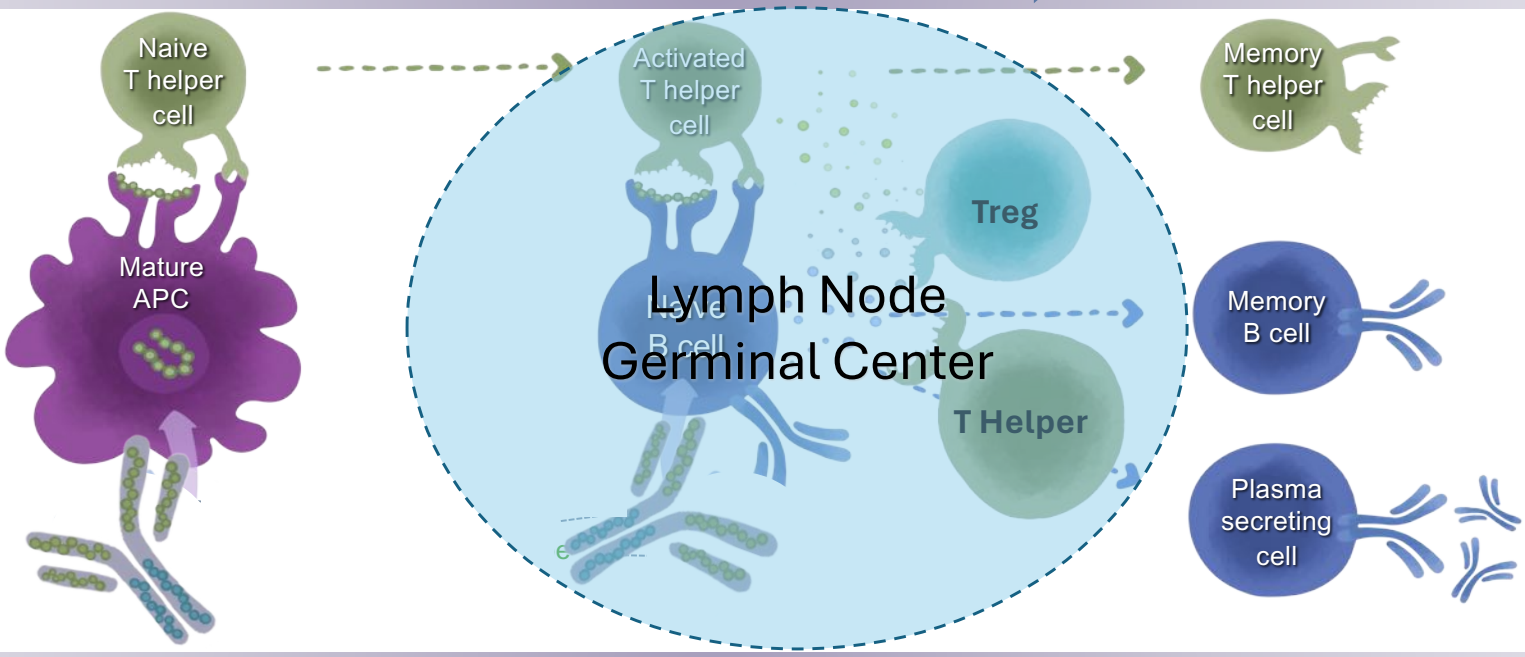
Jawa V et al. T-Cell Dependent Immunogenicity of Protein Therapeutics Pre-clinical Assessment and Mitigation-Updated Consensus and Review 2020. Front Immunol. 2020
and
De Groot A.S. and L. Moise. Prediction of immunogenicity for therapeutic proteins: State of the art. Current Opinions in Drug Development and Discovery. May 2007. 10(3):332-40.

Why T Cells? They help make antibodies in GC

Helper T cells (Follicular Th) drive ADA response

EpiVax

T Cell Activation → LEADS TO → B Cell Activation

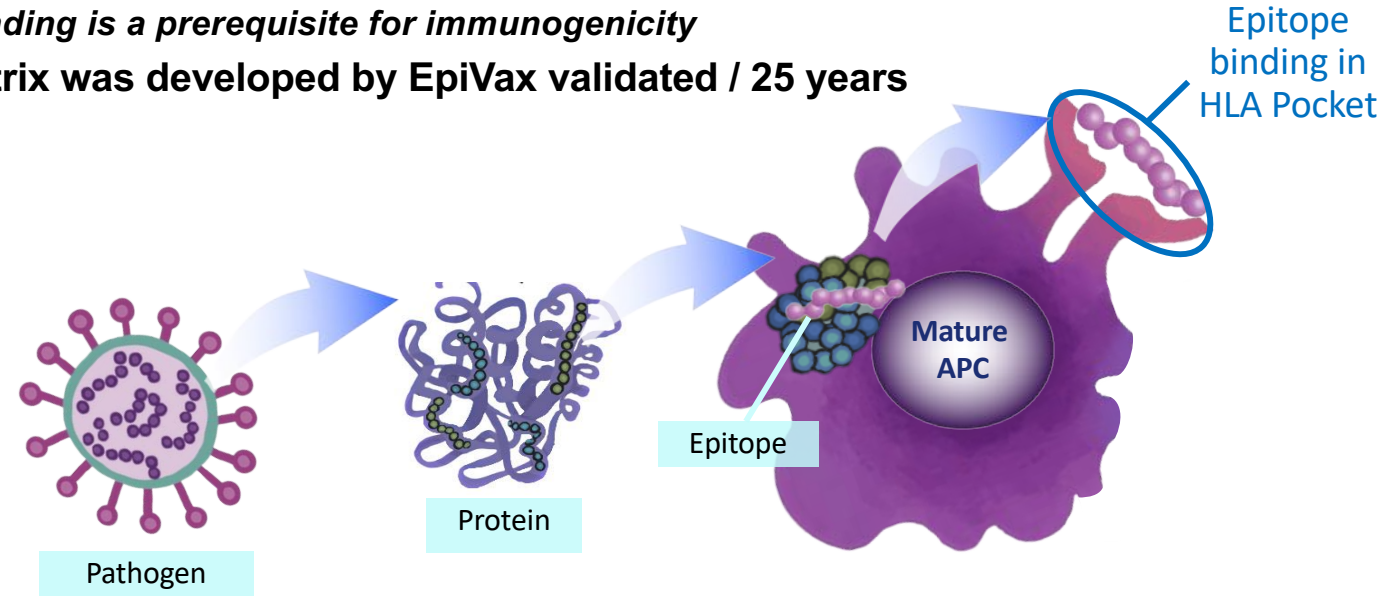


Activation of CD4 T cells and T-dependent antibody response

How computer algorithms identify T cell epitopes by sequence

EpiVax

- **Algorithms like EpiMatrix™ are used to predict T cell epitopes**
  - **Matrix-based algorithm predicting linear sequences that bind to HLA (and MHC)**
- **Can predict class I and class II HLA binding potential**
  - ***HLA binding is a prerequisite for immunogenicity***
- **The EpiMatrix was developed by EpiVax validated / 25 years**

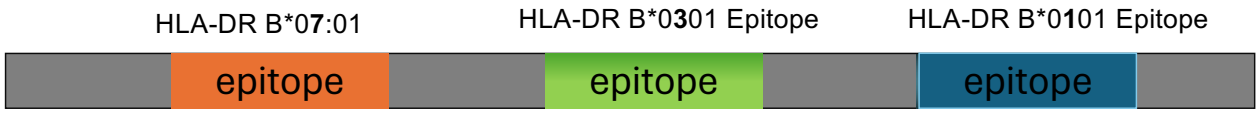


Algorithms consider HLA-specific binding

HLA Restriction of Immune Response (Zinkernagel and Doherty Nobel Prize)

EpiVax

Protein Therapeutic:



For a Global Population
Should we assess HLA DR ligands for
all individuals in population?
That could be complicated....

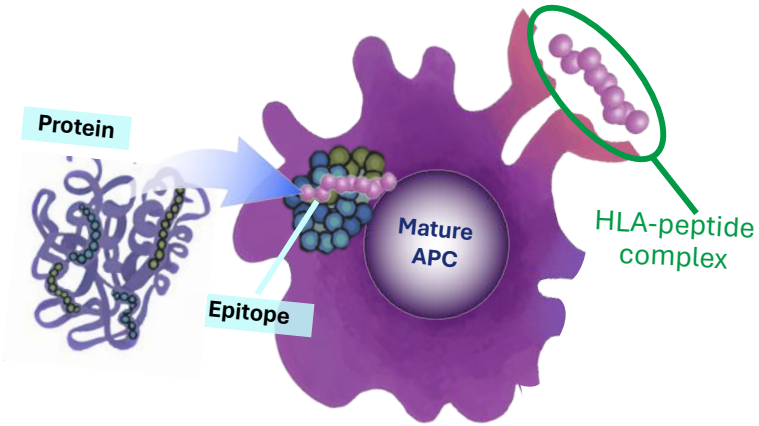
Many different HLA DR in Human Population:
How can we identify ligands for all of these individuals?

EpiVax

Human **L**eukocyte **A**ntigens

DR1*1102 DR1*1321 DR1*1506 DR1*0306 DR1*1307 DR1*1311 A*0201 DR1*0101 DR1*0408 DR1*1323 B*0702 DR1*0308
DR1*1506 DR1*0404 DR1*0102 DR1*0401 A*0301 DR1*0301 DR1*1502 DR1*1128
DR1*0801 DR1*1322 A*2402 DR1*1104 DR1*0813 DR1*0307 DR1*0802 DR1*0102
DR1*0806 DR1*1114 DR1*1328 DR1*1501 DR1*0804 DR1*1107 A*0101 DR1*0423
DR1*0311 DR1*1501 DR1*0309 DR1*0426 DR1*0817 DR1*1301 DR1*1106 DR1*1302 DR1*1121 B*4403 DR1*1327
DR1*1120 DR1*1305

Simplify global HLA binding Preferences to: 9 families for class II and 6 for class I

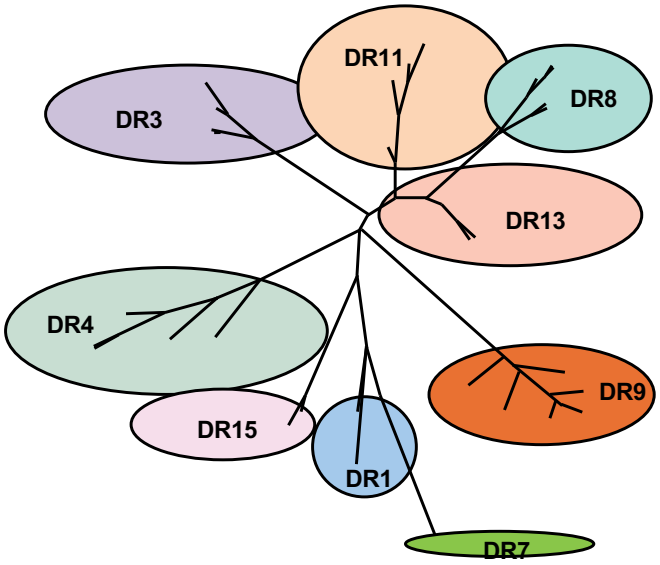


- Class I HLA (endogenous pathway) present epitopes that are 9 or 10 amino acids in length
  - Vaccines
  - Gene Therapy
- Class II HLA (exogenous pathway) bind peptides that are longer, but epitopes are 9 amino acids in length
  - Protein Therapeutics
  - Vaccines
  - Gene Therapy

Supertypes enable Broad HLA "Supertype" Coverage

EpiVax

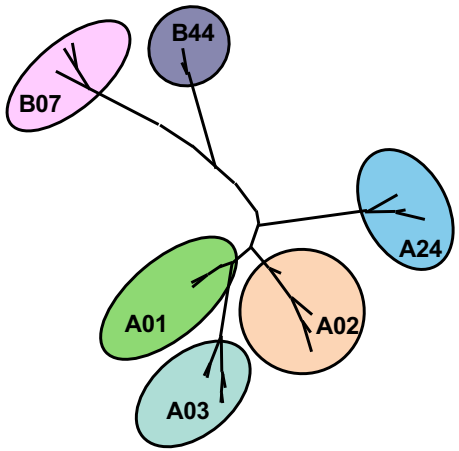
Class II HLA "Supertypes"



EpiVax tests for binding potential to the most common HLA molecules within each of the "supertypes"* shown to the left and right.

This allows us to provide results that are representative of >95% of human populations worldwide without needing to test each haplotype individually.**

Class I HLA "Supertypes"



*Lund et al. Definition of Supertypes for HLA Molecules Using Clustering of Specificity Matrices. Immunogenetics. 2004; 55(12):797–810.
**Southwood et al. Several Common HLA-DR Types Share Largely Overlapping Peptide Binding Repertoires. J Immunol. 1998; 160(7):3363–73.

Using Supertypes, Can Rank Proteins based on T cell epitope content

EpiVax

Total T cell epitope content = Overall immunogenic potential

Immunogenic potential increases with T cell epitope content

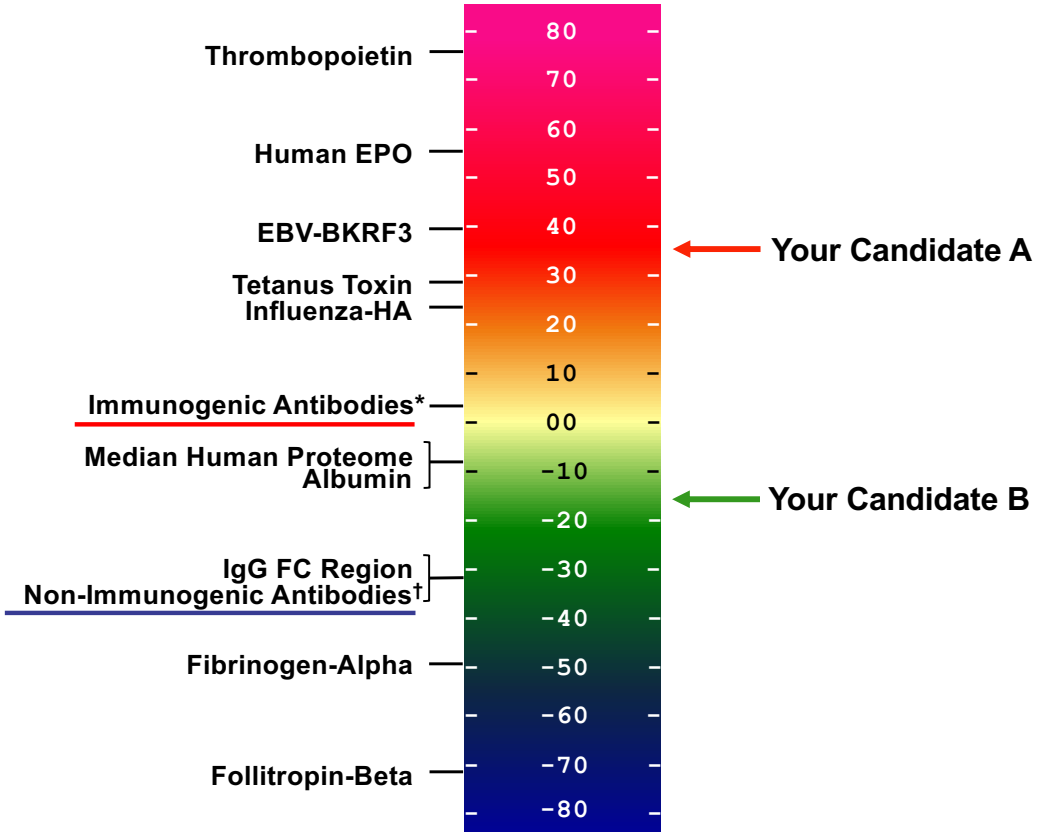
De Groot A.S. and L. Moise. Prediction of immunogenicity for therapeutic proteins: State of the art. Current Opinions in Drug Development and Discovery. May 2007. 10(3):332-40.

Immunogenicity Scale (Normalized)

Class II HLA Protein Immunogenicity Scale

EpiVax

Scale ranks antigens relative to a set of antigens with known immunogenicity

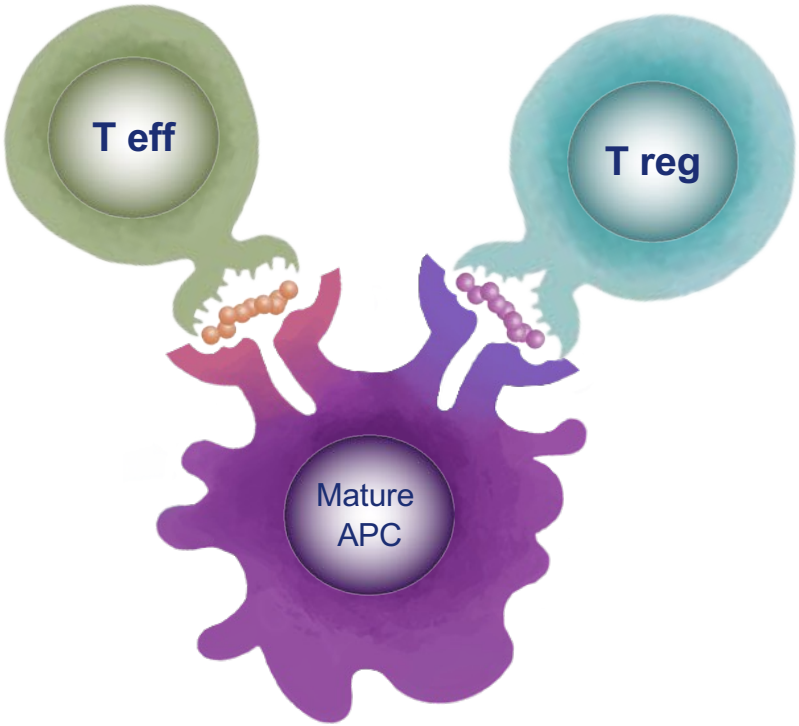


** Average of antibodies known to induce anti-therapeutic responses in more than 5% of patients*

† Average of antibodies known to induce anti-therapeutic responses in less than 5% of patients

But... Some T cell epitopes are not immunogenic
They are tolerated or actively tolerogenic

EpiVax



What epitopes trigger regulatory T cell responses?

TCR face must be relevant!

Relevance to Biologics

- Treg epitopes
  can be identified using in silico tools.
- Relevant to therapeutic *safety* and *efficacy*

How to find Treg epitopes: JanusMatrix
Tool for finding TCR-Cross-conserved ligands

EpiVax

Merci …
Sam Pine, Karen Heyninck (sanofi),
Annette Karle et al. (Novartis).... for revalidation of JanusMatrix

EXPERT REVIEW OF VACCINES, 2016
VOL. 15, NO. 5, 607–617
http://dx.doi.org/10.1586/14760584.2016.1123098

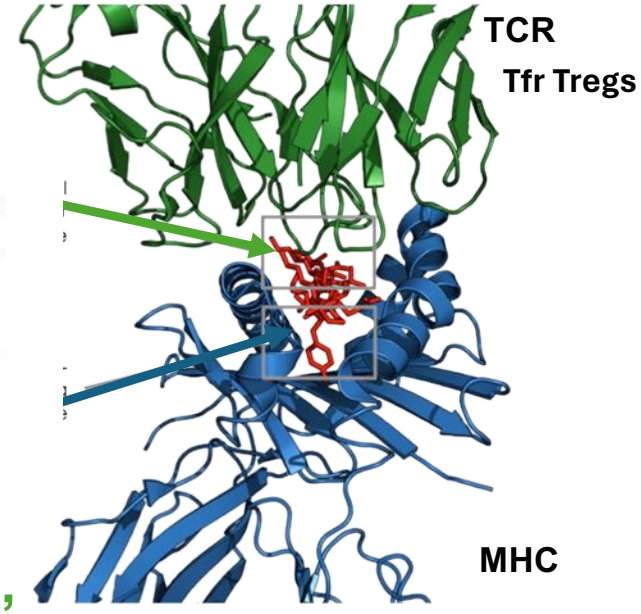
Taylor & Francis
Taylor & Francis Group

REVIEW

T cell epitope redundancy: cross-conservation of the TCR face between pathogens and self and its implications for vaccines and autoimmunity

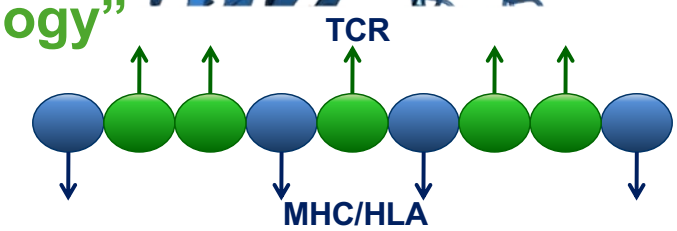
Leonard Moise[a,b], Sarah Beseme[a], Ryan Tassone[b], Rui Liu[b], Farzana Kibria[a], Frances Terry[a], William Martin[a] and Anne S. De Groot [a,b]

[a]EpiVax, Inc., Providence, RI, USA; [b]Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, USA



JanusMatrix – "High Human Homology"

EpiMatrix – HLA binding peptides



EpiMatrix, JanusMatrix, and Immunogenicity Analysis
Now Described in detail in the new publication by Mattei et al.

EpiVax

Immunogenicity Screening and Protein Re-engineering Interface:
Application to Monoclonal Antibodies
https://www.tandfonline.com/doi/full/10.1080/19420862.2024.2333729

New!

In silico methods for immunogenicity risk assessment and human homology screening for therapeutic antibodies

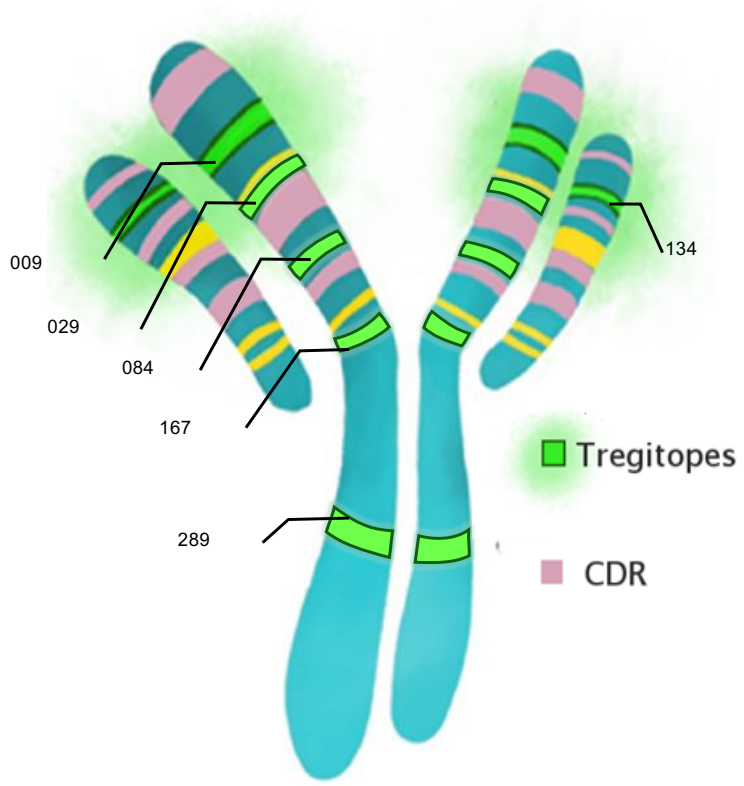
Aimee E. Mattei, Andres H. Gutierrez, Soorya Seshadri, Jacob Tivin, Matt Ardito, Amy S. Rosenberg, ...show all

Article: 2333729 | Received 04 Jan 2024, Accepted 19 Mar 2024, Published online: 27 Mar 2024

Cite this article https://doi.org/10.1080/19420862.2024.2333729 Check for updates

Treg Epitopes in IgG - Tregitopes
Regulatory T cell epitopes are naturally present in IgG

EpiVax

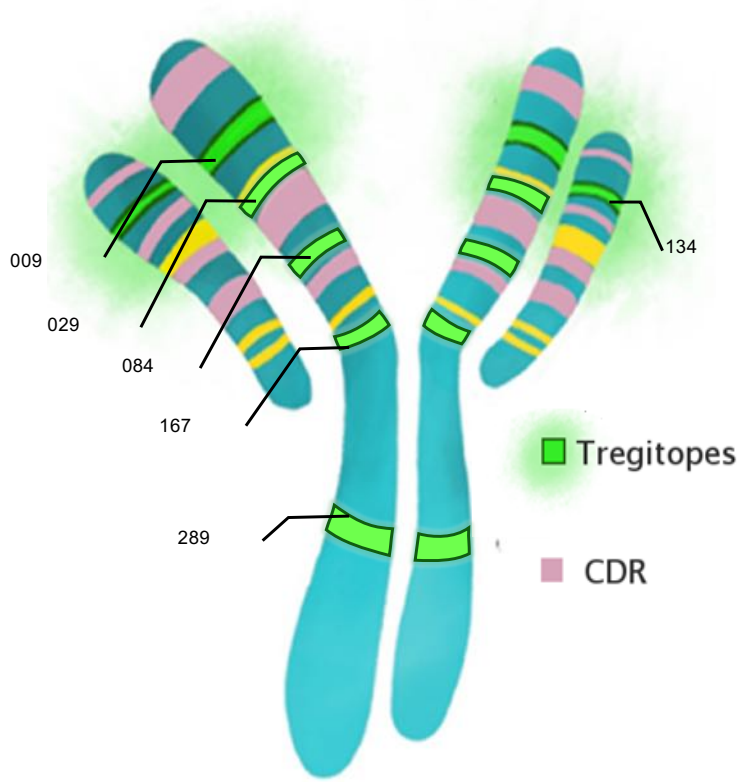


De Groot et al, Blood, 2008

- **15-20 amino acid peptides in conserved IgG regions**
- **Presentation of Tregitopes is HLA-restricted**
- **Tregitopes are presented by multiple HLA**
- **Highly conserved among IgG molecules**
- **Induce natural Tregs to modify immune response**

Tregitope	Sequence
009A	VQPGGSLRLSCAASG
029B	WVRQAPGKGL
084	FTLTISSLQ
088	YLQMNSLRAEDTAVY
134	FYPREAKVQWKVDNALQS
167	LQSSGLYSLSSVVTVPSSSL
289	YNSTYRVVSVLTVLH

Treg Epitopes in IgG - Tregitopes
Regulatory T cell epitopes are naturally present in IgG



De Groot et al, Blood, 2008

- Tregitopes **are among the most common, eluted epitopes** from IgG.
- Tregitopes **are adjacent and overlapping the CDR regions in the VH and VL**.
- **They reduce immune responses to nearby T effector epitopes.**
- **The presence of Tregitopes in monoclonals is inversely associated with ADA.**

167	LQSSGLYSLSSVVTVPSSSL
289	YNSTYRVVSVLTVLH

Tregitopes identified in IgG in 2008

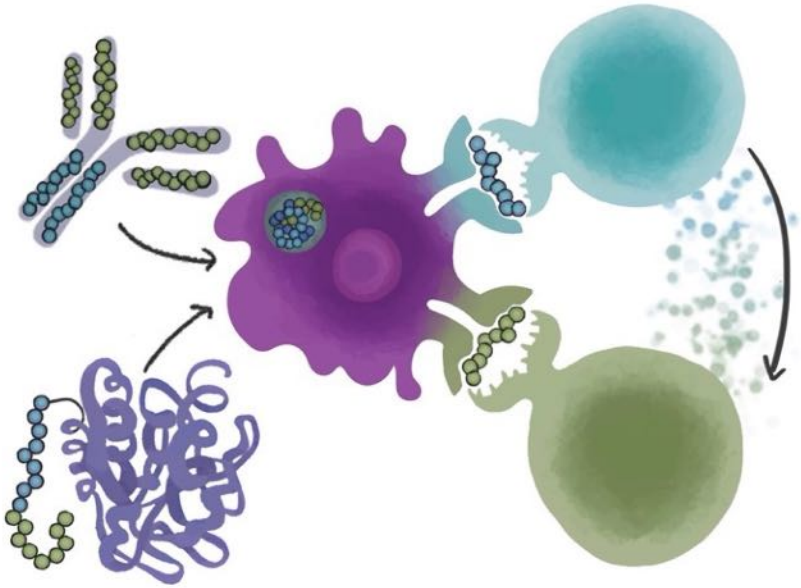
EpiVax

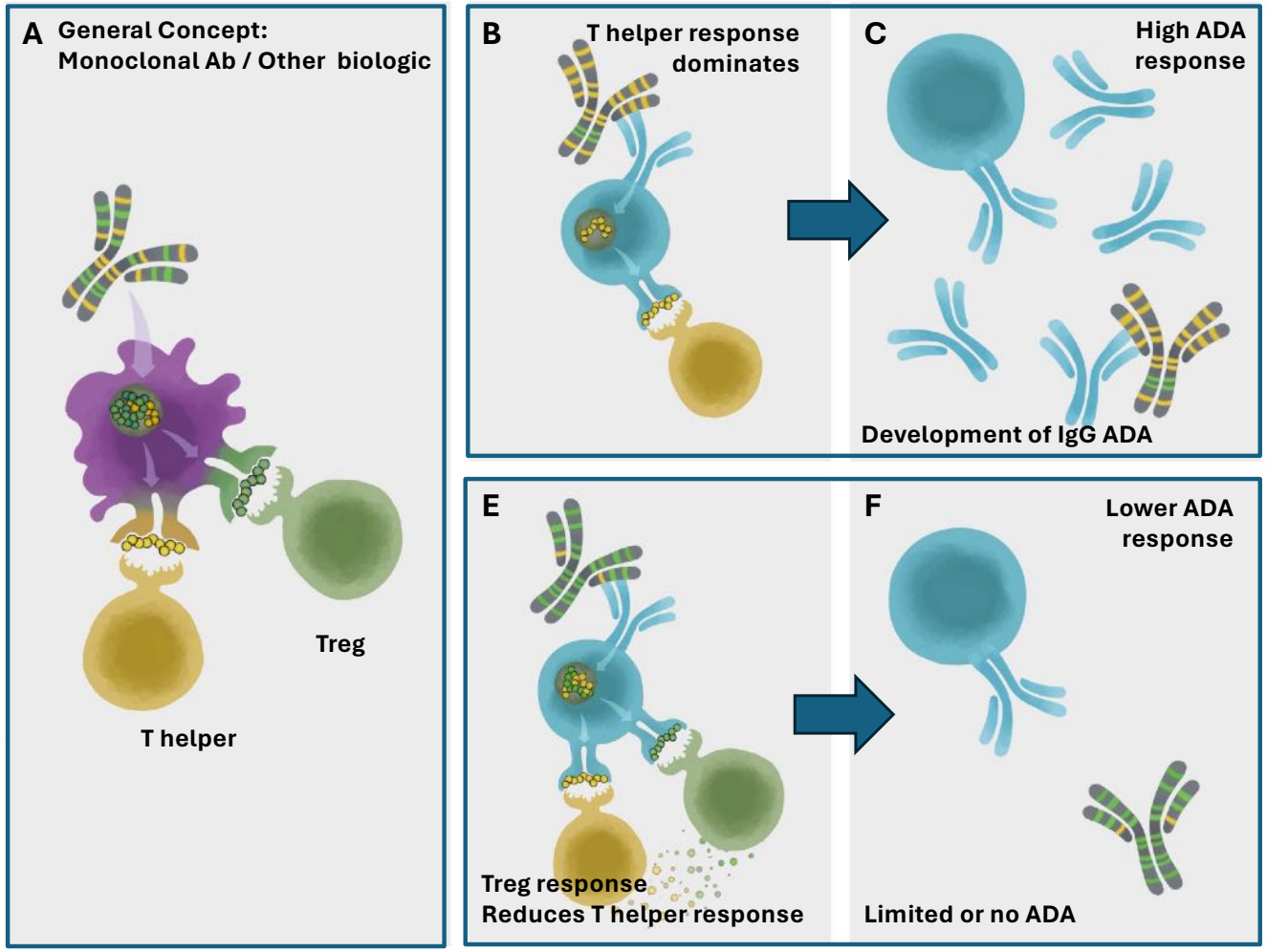
blood

Prepublished online Jul 25, 2008;
doi:10.1182/blood-2008-02-138073

Activation of natural regulatory T cells by IgG Fc-derived Peptide "Tregitopes"

Anne S. De Groot, Leonard Moise, Julie A. McMurry, Erik Wambre, Lawrence Van Overtvelt, Philippe Moingeon, David W. Scott and William Martin



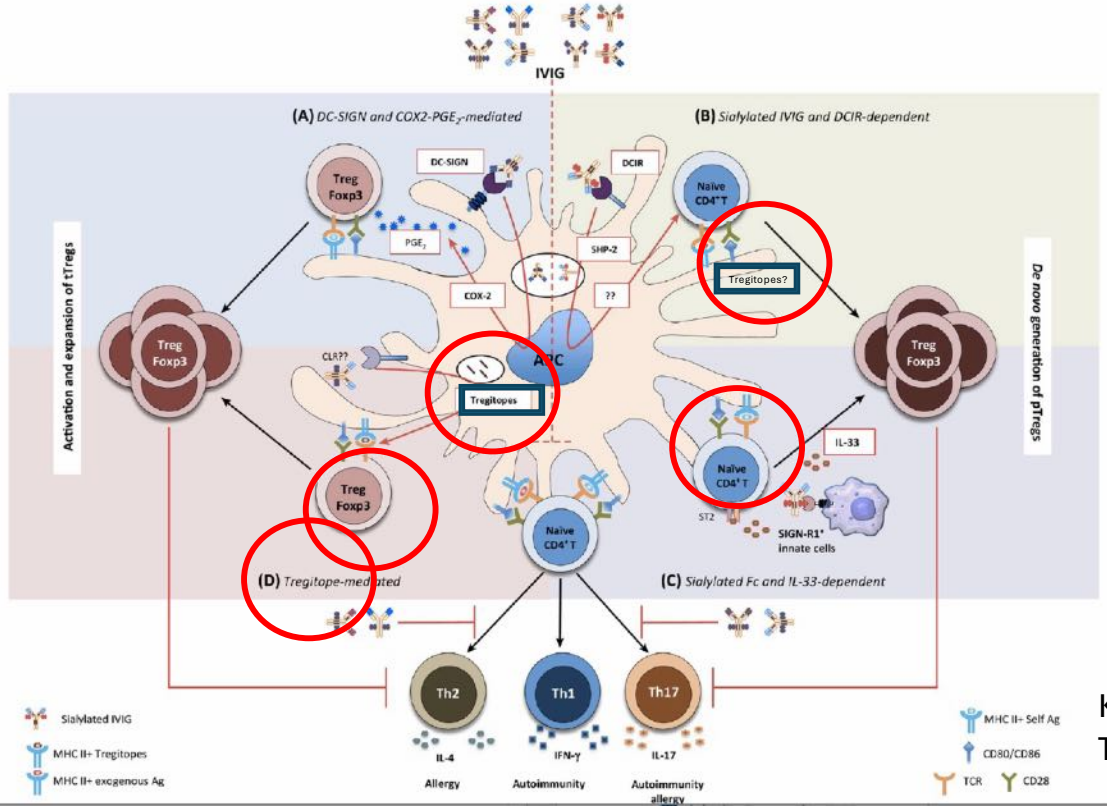


In general, the HLA-restricted T cell epitope content that drives immunogenicity may be moderated by the presence of Treg epitopes (Tregitopes) that are also HLA restricted.

From:
Jawa V et al. T-Cell Dependent Immunogenicity of Protein Therapeutics Pre-clinical Assessment and Mitigation-Updated Consensus and Review 2020. Front Immunol. 2020

Tregitope as (one of the) mechanism of action of IVIG

EpiVax



Kaveri and Bayry,
Trends in Immunology 2019

Tregitopes cause Treg to secrete IL-10
(Fc Tregitopes) re-validated by Franco (UCSD)) in 2015/22

Fc position	Sequence
21 - 35	TAALGCLVKDYFPEP
26 - 40	CLVKDYFPEPVTVSW
31 - 45	YFPEPVTVSWNSGAL
36 - 50	VTVSWNSGALTSGVH
51 - 65	TFPAVLQSSGLYSLS
56 - 70	LQSSGLYSLSSVVTV
61 - 75	LYSLSSVVTVPSSSL
66 - 80	SVVTVPSSSLGTQTY
121 - 135	SVFLFPPKPKDTLMI
126 - 140	PPKPKDTLMISRTPE
181 - 195	TYRVVSVLTVLHQDW
186 - 200	SVLTVLHQDWLNGKE
271 - 285	NNYKTTPPVLDSDGS
276 - 290	TPPVLDSDGSFFLYS
301 - 315	QGNVFSCSVMHEALH
306 -320	SCSVMHEALHNHYTQ

Not HLA-DR or individual HLA-Restricted

Tregitope 167
PAVLQSSGLYSL
SSVVTVPSSSLGTQ

Tregitope 289
EEQYNSTYRVV
SVLTVLHQDW

Not HLA-DR Restricted

Clinical and Experimental Immunology, 2022, **208**, 361–371
https://doi.org/10.1093/cei/uxac046
Advance access publication 10 May 2022
Research Article

Clinical & Experimental IMMUNOLOGY
OXFORD

Research Article

Intravenous immunoglobulin induces IgG internalization by tolerogenic myeloid dendritic cells that secrete IL-10 and expand Fc-specific regulatory T cells

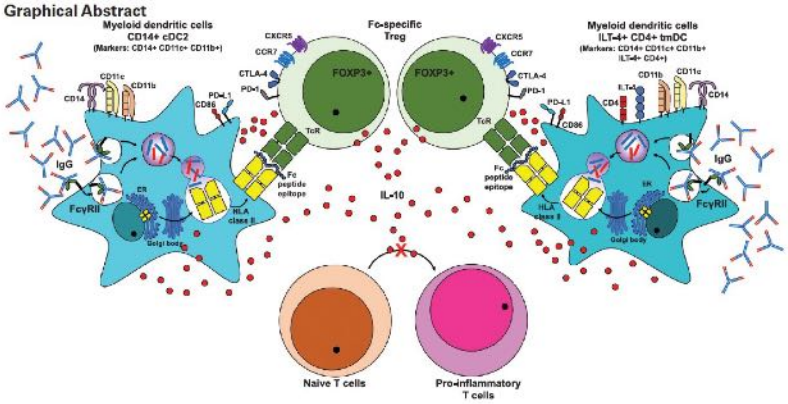
Li-En Hsieh[1], Jaeyoon Song[1], Adriana H. Tremoulet[1,2], Jane C. Burns[1,2], and Alessandra Franco[1,*]

[1]University of California San Diego, School of Medicine, Department of Pediatrics, La Jolla, CA 92093-0641, USA
[2]Rady Children's Hospital, San Diego, CA 92123, USA
*Correspondence: Alessandra Franco, 9500 Gilman Drive, La Jolla, CA 92093-0641, USA. Email: alfranco@health.ucsd.edu

Abstract

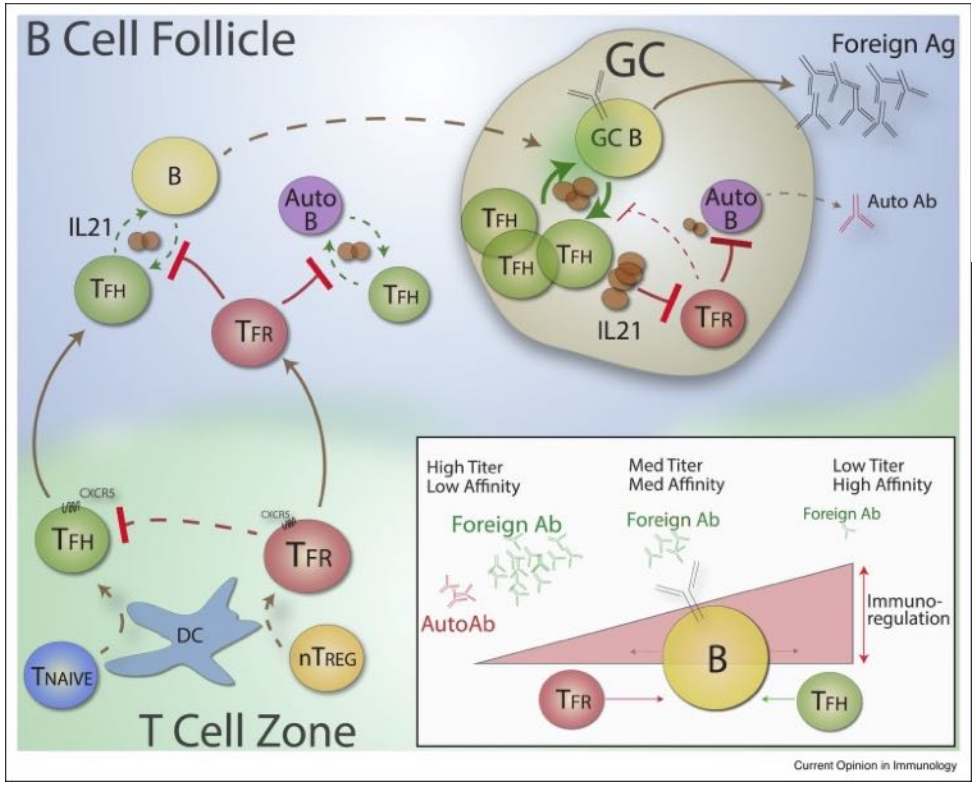
Intravenous immunoglobulin (IVIG) is used as an immunomodulatory agent in many inflammatory conditions including Multisystem Inflammatory Syndrome-Children (MIS-C) and Kawasaki disease (KD). However, the exact mechanisms underlying its anti-inflammatory action are incompletely characterized. Here, we show that in KD, a pediatric acute vasculitis that affects the coronary arteries, IVIG induces a repertoire of natural Treg that recognize immunodominant peptides in the Fc heavy chain constant region. To address which antigen-presenting cell (APC) populations present Fc peptides to Treg, we studied the uptake of IgG by innate cells in subacute KD patients 2 weeks after IVIG and in children 1.6–14 years after KD. Healthy adults served as controls. IgG at high concentrations was internalized predominantly by two myeloid dendritic cell (DC) lineages, CD14+ cDC2 and ILT-4+ CD4+ tmDC mostly through Fcγ receptor (R) II and to a lesser extent FcγRIII. Following IgG internalization, those two DC lineages secreted IL-10 and presented processed Fc peptides to Treg. The validation of IVIG function in expanding Fc-specific Treg presented by CD14+ cDC2 and ILT-4+ CD4+ tmDC was addressed in a small cohort of patients with MIS-C. Taken together, these results suggest a novel immune regulatory function of IgG in activating tolerogenic innate cells and expanding Treg, which reveals an important anti-inflammatory mechanism of action of IVIG.

Graphical Abstract



What do Tregitopes do IRL (in real life)?
They modulate antibody development in the LN

EpiVax



ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

The multifaceted functions of follicular regulatory T cells

Peter T Sage[1] and Arlene H Sharpe[2,3,4]

Studies with the **Tfr-DTR mouse demonstrated that Tfr cells potently regulate antibody responses** (Clement RL et al.).

- **Tfr Tregs broadly "inhibit" B cell response**
- **Lack of Tfr Tregs** leads to **low-affinity antibodies.**
- **Tfr Tregs in the Lymph Node:** may serve to restrain autoantibodies.

Clement et al.
TFR control IgG Maturation / Affinity in GCs

EpiVax

Follicular regulatory T cells control humoral and allergic immunity by restraining early B cell responses

Rachel L. Clement[1], Joe Daccache[1], Mostafa T. Mohammed[1], Alos Diallo[2], Bruce R. Blazar[3], Vijay K. Kuchroo[4,5,6], Scott B. Lovitch[7], Arlene H. Sharpe[2,4,7], Peter T. Sage[1,*]

[1]Transplantation Research Center, Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, 02115

[2]Department of Immunology, Harvard Medical School, Boston, MA, 02115

[3]Department of Pediatrics, Division of Blood and Marrow Transplantation, University of Minnesota Minneapolis, MN, 55455

[4]Evergrande Center for Immunologic Diseases, Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115

[5]Broad Institute, Cambridge, MA 02142

[6]Ann Romney Center for Neurologic Diseases, Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115

[7]Department of Pathology, Brigham and Women's Hospital, Boston, MA, 02115

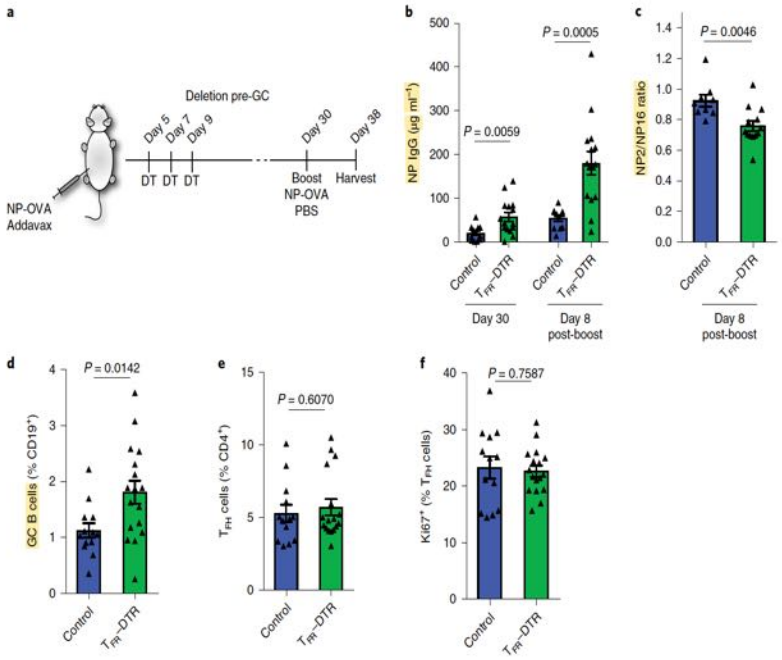


Fig. 4 | T_{FR} cells regulate antibody memory responses. a, Schematic of T_{FR} cell deletion to assess memory responses. T_{FR}-DTR or control mice were immunized with NP-OVA in Addavax and DT was given on days 5, 7 and 9 to delete T_{FR} cells before GC formation. Mice received a boost of NP-OVA without adjuvant at day 30. Mice were harvested on day 38. **b**, Analysis of NP-specific IgG levels before and after NP-OVA boost as in **a**. **c**, Quantification of the NP2/NP16 ratio in experiments as in **a**. **d**, Quantification of GC B cells (gated as CD19⁺GL7⁺FAS⁺) from dLNs of mice at day 38 as in **a**. **e**, Quantification of T_{FH} (gated as CD4⁺ICOS⁺CXCR5⁺FoxP3⁻CD19⁻) cells at day 38 as in **a**. **f**, Quantification of Ki67 expression in T_{FH} cells gated as in **e**. Column graphs represent the mean with error bars indicating s.e.m. The *P* value indicates a two-tailed Student's *t*-test. Data represent combined data from three independent experiments.

Confirmation: IgG-derived Treg epitopes (Tregitopes) Activate nTregs and suppress B cell response (Sette)

EpiVax

IgG Epitopes Processed and Presented by IgG$^+$ B Cells Induce Suppression by Human Thymic-Derived Regulatory T Cells

Li-En Hsieh,* John Sidney,† Jane C. Burns,* David L. Boyle,‡ Gary S. Firestein,‡ Yoav Altman,§ Alessandro Sette,† and Alessandra Franco*

We described a human regulatory T cell (Treg) population activated by IgG$^+$ B cells presenting peptides of the heavy C region (Fc) via processing of the surface IgG underlying a model for B cell–Treg cooperation in the human immune regulation. Functionally, Treg inhibited the polarization of naive T cells toward a proinflammatory phenotype in both a cognate and a noncognate fashion. Their fine specificities were similar in healthy donors and patients with rheumatoid arthritis, a systemic autoimmune disease. Four immunodominant Fc peptides bound multiple HLA class II alleles and were recognized by most subjects in the two cohorts. The presentation of Fc peptides that stimulate Treg through the processing of IgG by dendritic cells (DC) occurred in myeloid DC classical DC 1 and classical DC 2. Different routes of Ag processing of the IgG impacted Treg expansion in rheumatoid arthritis patients. *The Journal of Immunology*, 2021, 206: 1194–1203.

***Abstract**: The activation of natural regulatory T cells (nTreg) recognizing the heavy constant region (Fc) of IgG is an important mechanism of action of intravenous immunoglobulin (IVIG) therapy in Kawasaki disease (KD). Lack of circulating Fc-specific nTreg in the sub-acute phase of KD is correlated with the development of coronary artery abnormalities (CAA). Here, we characterize the fine specificity of nTreg in sub-acute (2- to 8-week post-IVIG) and convalescent (1- to 10-year post-IVIG) KD subjects by testing the immunogenicity of 64 peptides, 15 amino acids in length with a 10 amino acid-overlap spanning the entire Fc protein. About 12 Fc peptides (6 pools of 2 consecutive peptides) were recognized by nTreg in the cohorts studied, including two patients with CAA. To test whether IVIG expands the same nTreg populations that maintain vascular homeostasis in healthy subjects, we compared these results with results obtained in healthy adult controls. Similar nTreg fine specificities were observed in KD patients after IVIG and in healthy donors. These results suggest that T cell fitness rather than T cell clonal deletion or anergy is responsible for the lack of Fc-specific nTreg in KD patients who develop CAA. Furthermore, we found that adolescents and adults who had KD during childhood without developing CAA did not respond to the Fc protein in vitro, suggesting that the nTreg response induced by IVIG in KD patients is short-lived. Our results support the concept that peptide epitopes may be a viable therapeutic approach to expand Fc-specific nTreg and more effectively prevent CAA in KD patients.*

IgG-derived FC epitopes expand Tregs and suppress B cells

Franco/Sette peptides overlap with Tregitopes 167 and 289

EpiVax

Fc position	Sequence
21 - 35	TAALGCLVKDYFPEP
26 - 40	CLVKDYFPEPVTVSW
31 - 45	YFPEPVTVSWNSGAL
36 - 50	VTVSWNSGALTSGVH
51 - 65	TFPAVLQSSGLYSLS
56 - 70	LQSSGLYSLSSVVTV
61 - 75	LYSLSSVVTVPSSSL
66 - 80	SVVTVPSSSLGTQTY
121 - 135	SVFLFPPKPKDTLMI
126 - 140	PPKPKDTLMISRTPE
181 - 195	TYRVVSVLTVLHQDW
186 - 200	SVLTVLHQDWLNGKE
271 - 285	NNYKTTPPVLDSDGS
276 - 290	TPPVLDSDGSFFLYS
301 - 315	QGNVFSCSVMHEALH
306 -320	SCSVMHEALHNHYTQ

IgG1 Fc peptides tested for Treg recognition

IgG Epitopes Processed and Presented by IgG$^+$ B Cells Induce Suppression by Human Thymic-Derived Regulatory T Cells

Li-En Hsieh,* John Sidney,† Jane C. Burns,* David L. Boyle,‡ Gary S. Firestein,‡ Yoav Altman,§ Alessandro Sette,† and Alessandra Franco*

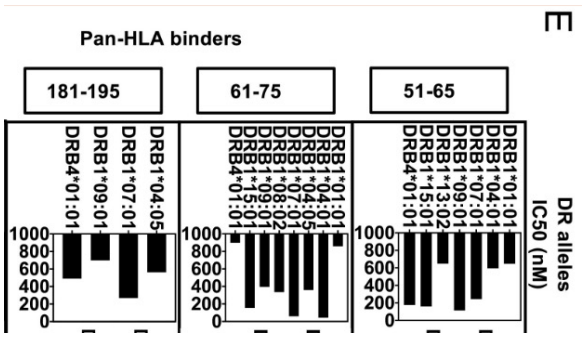
We described a human regulatory T cell (Treg) population activated by IgG$^+$ B cells presenting peptides of the heavy C region (Fc) via processing of the surface IgG underlying a model for B cell–Treg cooperation in the human immune regulation. Functionally, Treg inhibited the polarization of naive T cells toward a proinflammatory phenotype in both a cognate and a noncognate fashion. Their fine specificities were similar in healthy donors and patients with rheumatoid arthritis, a systemic autoimmune disease. Four immunodominant Fc peptides bound multiple HLA class II alleles and were recognized by most subjects in the two cohorts. The presentation of Fc peptides that stimulate Treg through the processing of IgG by dendritic cells (DC) occurred in myeloid DC classical DC 1 and classical DC 2. Different routes of Ag processing of the IgG impacted Treg expansion in rheumatoid arthritis patients. *The Journal of Immunology*, 2021, 206: 1194–1203.

Tregitope 167
PAVLQSSGLYSL
SSVVTVPSSSLGTQ

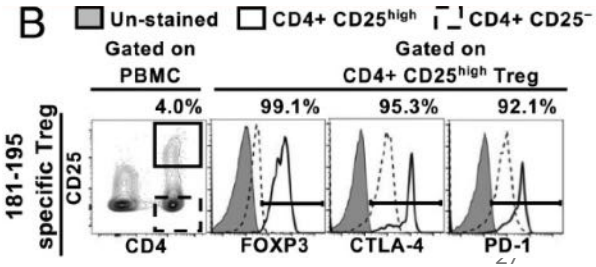
Tregitope 289
EEQYNSTYRVV
SVLTVLHQDW

Not HLA-DR or individual HLA-Restricted

Franco/Sette "Pan-HLA binders" Fig 1E

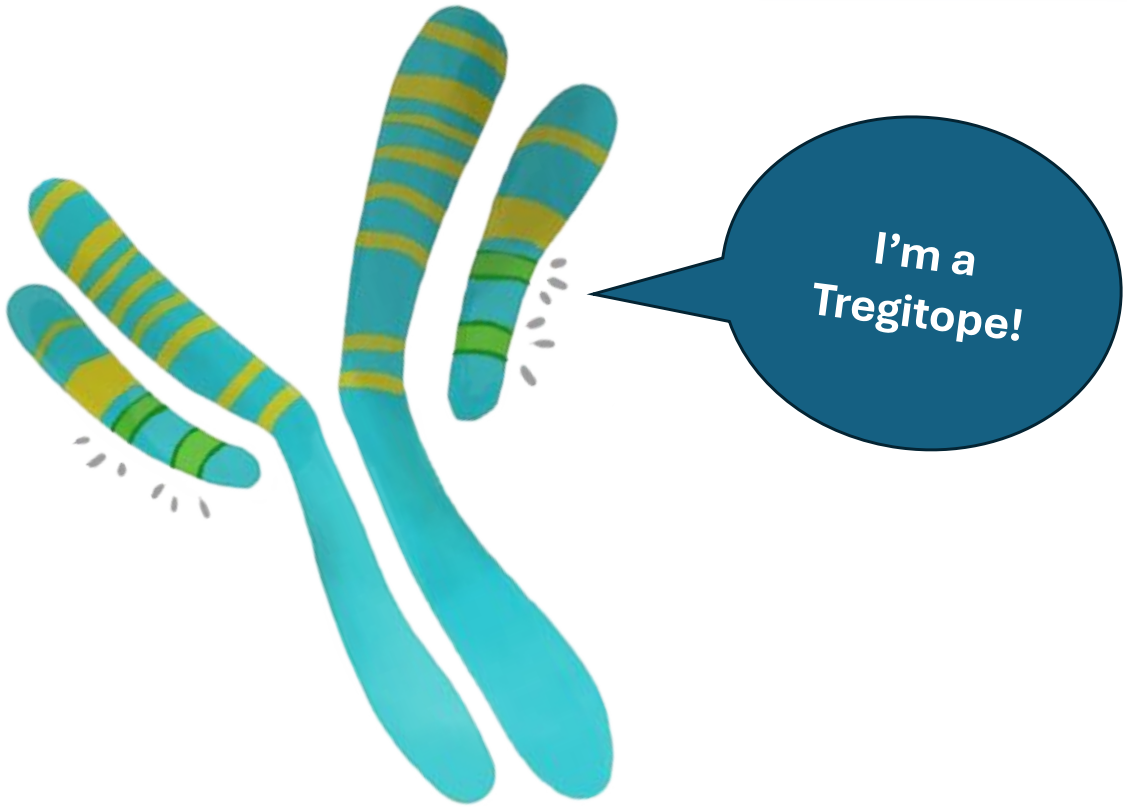


Franco/Sette "Expand Treg in vitro" Fig 1B



Now that we're friends, you can call me by my nickname!

EpiVax



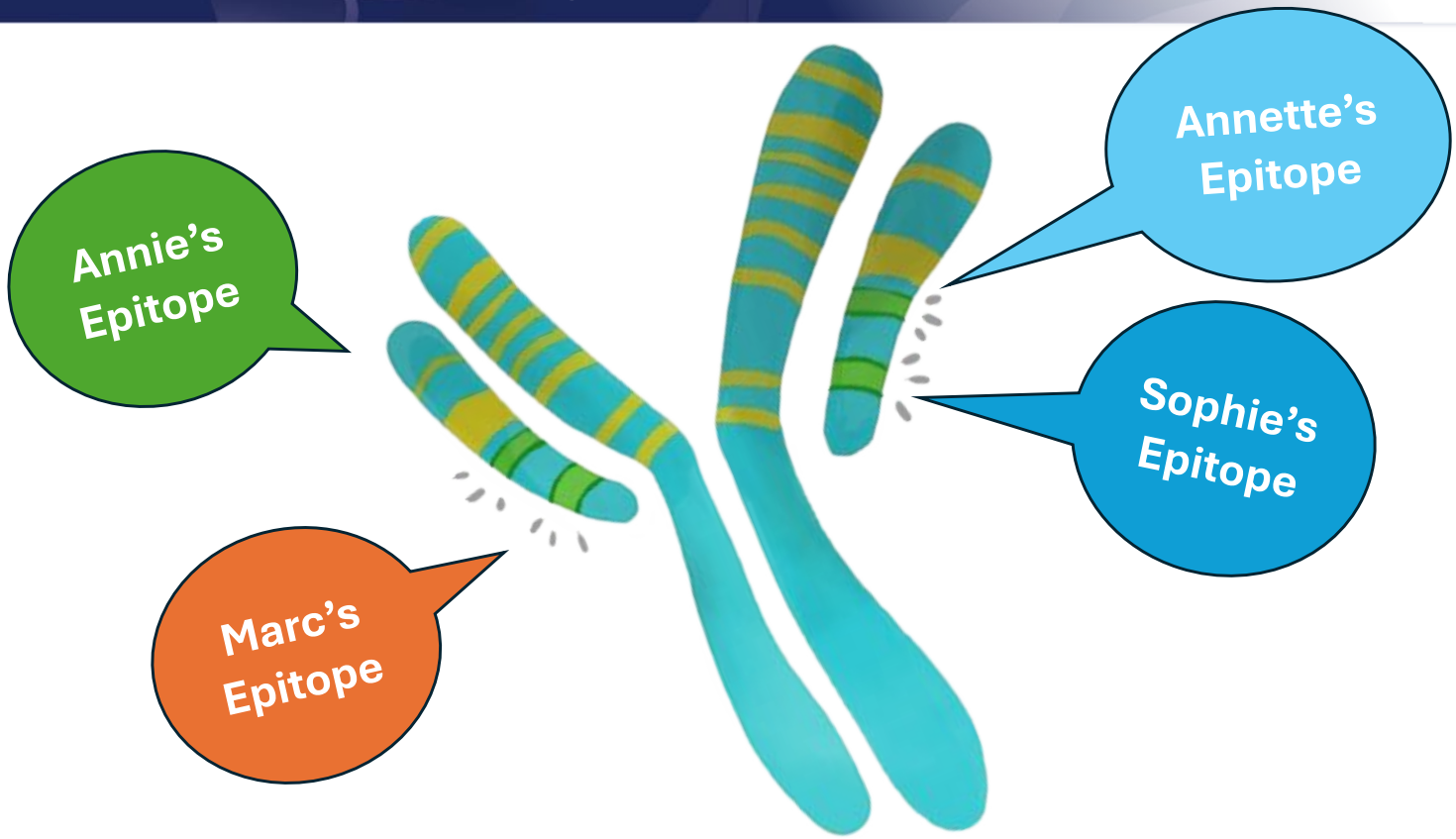
Outline

EpiVax

- Background **Enough Already!**
- **Individual differences in immunogenicity**
- Regional differences in immunogenicity
- HLA-restricted evolution of antibody affinity
- Conclusions

Can we predict individual responses based on HLA-restriction of Treg and Teff epitopes?

EpiVax



Individualized T cell Epitope Measure (iTEM)
Can be automated (website developed for Pompe)

EpiVax

Immunogenicity is

HLA Restricted

DRB1*0101 is predicted

to present this peptide more effectively

than **DRB1*1501**

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
								0
								0
								0
2.69	1.91	1.96	1.57		1.66	2.07	1.65	6
		1.77		1.58				1
2.15	1.8	2.14	2.19	1.77	1.72	1.75	1.61	7
								0
								0
								0
DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501	Total
2.69	1.91	2.14	2.19	1.77	1.72	2.07	1.65	--
4.84	3.71	5.87	2.19	1.77	3.38	3.82	1.65	27.23

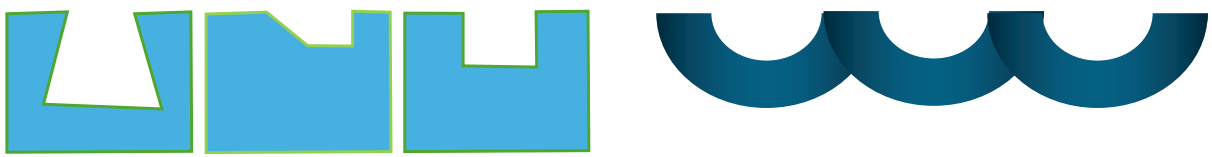
Different Immune Response Expected

HLA Restricts also Response to Treg epitopes: Do individuals respond differently?

EpiVax

Treg epitopes can also be HLA-restricted:

Different HLA,
Different Immune Response



HLA-DR B*0301 HLA-DR B*0101

iTEM (Individual T cell Epitope Measure) with JMX
"J-ITEM" Heat Map by HLA DR allele haplotype/Treg epitopes

EpiVax

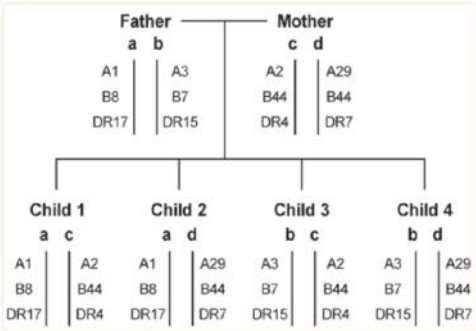
"iTEM" Heat map of HLA DR-specific T cell responses

Moving from global immune response … to individual response

iTEM/J-iTEM scores ranging from **-62.99** to **8.63**

iTEM score **low** **or** **high** immunogenic potential

Sequence: Your Protein



	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501
HLA freq.*	10.4	11.9	24.4	14	15.1	15.5	15.1	20.5
DRB1*0101		--	--	--	--	--	--	--
DRB1*0301			--	--	--	--	--	--
DRB1*0401				--	--	--		--
DRB1*0701					--			
DRB1*0801						--		--
DRB1*1101								--
DRB1*1301								--
DRB1*1501								

Strong

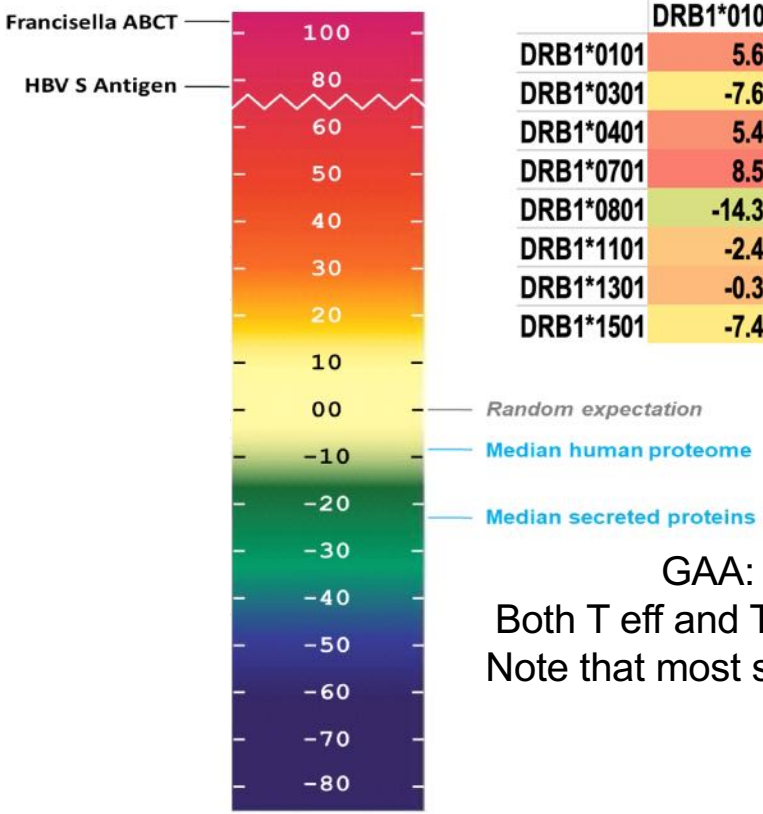
Weak

Strength of iTEM score: Low Medium High

* Southwood et al., J. Immunol. 1998;160;3363-3373

iTEM I(Individualized Immunogenicity Risk) Report for GAA – by HLA DR allele

EpiVax



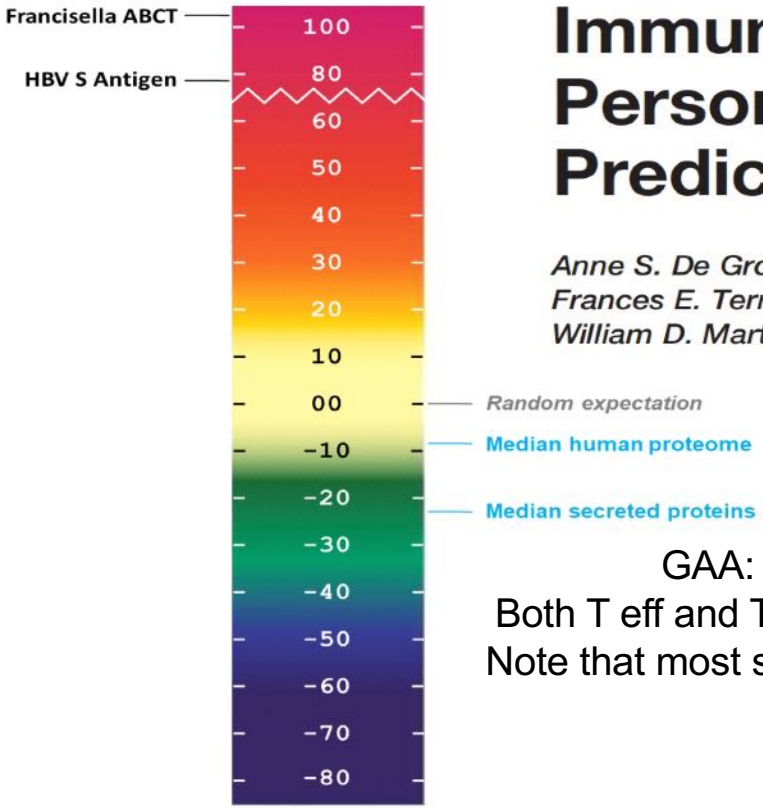
	DRB1*0101	DRB1*0301	DRB1*0401	DR		B1*1101	DRB1*1301	DRB1*1501
DRB1*0101	5.64	--	--	--			--	--
DRB1*0301	-7.62	-20.88	--	--		--	--	--
DRB1*0401	5.48	-7.78	5.32	--	--	--	--	--
DRB1*0701	8.52	-4.74	8.36	11.41	--	--	--	--
DRB1*0801	-14.31	-27.57	-14.47	-11.43	-34.26	--	--	--
DRB1*1101	-2.46	-15.72	-2.62	0.42	-22.41	-10.56	--	--
DRB1*1301	-0.36	-13.62	-0.51	2.53	-20.3	-8.45	-6.35	--
DRB1*1501	-7.48	-20.73	-7.63	-4.59	-27.42	-15.57	-13.47	-20.59

High iTEM
High Risk

GAA: Look for immune tolerance to tolerogenic epitopes
Both T eff and Treg are relevant to ADA development and can be predicted.
Note that most scores are above **negative 23**, the median for Hu Secretome

iTEM I(Individualized Immunogenicity Risk) Report for GAA – by HLA DR allele

EpiVax



Immune Tolerance-Adjusted Personalized Immunogenicity Prediction for Pompe Disease

Anne S. De Groot[1,2], Ankit K. Desai[3], Sandra Lelias[1], S. M. Shahjahan Miah[1], Frances E. Terry[1], Sundos Khan[1], Cindy Li[3], John S. Yi[4], Matt Ardito[1], William D. Martin[1] and Priya S. Kishnani[3]*

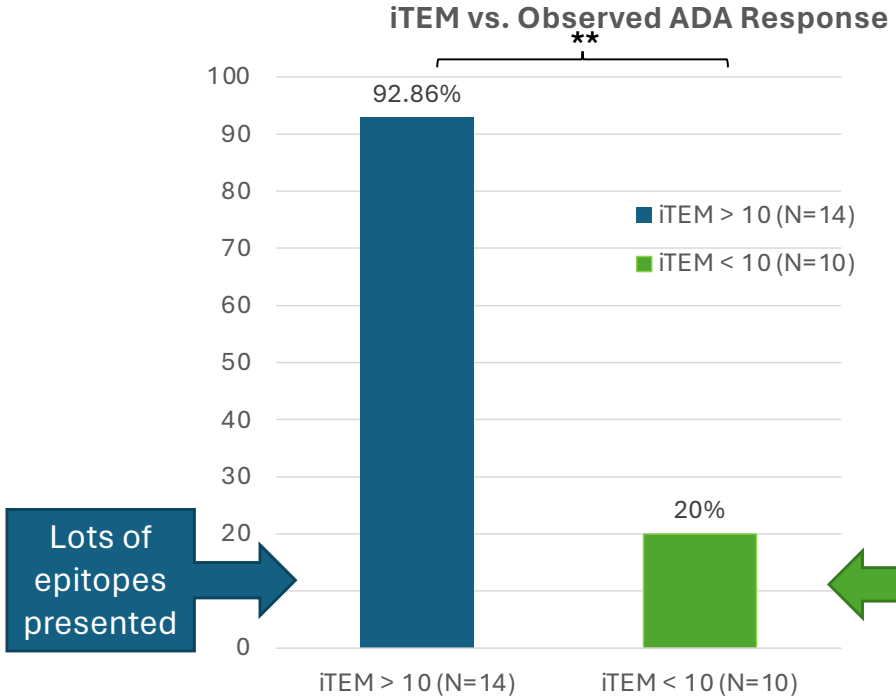
GAA: Look for immune tolerance to tolerogenic epitopes

Both T eff and Treg are relevant to ADA development and can be predicted.

Note that most scores are above **negative 23**, the median for Hu Secretome

Results of iTEM Analysis for Pompe Patients

Complete Cohort – CRIM-Positive & CRIM-Negative



Lots of epitopes presented

Few epitopes presented

For All Patients (n=24)	
Odds Ratio (Diff iTEM >10 vs Diff iTEM <10)	52

Odds of developing high ADA are 52 times higher in iTEM>10 patients compared to those with iTEM<10

p value = 0.0005
(Fisher's exact test)

High iTEM High Risk

De Groot et al. 2019

Also relevant to T cell assays in vitro

Does presence of Treg epitopes modulate response?

EpiVax

Characterization of CD4 T Cell Epitopes of Infliximab and Rituximab Identified from Healthy Donors

*Moustafa Hamze[1], Sylvain Meunier[1], Anette Karle[2], Abdelaziz Gdoura[1], Amélie Goudet[1], Natacha Szely[3], Marc Pallardy[3], Franck Carbonnel[4], Sebastian Spindeldreher[2], Xavier Mariette[5], Corinne Miceli-Richard[5] and Bernard Maillère[1]**

[1]CEA-Saclay, Institut de Biologie et Technologies, Université Paris-Saclay, Gif sur Yvette, France, [2]Novartis Pharma AG, Basel, Switzerland, [3]INSERM UMR 996, Faculté de Pharmacie, Université Paris-Sud, Chatenay Malabry, France, [4]Service de gastro-entérologie, Hôpitaux Universitaires Paris-Sud, Le Kremlin-Bicêtre, France, [5]INSERM UMR 1184, Assistance Publique-Hôpitaux de Paris, Service de Rhumatologie, Hôpitaux Universitaires Paris-Sud, Université Paris-Sud, Le Kremlin-Bicêtre, France

Hamze 2017 RTX/IFX Personalized HLA Analysis (iTEM) IFNg ELISpot - JanusMatrix Predicts Outcomes

EpiVax

Population level threshold for peptide immunogenicity

Considering responding donor HLA, we can explain 5 of 9 positive responses at a strict EpiMatrix threshold of 5%, and 8 of 9 at a more relaxed threshold of 10%.

Rituximab	TP	FP	FN	TN	Accuracy	Odds Ratio	Fisher's Exact (2 tailed)
EpiMatrix Cluster Score≥10	2	3	7	33	78%	>1	0.57
Accounting for High human cross-conservation	2	2	7	34			
Considering patient HLA (5%)	5	2	4	34			
Considering patient HLA (10%)	8	2	1	34	93%	>1	P<0.01

Using JanusMatrix Algorithm, adjust for human cross-conservation (tolerated epitopes) and improve True Negative count

Infliximab	TP	FP	FN	TN	Accuracy	Odds Ratio	Fisher's Exact (2 tailed)
EpiMatrix Cluster Score≥10	3	1	6	36	85%	>1	0.02*
Accounting for High human cross-conservation	3	0	6	37			
Considering patient HLA (5%)	8	0	1	37			
Considering patient HLA (10%)	8	0	1	37	98%	>1	P<0.01

Most IFX positive responses were explained by donor HLA at EpiMatrix standard threshold. JanusMatrix reclassified one FP to TN.

Individualized Immunogenicity Risk (iTEM)

EpiVax

Post hoc T cell assay Analysis (Hamze et al.) Conclusion

- **With EpiMatrix /JanusMatrix analysis**, overall, correlation with T cell response ranges from **93% to 98%*** for Rituximab and Infliximab, respectively.

- False Positive and False Negative correlations are due to **HLA-specificity**; **post-hoc evaluation accounting for HLA restrictions** in the results improves correlations as can be expected.

Take Aways from "Individual" studies:

- **Tolerated or ... Treg epitopes** appear to modulate immune responses as measured in in vitro assays (Hamze, others) and in vivo (Pompe).

- **(Personal) HLA-restricted immune response to T effector and T reg epitopes drives overall immunogenicity.**

Outline

EpiVax

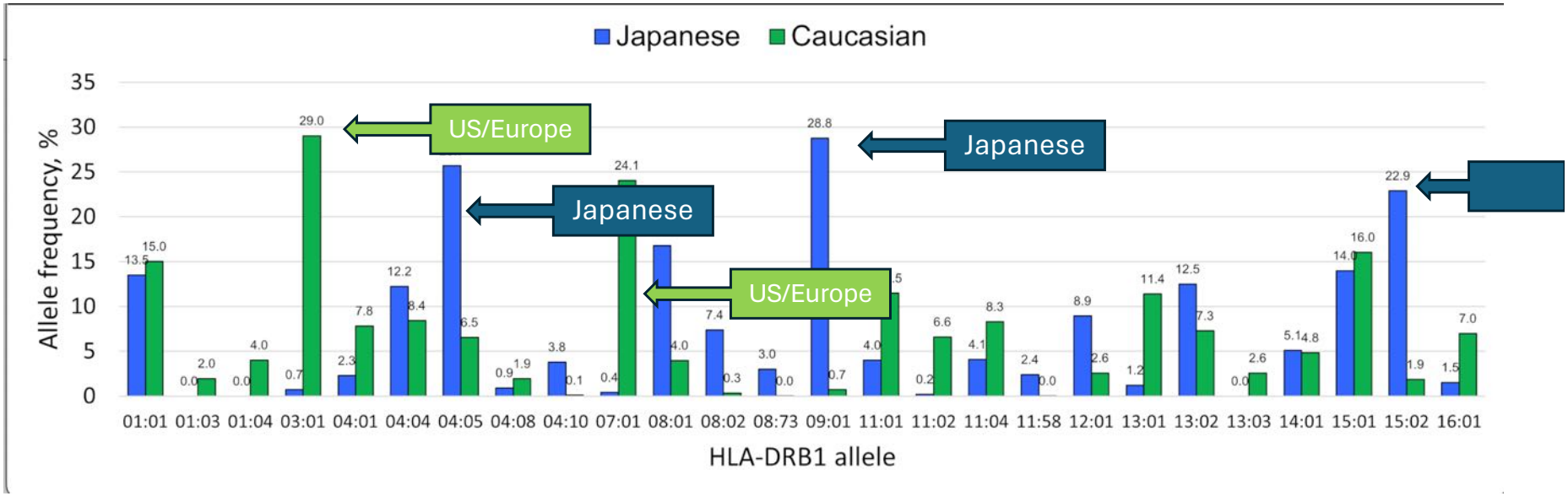
- Background
- Individual differences in immunogenicity **in vivo and in vitro!**
- **Regional differences in immunogenicity**
- HLA-restricted evolution of antibody affinity
- Conclusions

If individual HLA prevalence differs in regions of the world, Does ADA risk vary by regional location?

EpiVax

Hypothesis: Regional HLA DR Differences impact immunogenicity in Subject Populations

Since ADA responses can interfere with the efficacy of the DMARDS, and HLA-DR-restricted epitopes are the root cause of the ADA, we hypothesize that population-specific HLA-DR distributions may help to explain observed differences in immunogenicity between global patient groups.



Accepted for Publication (Frontiers)!

Individual and population-level variability in HLA-DR associated immunogenicity risk of biologics used for the treatment of Rheumatoid Arthritis

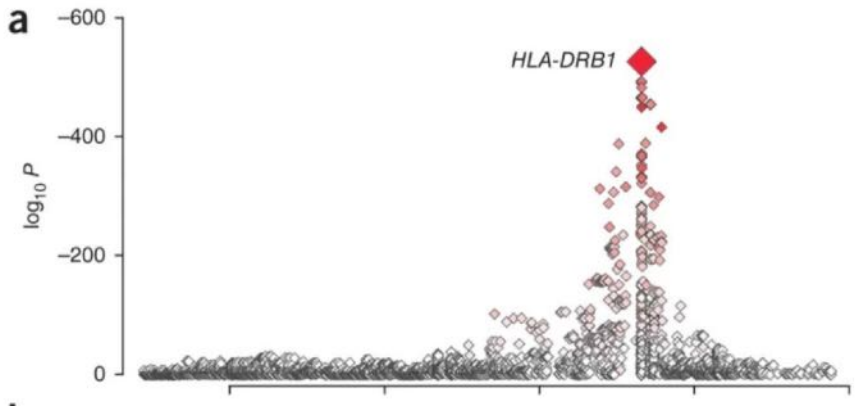
Naonobu Sugiyama, Frances E. Terry, Andres H. Gutierrez, Toshitaka Hirano , Masato Hoshi, Yasushi Mizuno, William Martin, Shin'ichiro Yasunaga, Hiroaki Niiro, Keishi Fujio**, Anne S. De Groot**

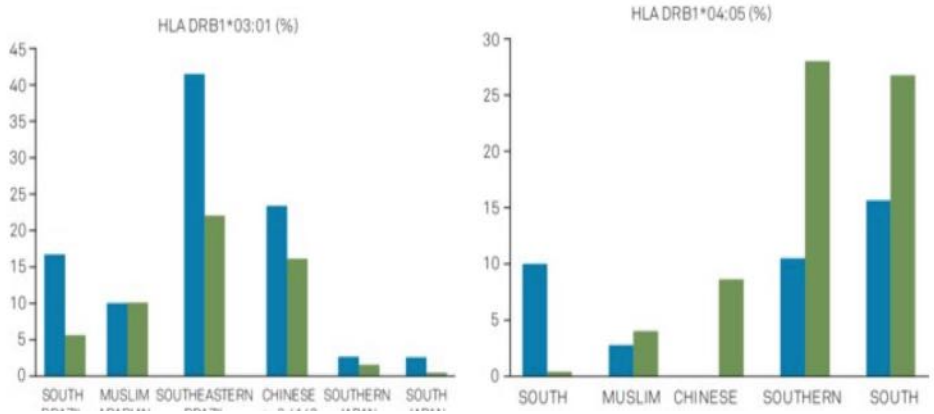
Pfizer, Inc. Fukuoka University, Kyushu University, University of Tokyo, and EpiVax Inc. (**Thank you Sophie T**)

Example of HLA DR Differences and Resources for data on Global Prevalence

Example of observed differences: HLA DRB1 0301, 0405

Figure 1: Association tests within the MHC to rheumatoid arthritis.





Five amino acids in three HLA proteins explain most of the association between MHC and seropositive RA
Soumya Raychaudhuri … Paul I W de Bakker ,Nature Genetics volume 44, pages 291–296 (2012)

Front Genet. 2023 Mar 23;14:866407. doi: 10.3389/fgene.2023.866407. eCollection 2023.

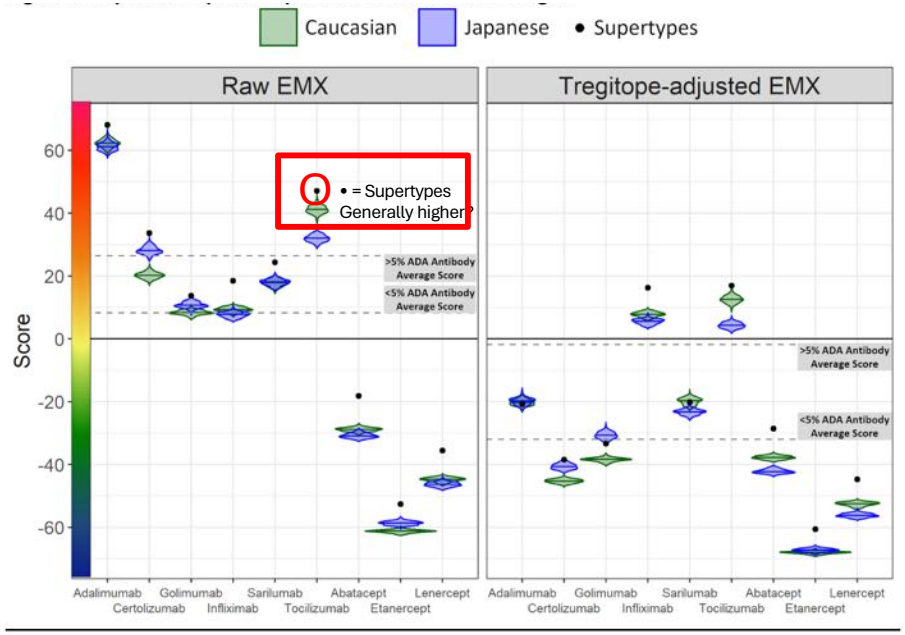
An HLA map of the world: A comparison of HLA frequencies in 200 worldwide populations reveals diverse patterns for class I and class II

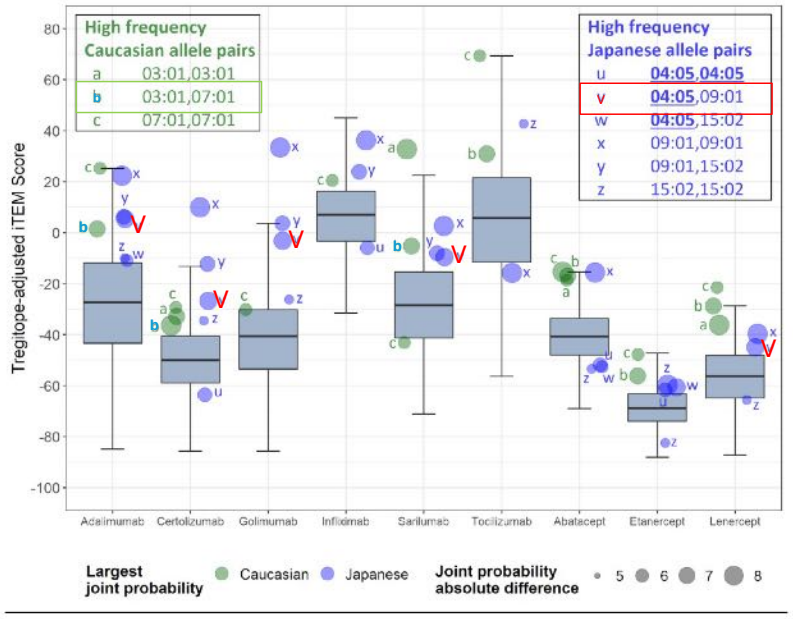
Esteban Arrieta-Bolaños [1] [2], Diana Iraíz Hernández-Zaragoza [3], Rodrigo Barquera [4]

Regional Differences in Potential Immunogenicity of DMARDS for RA are driven by HLA DR Allele Prevalence

EpiVax

Using COMBINATIONS of HLA DR ALLELES found in Japanese/Asian and … Caucasian/European
Allele frequency-weighted scores identify significant differences in * regional immunogenicity risks for DMARDS





Different distributions of risk are due to ….COMBINATIONS of HLA DR that are more frequent in certain populations

Publication for More Details
https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2024.1377911/abstract

EpiVax

ORIGINAL RESEARCH article

Front. Immunol.
Sec. B Cell Biology
Volume 15 - 2024 |
doi: 10.3389/fimmu.2024.1377911

This article is part of the Research Topic
The Immune Response to Therapeutic Antibodies
View all 7 articles >

Individual and population-level variability in HLA-DR associated immunogenicity risk of biologics used for the treatment of Rheumatoid Arthritis

Provisionally accepted

Naonobu Sugiyama [1*] Frances E. Terry [2] Andres H. Gutierrez [3]
Toshitaka Hirano [1] Masato Hoshi [1] Yasushi Mizuno [1]
William Martin [3] Shin'Ichiro '. Yasunaga [4*] Hiroaki Niiro [5]
Keishi Fujio [6] Anne S. De Groot [7*]

Conclusions from Publication:

(1) Analysis of HLA-DR allele haplotypes in RA patient populations could improve the selection of DMARDs.

(2) Certain HLA-DR allele combinations might predispose individuals to a heightened immune response towards specific biologic DMARDs.

(3) Differences in the frequencies of higher risk HLA pairs in regional populations could also explain any differences in the immunogenicity of biologics that are observed in regional cohorts participating in studies that measure ATA.

(4) In clinical practice, this information could guide personalized therapeutic decisions and the selection of one biological DMARD over another.

Outline

EpiVax

- Background
- Individual differences in immunogenicity
- Regional differences in immunogenicity
- **HLA-restricted evolution of antibody affinity**
- Conclusions

Why is 'individual' immune response relevant to individual antibody maturation?

- T effector and Treg epitopes are key to individual immunogenicity risk
- As antibodies mature, T cell epitopes disappear in an HLA-restricted manner (Dekosky, 2021).
- HLA restriction determines epitope removal.
- HLA-restricted epitopes that change are … Tregitopes!

Published Data showing that T cell epitopes (Tregitopes) are deleted in an HLA-restricted manner (collaboration with Brandon Dekosky)

EpiVax

Cold Spring Harbor Laboratory

bioRχiv

THE PREPRINT SERVER FOR BIOLOGY

HOME | ABC
| ALERTS / RS

Search

New Results

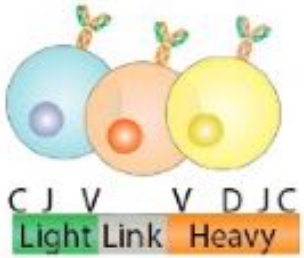
Comment on this paper

Human antibody immune responses are personalized by selective removal of MHC-II peptide epitopes

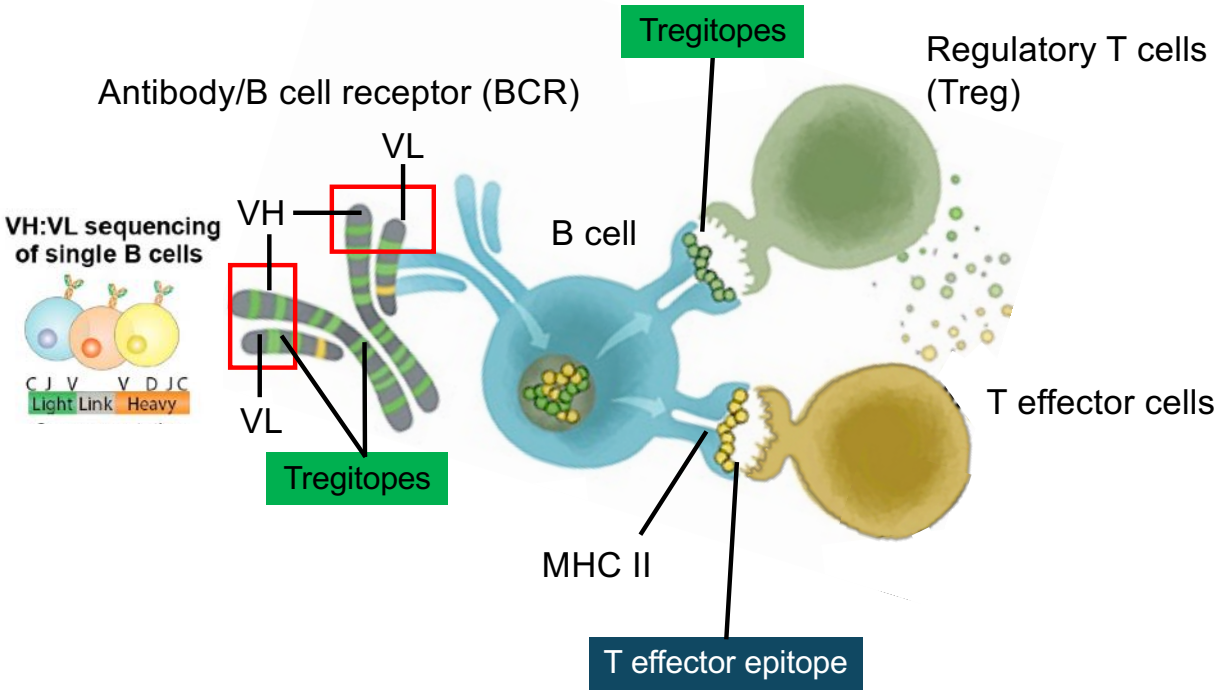
Highlights

- Antibody somatic hypermutation selectively removes MHC-II peptide epitopes from B cell receptors.
- Antibodies with lower MHC-II epitope content show evidence of greater T cell help, including class-switching.
- MHC-II peptide epitope removal from a BCR is linked to long-term antibody secretion in serum.
- MHC-II genotype provides a personalized selection pressure on human antibody development.

VH:VL sequencing of single B cells



Hypothesis: T-cell dependent Antibody Maturation is modulated by Tregitopes

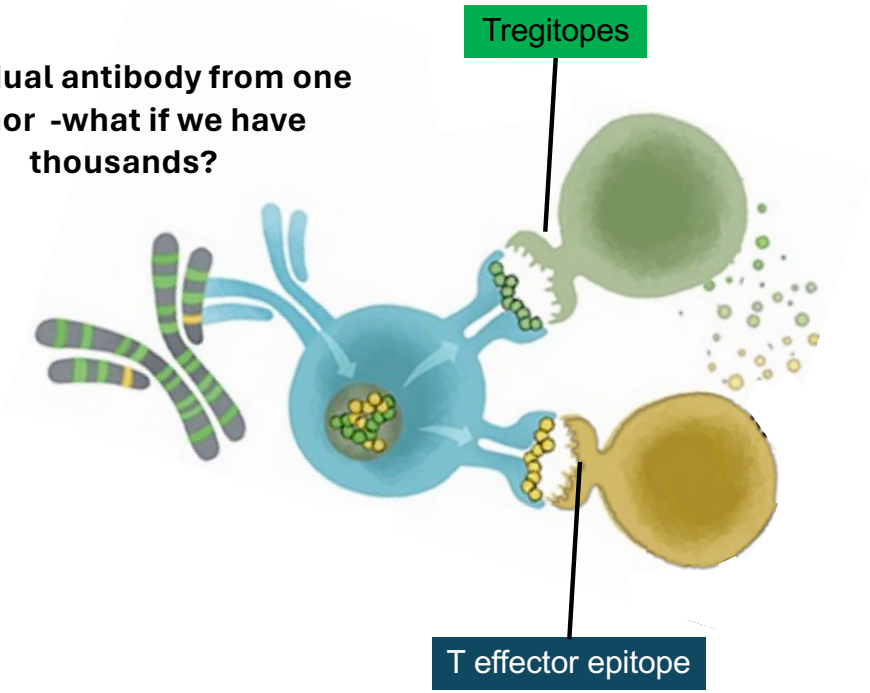


- Likely targets of T follicular regulatory cells (among other self epitopes).
- Tregitopes **are among the most common, eluted epitopes** from IgG.
- Tregitopes **are adjacent and overlapping the CDR regions in the VH and VL**.
- They reduce immune responses to nearby T effector epitopes.
- The presence of Tregitopes in monoclonals is inversely associated with ADA.

T-cell dependent Antibody Maturation – Analysis of Jaffee Dataset
Predicting the phenotype of T cells responding to the BCR epitopes

EpiVax

individual antibody from one donor -what if we have thousands?



VH QSVLTQPPSVSAAPGQKVTISCSGSSSNIGKYSV…

VL QVQLQESGPGLVKPSETLSLTCTVSGGSISSNYW…

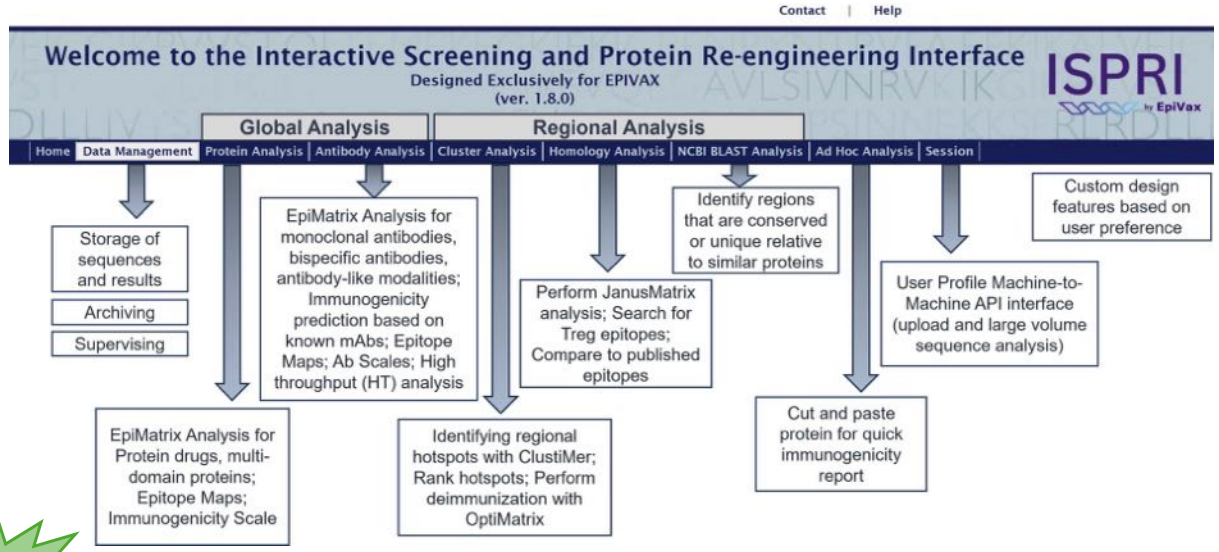
EpiMatrix

T cell epitope content
Donor-specific HLA ligands

Can we analyze thousands of antibodies generated in the germinal center from individual donors and determine whether their **HLA-restricted versions of Tregitopes are preserved or lost during antibody maturation** as measured by Somatic Hypermutation (SHM)?

ISPRI Toolkit

EpiVax



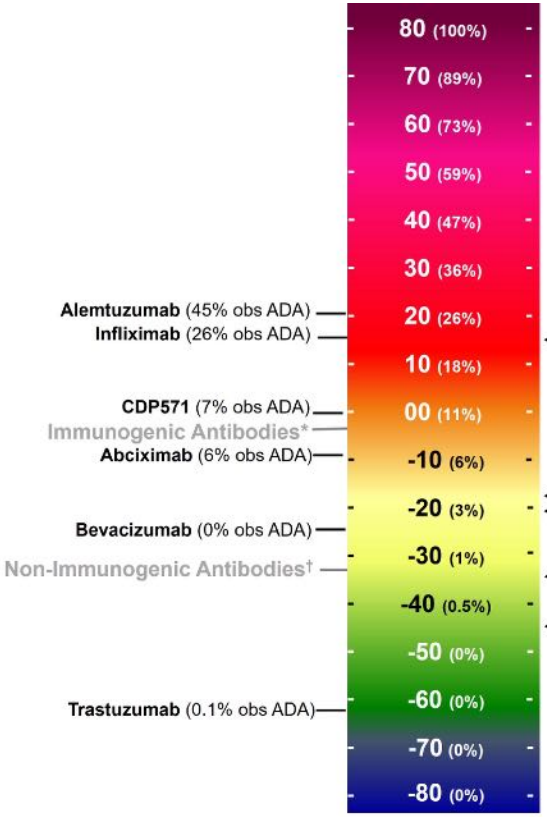
New!

In silico methods for immunogenicity risk assessment and human homology screening for therapeutic antibodies

Aimee E. Mattei, Andres H. Gutierrez, Soorya Seshadri, Jacob Tivin, Matt Ardito, Amy S. Rosenberg, ...show all

Article: 2333729 | Received 04 Jan 2024, Accepted 19 Mar 2024, Published online: 27 Mar 2024

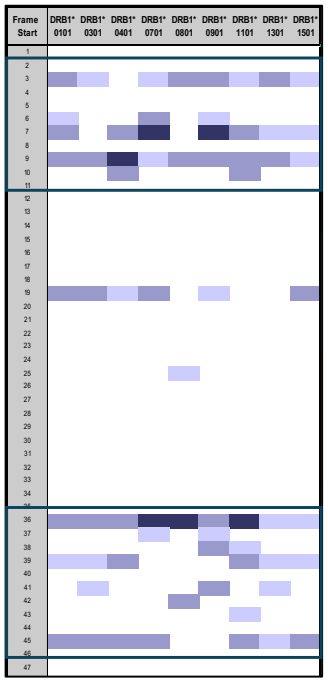
Cite this article https://doi.org/10.1080/19420862.2024.2333729 Check for updates



Analysis of > 2M sequences per year

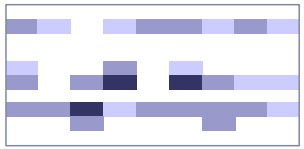
EpiVax

Global

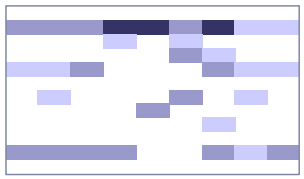


Neutral Protein

Regional

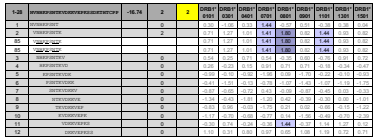


Promiscuous epitope X

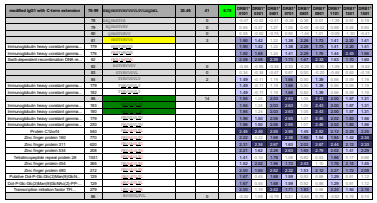


Promiscuous Epitope Y

Humanness



LOW DEPTH OF COVERAGE/ CROSS-CONSERVATION WITH HUMAN PROTEOME

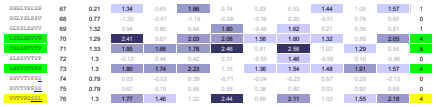


HIGH DEPTH OF COVERAGE/ CROSS-CONSERVATION WITH HUMAN PROTEOME

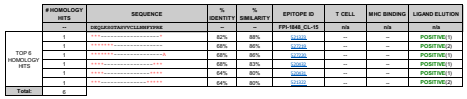
Regulatory/Published Epitopes

No putative regulatory epitopes (Tregitopes) = No score adjustment

Tregitopes are highlighted in **green**

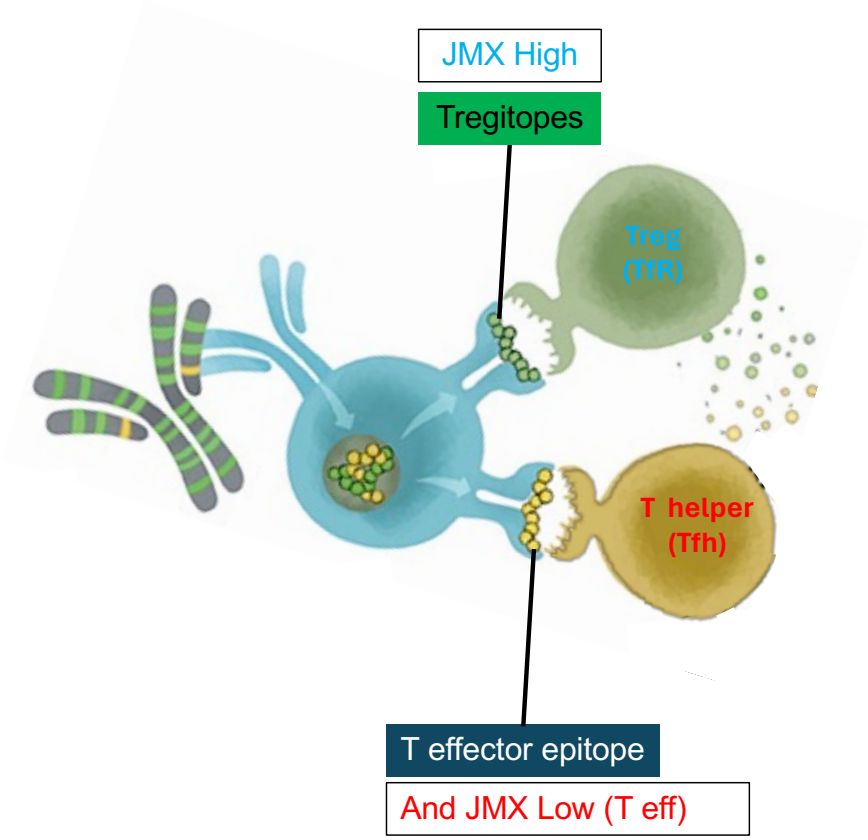


Alignment to published IEDB epitopes



Evaluate SHM vs. T cell epitope content in large dataset
Classify phenotype of epitopes in the BCR sequences

EpiVax



VH QSVLTQPPSVSAAPGQKVTISCSGSSSNIGKYSV…

VL QVQLQESGPGLVKPSETLSLTCTVSGGSISSNYW…

EpiMatrix

T cell epitope content
Donor-specific HLA ligands

JanusMatrix

Regulatory		Effector
Tregitope content Known Tregitopes	**JMX high** High cross-conservation with human proteins	**JMX low** Low cross-conservation with human proteins
Tregitope	Potentially tolerated or actively tolerogenic	Potential T effector epitopes

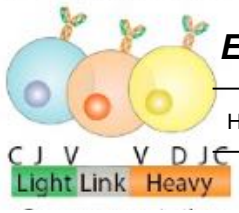
Welcome to the ISPRI Web Site

Designed Exclusively for EPIVAX
(ver. 1.8.0)

EpiVax

rotein Analysis | Antibody Analysis | Cluster Analysis | Homology Analysis | NCBI BLAST Analysis | Ad Hoc Analysis | ISPRI HT Analysis | Session

VH:VL sequencing of single B cells



EpiMatrix

Hits: Top 1% | Top 5% | Top10%

Class II EpiMatrix Report
Sequence: VH

Frame Start	AA Sequence	DRB1*1301 Z-Score	DRB1*1502 Z-Score
1	XXXXXXXXX	-0.11	0.07
2	XXXXXXXXX	0.92	2.28
3	XXXXXXXXX	-0.68	0.64
4	XXXXXXXXX	-0.07	1.35
5	XXXXXXXXX	0.47	0.83
6	XXXXXXXXX	-1.54	-1.77
7	XXXXXXXXX	-1.52	-0.81
8	XXXXXXXXX	-0.37	-1.09
9	XXXXXXXXX	-0.92	0.16
10	XXXXXXXXX	0.08	0.11
11	XXXXXXXXX	2.37	0.82
12	XXXXXXXXX	1.56	2.41
13	XXXXXXXXX	-0.67	-0.54
14	XXXXXXXXX	0.01	0.45
15	XXXXXXXXX	0.68	0.96
16	XXXXXXXXX	0.18	0.19
17	XXXXXXXXX	0.78	-0.08
18	XXXXXXXXX	2.29	1.75
19	XXXXXXXXX	0.43	-0.62
20	XXXXXXXXX	-0.55	1.74
21	XXXXXXXXX	-0.12	0.18
22	XXXXXXXXX	-0.45	-0.89
23	XXXXXXXXX	-0.42	0.5
24	XXXXXXXXX	0.32	-0.6
26	XXXXXXXXX	0.24	0.9
28	XXXXXXXXX	-1.61	0.07
29	XXXXXXXXX	0.79	0.13
30	XXXXXXXXX	-1.09	-0.25
31	XXXXXXXXX	-0.47	0.25
32	XXXXXXXXX	0.81	-0.56
33	XXXXXXXXX	-0.81	-0.69
34	XXXXXXXXX	1.33	0.31
35	XXXXXXXXX	-0.2	-0.13
36	XXXXXXXXX	0.98	2.24
37	XXXXXXXXX	1.26	1.61

Tregitope (rows 11–12)

Tregitope (rows 18–20)

Tregitope (rows 36–37)

Typical analysis of one individual antibody from one donor

Typical analysis of one individual antibody from one donor

- **four donors** in Jaffe publication

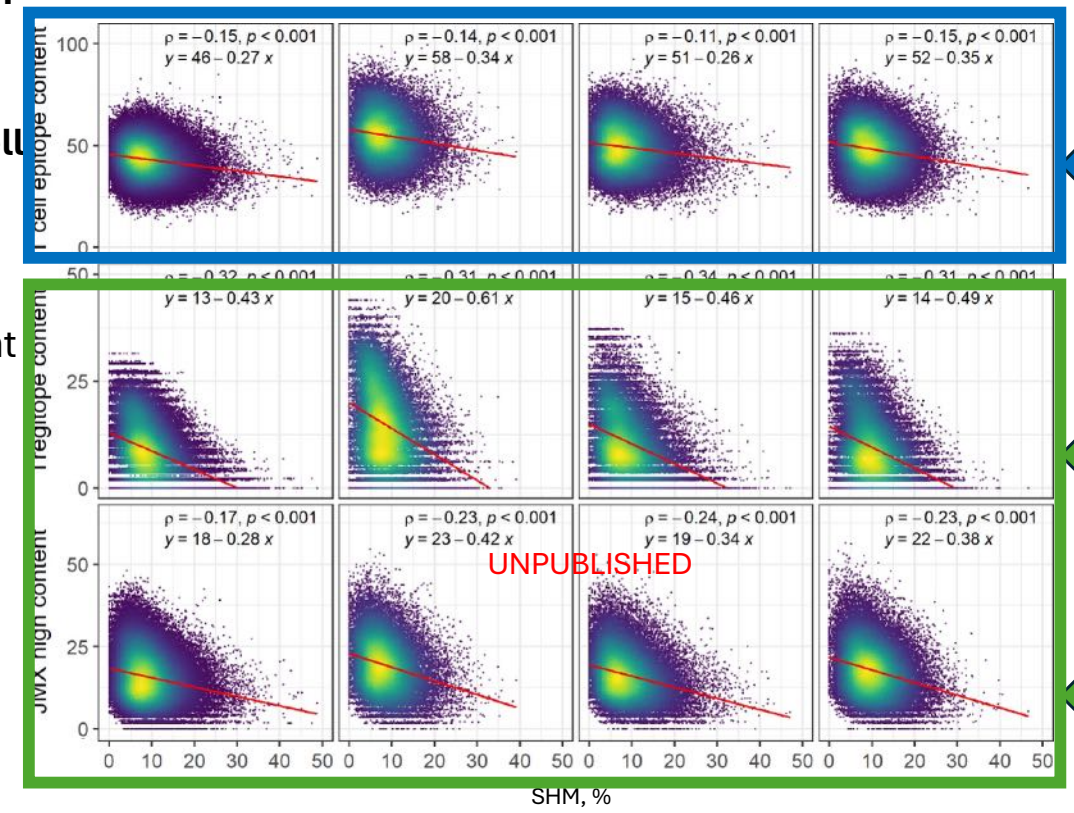
Donor	Vendor	Catalog #	Relevant immune history (vendor donor ID)	Sex	Age	HLA type	Blood type	Known serologies
1	Cellero	10052	SARS-CoV-2 (523)	F	45	A*03:01/68:01; B*15:01/35:03; C*03:03/04:01; DRB1*13:01/16:01	O+	SARS-CoV-2
2	Cellero	1146	SARS-CoV-2 (527)	F	35	A*02:01/02:01; B*44:02/51:01; C*02:02/05:01; DRB1*01:01/15:01	O+	SARS-CoV-2
3	Cellero	1132	Type 1 Diabetes (607)	F	38	A*02:01/24:02; B*39:06/45:01; C*07:02/16:01; DRB1*04:04/11:01	O+	–
4	Cellero	10050	Celiac's disease (649)	F	50	A*02:01/30:02; B*18:01/51:01; C*02:02/05:01; DRB1*03:01/15:01	O+	CMV

Jaffe, D.B., Shahi, P., Adams, B.A. *et al.* Functional antibodies exhibit light chain coherence. *Nature* **611**, 352–357 (2022).

What about Tregitopes?
And epitopes with high human homology (JMX high)

EpiVax

Donor-specific HLA-DRB1 T cell epitope content, Tregitope content, and potentially tolerated T cell epitope content in antibody sequences decline with SHM, while potential T effector content increases.



Memory B cells

Total T cell epitopes decline

Tregitopes Decline

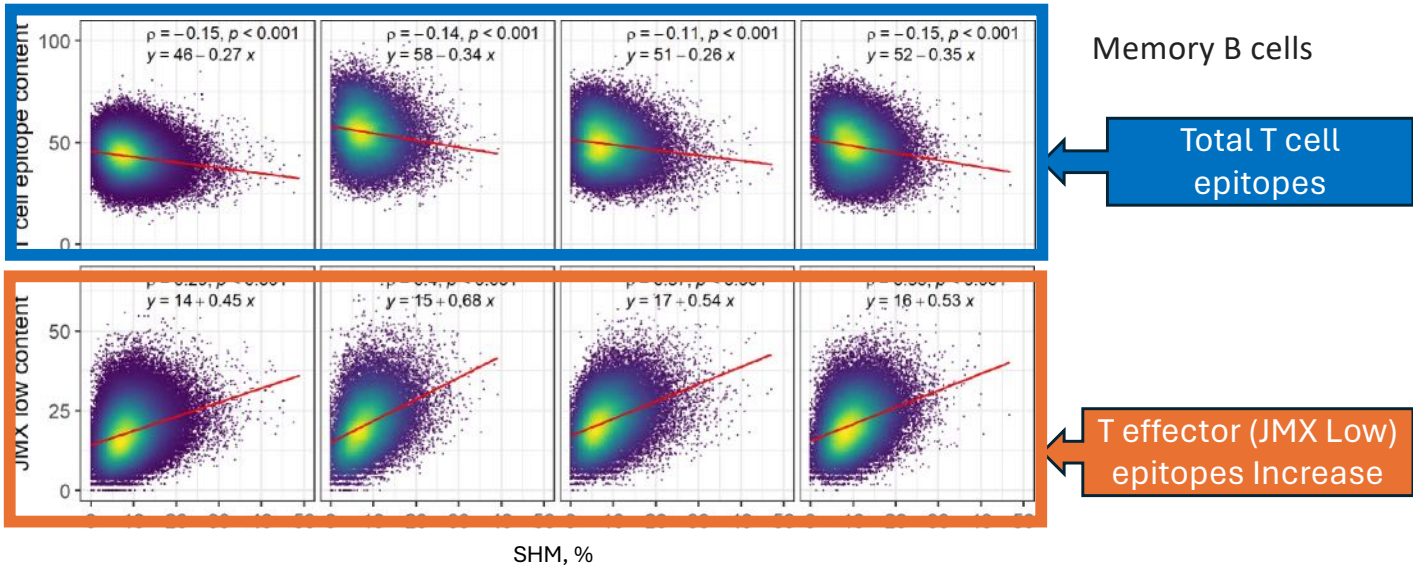
JMX high epitopes Decline

What about T effector
And epitopes with high human homology (JMX high)

EpiVax

Donor-specific HLA-DRB1 T cell epitope content, Tregitope content, and potentially tolerated T cell epitope content in antibody sequences decline with SHM, **while potential T effector content increases.**

UNPUBLISHED



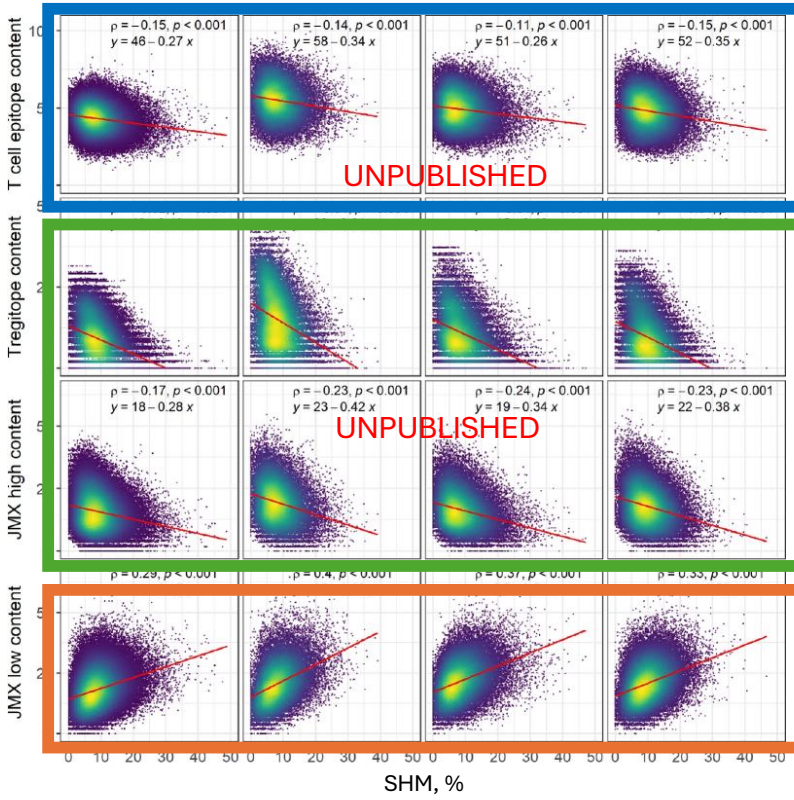
UNPUBLISHED

Donor-specific HLA-DRB1 T cell epitope content, Tregitope content, and potentially tolerated T cell epitope content in antibody sequences decline with SHM, while potential T effector content increases. Scatter plot of (A) T cell epitope content and subsets of (B) Tregitope content, (C) JMX high (potentially tolerated) content, and (D) JMX low (potential T effector) content vs. SHM. SHM percentages were calculated based on the identify percentage between heavy and light chain V-genes and their corresponding germlines using IgBLAST. Each point represents one antibody sequence; points are colored by data density from low (purple) to high (yellow). The number of antibodies per donor is shown at the top of the figure. Spearman ρ correlation and p-values are indicated. Linear regression equations and lines (red) are also shown.

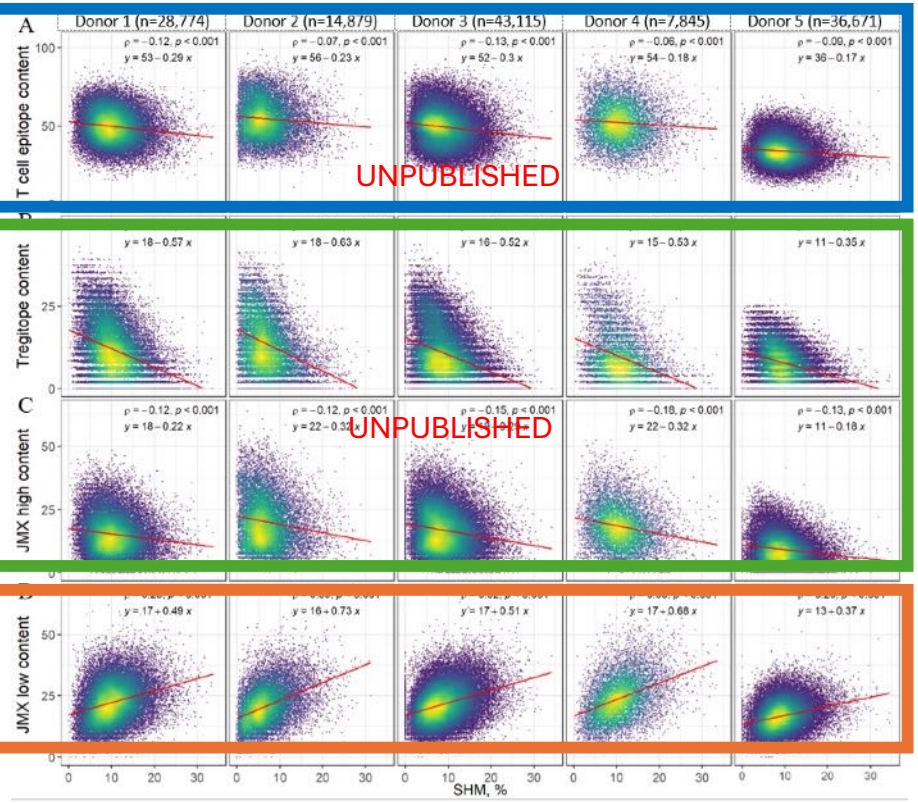
Two Independent Datasets: Nearly Identical Results
Different donors, and different HLA restrictions

EpiVax

Current analysis - Jaffe (n=249,958)



Previous analysis - Dekosky (n=123,439)



Outline

EpiVax

- Background
- Individual differences in immunogenicity
- Regional differences in immunogenicity
- HLA-restricted evolution of antibody affinity
- **Conclusions**

Conclusions – relevant to EIP Conference

EpiVax

- Both Treg (Tregitopes) and T effector (T helper) cells modulate ADA.
- In vitro studies should consider impact of Treg epitopes on outcomes.
- Because T cell response is (HLA) individualized, risk is also individual;
- Because HLA differences are regional, regional differences may occur.
- Treg deletion (impairment) affects evolution of antibody affinity and titer.
- In silico analysis enables assessment of likely T cell response.
- Websites can be developed to estimate personal risk of biologics.

The future is calling: Personalized Immunogenicity Risk Assessment: It's Personal

EpiVax

Contact | Help

Welcome to the EpiVax PIMA Web Site

Designed Exclusively for Duke
(ver. 1.0.0)

EpiVax

Home | Data Management | Session

Data Management

Patient Manager
Manage all of the Patients Currently Loaded within the Platform

Add New Patient
Upload New Patient Information

Archive Manager
Manage and Restore all Archived Patient Data

frontiers in Immunology

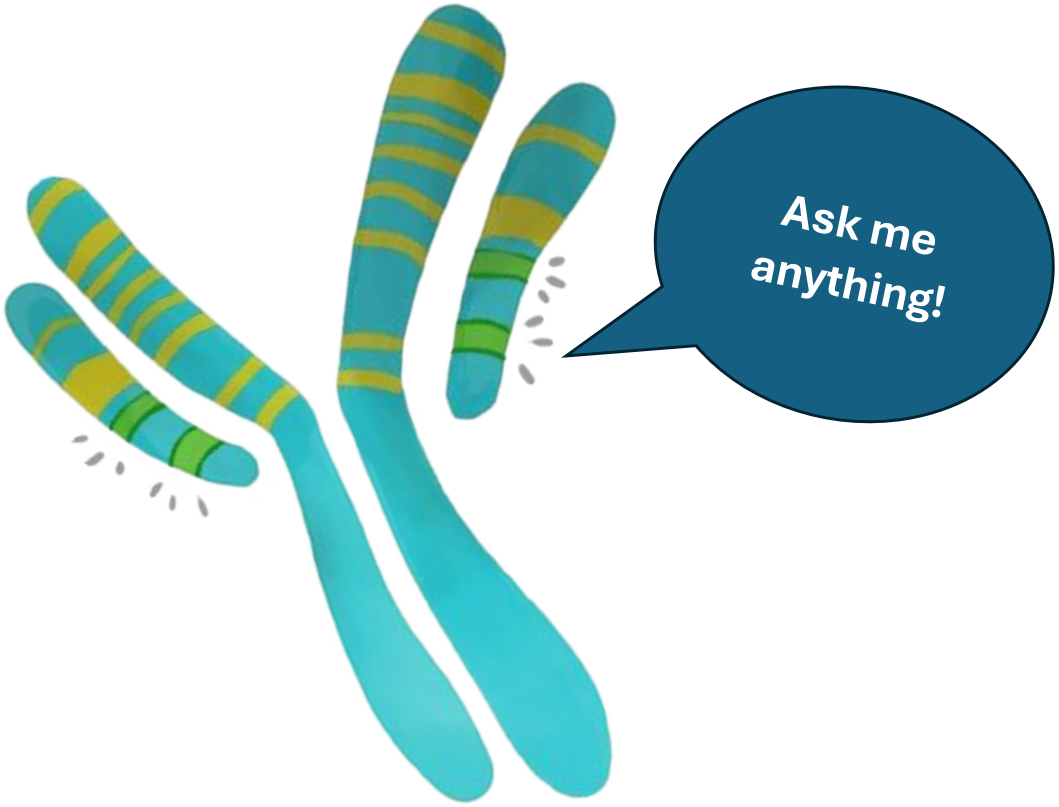
ORIGINAL RESEARCH
published: 16 June 2021
doi: 10.3389/fimmu.2021.636731

Immune Tolerance-Adjusted Personalized Immunogenicity Prediction for Pompe Disease

Anne S. De Groot[1,2], Ankit K. Desai[3], Sandra Lelias[1], S. M. Shahjahan Miah[1], Frances E. Terry[1], Sundos Khan[1], Cindy Li[3], John S. Yi[4], Matt Ardito[1], William D. Martin[1] and Priya S. Kishnani[3]*

Thank you for your attention!

EpiVax




EpiVax
| www.epivax.com | Providence, RI | 401.272.2123 |