The Two-Faced T Cell Epitope: Examining the Host-Microbe Interface with JanusMatrix

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Abstract

We leveraged computing power advances, the availability of terabytes of genomic sequences from organisms to which humans are exposed, and advanced immunoinformatics tools to explore the intersection of commensals, pathogens, and the human genome at the T cell epitope level. We developed JanusMatrix, which examines cross-reactive T cell epitopes from both HLA binding and TCR-facing sides to allow comparison across large genome sequence databases including common human pathogens (HP), the human gut microbiome (HM), and the human genome (HG). Initial study reveals different levels of HG, HM, and HP cross-reactivity (XR) for known Treg and Teff epitopes. In Hand Foot Mouth Disease (HFMD), extensive XR with HM seems to predict immunodominance; more limited XR with enteroviruses (e.g., polio) may protect against severe HFMD. For common Teff epitopes, HG XR is more limited than HM XR. For Treg epitopes defined in HCV disease and for Tregitopes, HG XR is larger. Greater XR with HG compared to HM seems to distinguish defined Treg and T effector epitopes. While predicting all influences on immune response may be impossible, the vast availability of human pathogen and commensal organism sequences now allows T cell epitope comparisons in these large datasets. Startling discoveries relevant to vaccine development and T cell response phenotype understanding are emerging as we apply this powerful technology.

Cross-Reactivity

- Intrinsic characteristic of TCR, i.e., each single TCR can potentially interact with different peptide-MHC complexes.
- Critical to many aspects of T cell biology, including positive and negative selection.
- Involved in heterologous immunity. Both human and murine immune responses to antigens can be modified as a consequence of T cell cross-reactivity.
- Can have positive or negative (e.g., leading to pathology) effects.

JanusMatrix

In Roman mythology, Janus is the god of beginnings and transitions, of gates, doors, doorways, endings, and time. He is usually portrayed as a two-faced god, looking both to the future and the past.

TCR cross-reactivity prediction:

- Given a protein or peptide, T cell epitopes are identified using EpiMatrix.
- JanusMatrix searches for potentially XR TCR by dividing each predicted epitope into TCR-facing residues and MHC-binding residues, and searching for matching sequences in preloaded reference databases.

Reference databases available include:

- human pathogens (HP; bacteria and viruses)
- human gut microbiome (HM)
- human genome (HG)

Cross-reactive peptides:

- Predicted to bind the same MHC allele.
- Same/similar T cell-facing residues (epitope).
- MHC-facing residues (agretope) can differ.

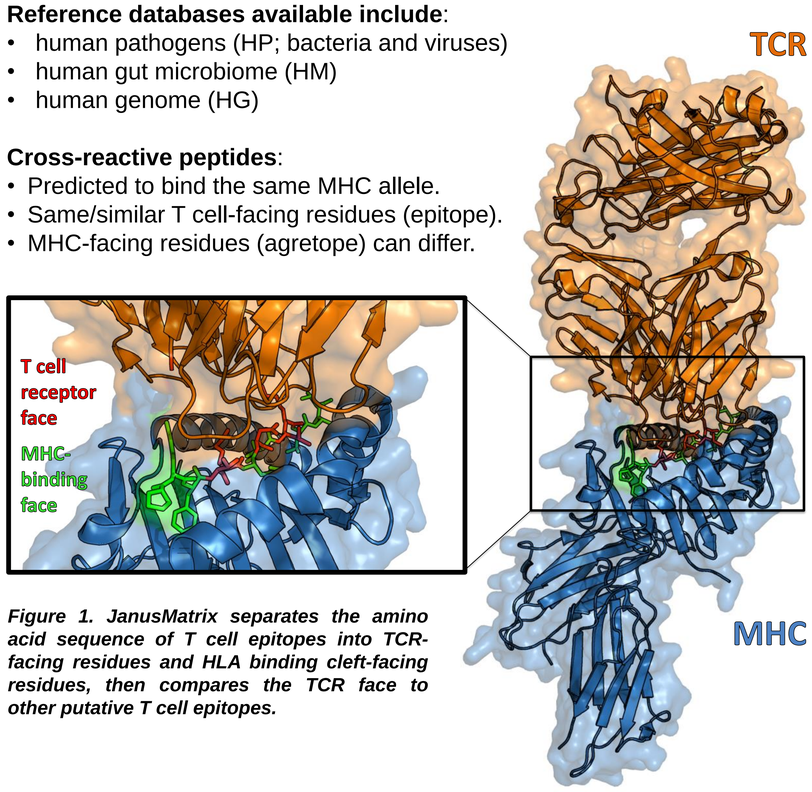


Figure 1. JanusMatrix separates the amino acid sequence of T cell epitopes into TCR-facing residues and HLA binding cleft-facing residues, then compares the TCR face to other putative T cell epitopes.

JanusMatrix Website

- JanusMatrix: Web-based interface with query and analysis tools.
- Integrates genomic sequences, immunological data, and applied computational epitope prediction and analysis.
- Predicts Class I and Class II epitopes using EpiMatrix, compares TCR-facing residues of the predicted epitopes against databases defined by the user.
- Generates multiple outputs including summaries, predicted epitopes, cross-reactivity across multiple databases, cross-reactivity annotation, among others.
- Outputs are interactive and can be downloaded.
- Cytoscape-based approach to visualize related epitope networks.

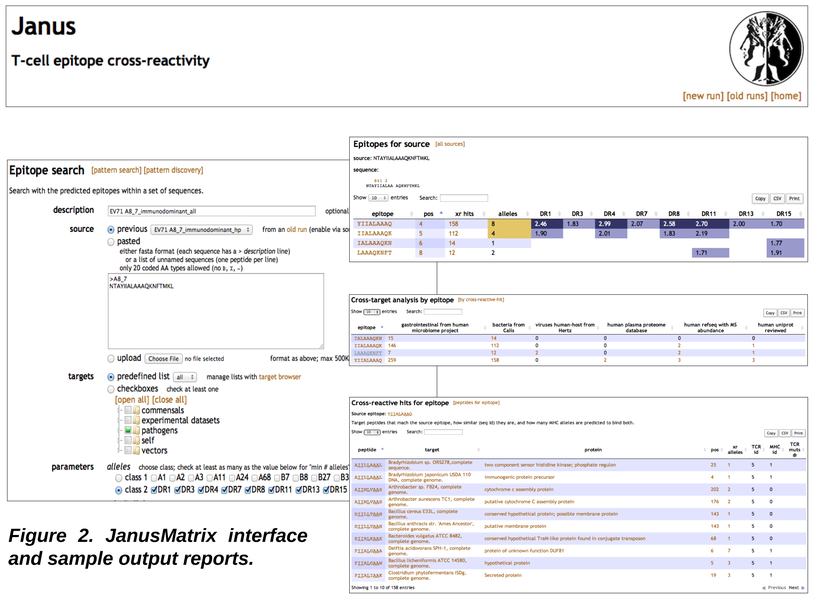
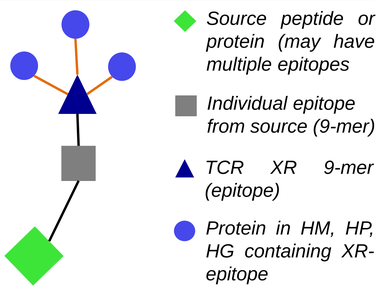


Figure 2. JanusMatrix interface and sample output reports.

Epitope Network Visualization

- XR epitopes described in tabular format, patterns visualized with "epitope networks" using Cytoscape.
- Epitope networks: nodes for epitopes and the proteins that contain them, lines between XR epitope pairs and between proteins and their constituent epitopes.



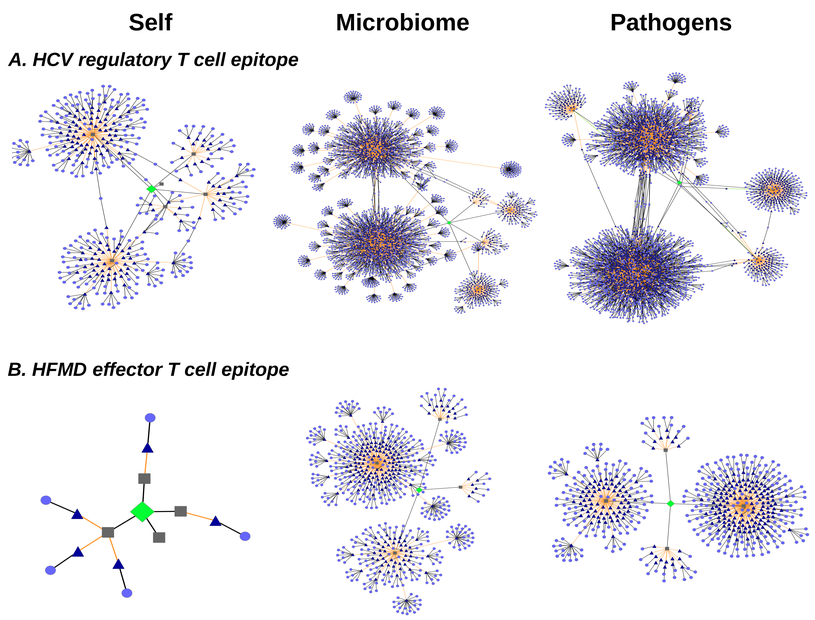


Figure 3. TCR-Epitope Networks (using Cytoscape) for regulatory T cell epitopes (A) and effector T cell epitopes (B). *Epitopes with TCR-facing residues similar in protein sequences from the HG (left), HM (center), and human viral and bacterial genomes (HP; right) databases.*

JanusMatrix patterns

- Mapped groups of peptides experimentally shown to induce defined T cell response (Teff, Treg, or no response).
- Searched for trends in number of XR TCR-facing epitopes across HG, HM, and HP databases.
- Set of 10,000 random nine-mers also evaluated for comparison.

Database	Median cross-reactive hits (ratio, 1x10[6])[a]					Number of:		
	T eff epitopes		Treg epitopes		Random[b]	Genomes	Proteins per database	Amino acids per database
	CEFT	EV71	Tregs	HCV				
Self (HG)	2 (0.18)	5 (0.44)	8.5 (0.75)	23.5 (2.08)	1 (0.09)	1	20,248	11,301,336
Microbiome (HM)	29 (0.13)	427 (1.95)	31 (0.14)	103 (0.47)	14 (0.06)	204	705,684	218,452,796
Pathogens (HP)	17 (0.12)	296 (2.02)	19 (0.13)	107.5 (0.73)	10 (0.07)	221	455,237	146,398,849

A

Epitope type	HG	HM	HP
Treg vs. CEFT	**0.00**	0.63	0.62
CEFT vs. Random	**0.00**	**0.05**	0.11
Treg vs. Random	**0.00**	**0.01**	**0.02**

B

Database	CEFT	Treg	Random
HG vs. HM	0.10	**0.00**	**0.00**
HG vs. HP	0.24	**0.00**	**0.00**
HM vs. HP	0.50	0.15	**0.00**

[a]Ratio of XR hits per number of amino acids in the comparison database.
[b]Nine-mer predicted to be an epitope.

A) P-values of comparisons between ratios across three types of epitopes by database.
B) P-values of comparisons between ratios across databases by type of epitope.

XR Pattern Illustration

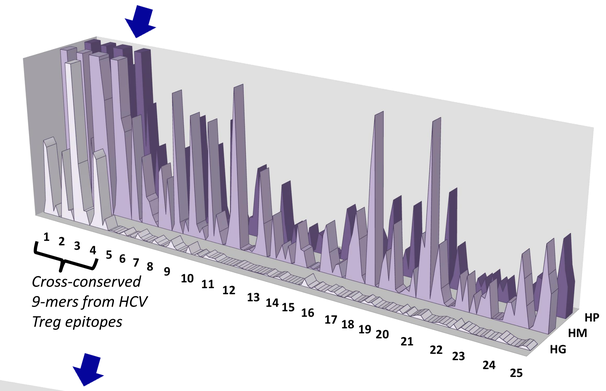


Figure 4. Hepatitis C virus (HCV) HM/HP epitope conservation and immunodominance. *For Treg epitopes in HCV disease, more extensive HG XR. Overall, greater HG XR.*

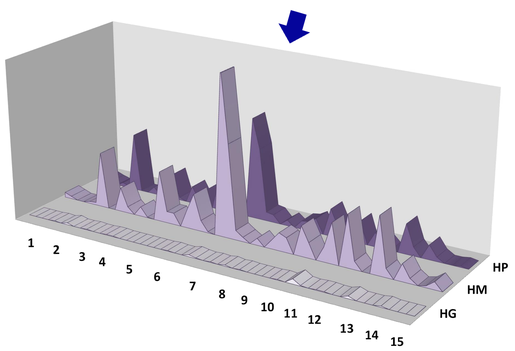


Figure 5. Hand-Foot-Mouth Disease (HFMD) HM/HP epitope conservation and immunodominance. *Likely importance of XR between microbial genomes to immunodominance of a given epitope.*

Both figures: Y axis, number of XR hits in database; X axis, individual epitopes (and 9-mers within epitopes).

Discussion

Using JanusMatrix, we have begun to expand information available on the role of T cell epitopes conserved between the HM, HG, and HP, and to understand how these epitopes influence the immune response by:

- Examining whether human commensal exposure alters subsequent T cell responses to common human pathogens.
- Re-examining prevailing autoreactive T cell paradigms.
- Defining microbial T cell epitopes contributing to host-microbial homeostasis.
- Defining homologous T cell epitopes among variant strains or serotypes of the same pathogen (e.g., dengue).
- Exploring immune-mediated favorable or adverse vaccination responses, as distinct XR patterns may have important implications for vaccine development, safety, efficacy.
- Uncovering important similarities between HIV and the human genome.

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