Modeling HLA binding and "self" conservation using in silico tools predicts immunogenic T cell epitopes in vaccinated individuals

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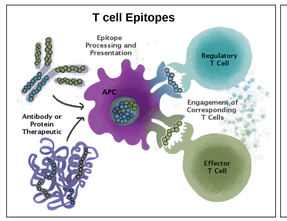
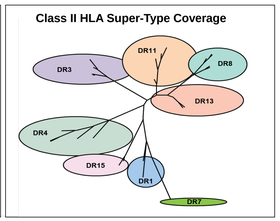
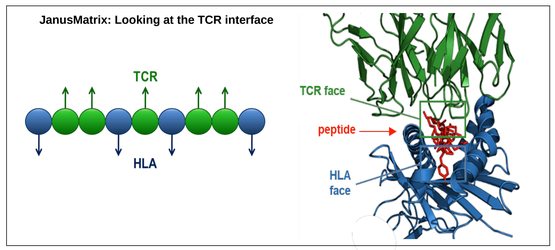
PURPOSE

The ability of the immune system to develop a response against pathogens relies on the presentation of peptide antigens to CD4+ T-cells in the context of class II Human Leukocyte Antigen (HLA-II) molecules found on the surface on antigen presenting cells. Peptide binding to HLA is dependent on the position of specific amino acid "anchor residues" that interact with complementary binding pockets found within the HLA binding groove. Through the work of several laboratories, allele-specific binding profiles have been elucidated allowing for the development of in silico-based prediction tools, such as EpiMatrix developed by EpiVax Inc. Since peptide binding to HLA is a prerequisite for immunogenicity, the ability to predict which peptides within a given protein will bind HLA, provides an important first step in immunogenicity screening.





Furthermore, we also evaluate epitope similarity to the human proteome at the T cell receptor (TCR) interface, which may induce regulatory T cell responses, using the JanusMatrix algorithm[1]. The combination of in silico predictions validated by in vitro assays provides a powerful method whereby targeted vaccines can be developed on demand in response to an outbreak. By screening a pathogen's proteome, we can rapidly narrow down the search for target epitopes allowing for focused vaccine design.



Case Study 1: Malaria

METHODS

- EpiMatrix was used to identify class II epitopes providing broad HLA coverage in RH5, a highly conserved *Plasmodium falciparum* blood-stage antigen that has recently been assessed in a Phase I clinical trial with controlled human malaria infection (CHMI)
- JanusMatrix was used to analyze epitope similarity to human proteome at TCR interface
- Predicted epitopes were synthesized and validated in interferon gamma (IFNγ) ELISpot assays using PBMC from clinical trial vaccinees administered RH5.1, a full-length recombinant RH5 protein vaccine.
- For each vaccinee's HLA-DR haplotype we calculated:
  - an individualized T cell epitope measure (iTEM) score[2]
  - score adjusted for human cross-conservation using JanusMatrix (J-iTEM)

Example of iTEM Scores

	DRB1*0101	DRB1*0301	DRB1*0401	DRB1*0701	DRB1*0801	DRB1*1101	DRB1*1301	DRB1*1501
HLA freq.[3]	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								

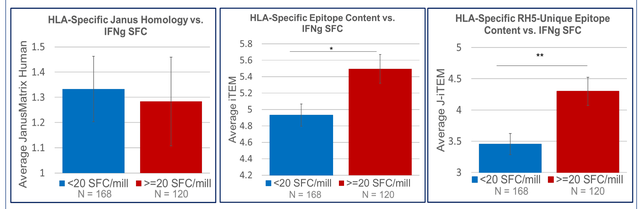
Strength of iTEM score: Low — Medium — High

RESULTS

JanusMatrix: As "selfness" decreases, average inflammatory response increases.

iTEM: HLA DR type that matches epitope binding predictions predicts response in vitro

J-iTEM: combining HLA-specific epitope content and selfness improves prediction of immunogenicity over either metric alone.

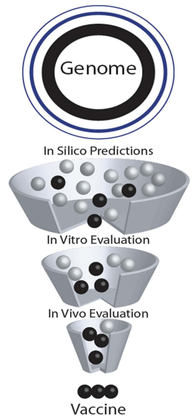


Peptides inducing positive responses were shown to have higher iTEM and J-iTEM scores ($p<0.05$ and $p<0.01$, respectively) for each vaccinee than negative peptides. **Indicating that volunteers could present the peptides and respond with IFNγ if the peptide was unique to malaria and non-tolerogenic.**

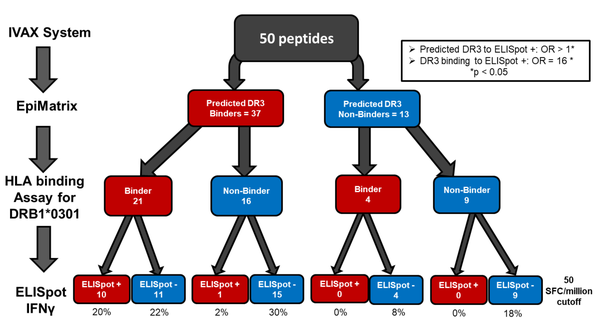
Case Study 2: Q Fever

METHODS

- *Coxiella burnetii* (Cb), the causative agent of Q fever, is a Gram-negative intracellular bacterium transmitted via aerosol.
- The EpiMatrix and JanusMatrix algorithms were used to identify 50 promiscuous Class II epitopes from Cb antigens that are known B and T cell targets.
- These putative epitopes were tested in HLA binding assays and screened for immunogenicity in HLA-DR3 transgenic mice (tgHLA-DR3) that were subjected to heterologous DNA/DNA/peptide/peptide prime-boost immunizations. Epitope-specific responses in the tgHLA-DR3 mice were evaluated using IFNγ ELISpot assays.
- Significant epitope-specific IFNγ responses (compared to vehicle-immunized mice) were found for 11/50 peptides, *all of which are predicted HLA-DRB1*0301 epitopes* (Fisher's exact p-value: 0.023). All but one of these epitopes were confirmed binders to DRB1*0301 *in vitro*.



RESULTS

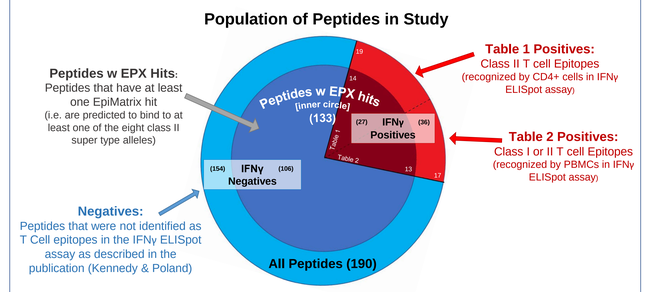


These findings further validate the use of *in silico* tools to prospectively identify potential vaccine targets.

Case Study 3: Smallpox

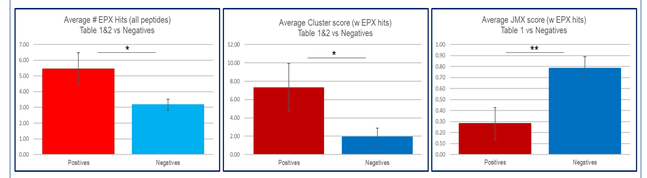
METHODS

- Published HLA class II-restricted T cell epitopes from vaccinia virus membrane proteins[4] were retrospectively analyzed using EpiMatrix and JanusMatrix
- An overlapping peptide library of four vaccinia membrane proteins known to induce a humoral response in vaccinated individuals was synthesized and tested in IFNγ ELISPOT assays using the PBMCs of 29 recent smallpox vaccine recipients.
- We analyzed the peptides using EpiMatrix and JanusMatrix algorithms.



RESULTS

- We found that the peptides identified as T cell epitopes were predicted to bind to class II HLA super-type alleles by EpiMatrix more often than the remainder of the overlapping peptide library ($p<0.03$)
- We found that the peptides identified as class II T cell epitopes had lower JanusMatrix Human Homology scores than the remainder of the overlapping peptide library ($p=0.005$).



This is consistent with the identification of T cell epitopes as described by Kennedy & Poland suggesting that **in silico binding predictions correlate to T cell responses in vitro.**

CONCLUSIONS

- **EpiMatrix and JanusMatrix algorithms efficiently identify putative T cell epitopes, distinguish likely inflammatory peptides from regulatory peptides, and are adaptable to a patient HLA-specific level of assessment.**
- **Combining HLA-specific epitope content and "selfness" improves prediction of immunogenicity over either metric alone**
- **Applying both tools in the early stages of vaccine design, antigen selection and engineering will result in the advancement of next generation vaccines where the minimal essential components of protection can be delivered without off-target or unintentionally suppressive signals deleterious to vaccine efficacy.**

REFERENCES AND ACKNOWLEDGEMENTS

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[2]Cohen et al., J Biomed & Biotech; 2010; A Method for Individualizing the Prediction of Immunogenicity of Protein Vaccines and Biologic Therapeutics: Individualized T Cell Epitope Measure (iTEM)
[3]Southwood et al., J. Immunol. 1998; 160: 3363-3373
[4]Kennedy & Poland, Virology 2010; 408(2): 232-240

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For questions regarding in silico antigen screening and vaccine design, please contact: Katie Porter at 401-272-2123, ext. 115; or at info@epivax.com

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