Structure-guided Immunogen Design: Modulating B cell and T cell Immunogenicity of First and Second Generation H7N9 HA Vaccines

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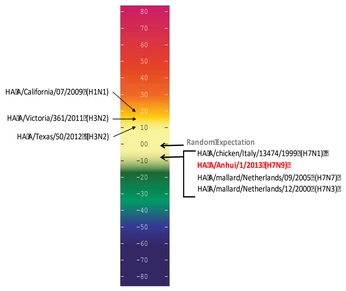
H7N9 Influenza is Poorly Immunogenic

A new avian-origin influenza virus emerged near Shanghai in February 2013. Human-to-human transmission of avian-origin H7N9 influenza A has been limited to a few clusters, but the high mortality rate (~30%) associated with human infection has raised concern about the potential for this virus to become a significant human pathogen.

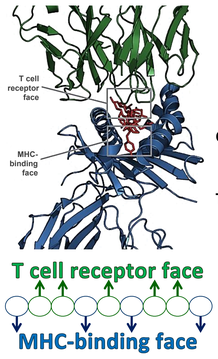
Immunogenicity Potential



- We used well-established immunoinformatics tools to estimate the immunogenic potential of H7N9 proteins.
- HA proteins derived from human-derived H7N9 strains isolated in 2013 contain fewer T cell epitopes than most other circulating strains of influenza.
- H7N9 HA contains a predicted Treg-inducing epitope.
- Conservation of T cell epitopes with other strains of influenza was very limited.
- Based on our analysis, avian-origin H7N9 2013 appears to be a "stealth" virus.
- Protective antibody responses in infection and vaccination are reported to be delayed and weak.

➢ To prepare for an H7N9 pandemic, vaccine strategies that overcome the poor immunogenicity of H7N9 HA are needed.

Identification of Treg-Inducing Epitopes

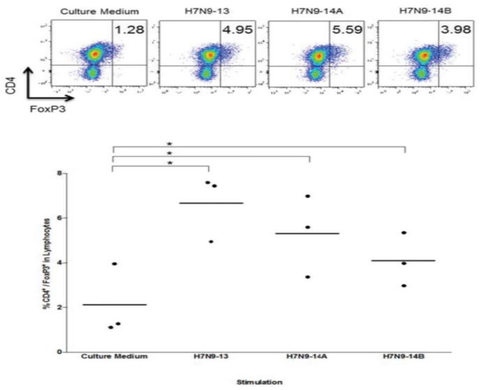


JanusMatrix separates the amino acid sequence of T cell epitopes into TCR-facing residues (epitope) and HLA binding cleft-facing residues (agretope), then compares the TCR face to other putative T cell epitopes.

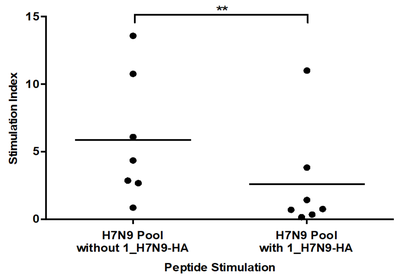
Cross-reactive peptides:
- Are predicted to bind the same MHC allele.
- Share same/similar T cell-facing residues.

TCR cross-reactivity prediction:
- Given a protein or peptide, T cell epitopes are identified based on MHC contacts using EpiMatrix.
- JanusMatrix searches for potentially cross-reactive TCR by screening TCR-facing residues against a preloaded, EpiMatrix-processed reference database.
- Peptides with high cross-reactive potential are associated with reduced IFNγ secretion in PBMCs of healthy donors (Liu *et al.* Human Vaccines & Immunotherapeutics 2015).

H7N9 epitopes with high potential for self cross reactivity elevate Treg frequency in-vitro



H7N9 HA Treg activating epitope actively suppresses bystander immune response



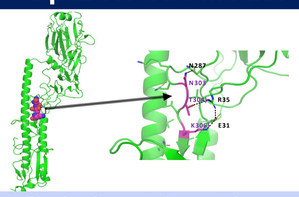
Design of Treg Epitope-Deleted H7N9 HA

Optimized H7-HA Design

	297	309
H7-HA	PRYVKQRSLLAT	
	306	318
H3-HA	PRYVKQNTLKLAT	
	297	309
H7-HA-Opt1	PRYVKQNTLKLAT	

The Treg-inducing epitope in H7-HA is replaced with a broadly reactive and highly conserved H3-HA epitope at the corresponding position.

Global HA fold preserved in Opt1 H7-HA Model

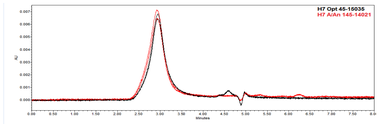


Close-up of local environment where mutations were made (right) shows stabilizing hydrogen and ionic bonding contacts between introduced amino acids (pink labels) and H7-conserved wild type amino acids (black labels).

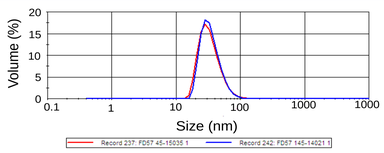
Production and Testing of Treg Epitope-Deleted H7N9 HA

Opt1 rH7-HA biophysical properties comparable to wild type

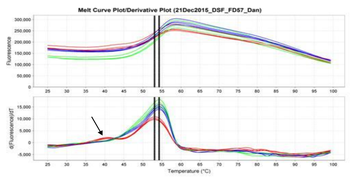
Size Exclusion Chromatography Retention Time



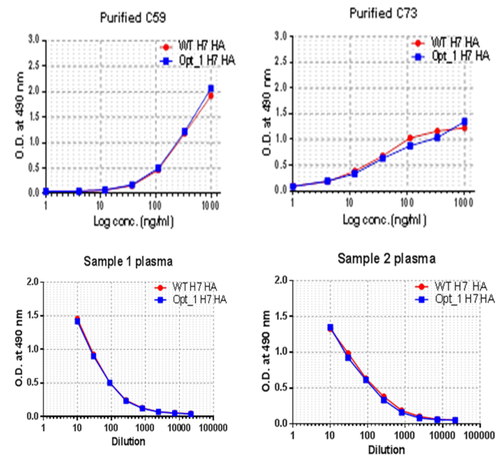
Particle Size by Dynamic Light Scattering



Thermal Stability by Differential Scanning Fluorimetry



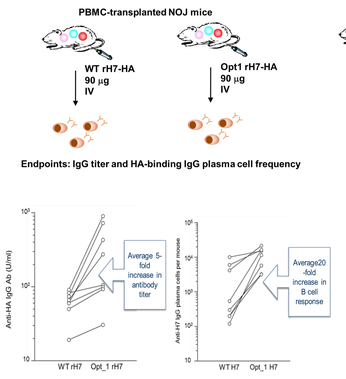
Opt1 rH7-HA maintains antigenic structures



Previously identified monoclonal antibodies against WT rH7-HA (top panel) and antibodies from two patients with anti H7-HA antibodies (bottom panel) recognize Opt1 rH7-HA with affinity equivalent to WT rH7-HA.

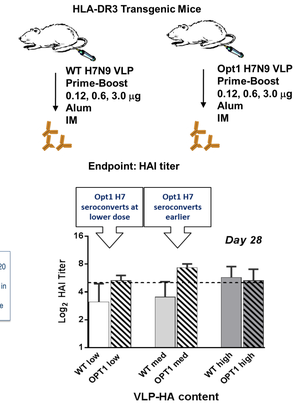
In-vivo Immunogenicity: Humanized Mice

rHA Vaccine



Opt1 rH7-HA stimulates higher anti-H7-HA IgG titers and higher frequencies of anti-H7-HA plasma cells than mice immunized with wild type protein.

VLP Vaccine



Opt1 H7N9 VLP vaccine may protect against H7N9 infection faster and at lower doses than WT vaccine. Modifications of H7-HA in Opt1 preserve neutralizing epitopes.

Second Generation H7N9 HA Engineered to Induce CD4+ T Cell Memory

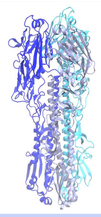
Seasonal Influenza Memory CD4+ T Cell Epitope Selection Utilizing Immunoinformatics Tools

FILTER	TOOL	SELECTION CRITERIA	H1 34,026	H3 38,733
Conservation in H1 and H3	Conservatrix	Conservation in 50% of subtype-specific isolates	528	528
Potential for broad reactivity	EpiMatrix	Potential to bind 4 or more 9 HLA class II supertype alleles	36	29
Reported T cell activity	JanusMatrix	9-mer cross-conservation against IEDB for published results	9	12
Sequence similarity to H7N9	Homology search	>40% identity with corresponding 9-mers in 803 H7N9 HA human isolates identified between 2013 and 2017	8	12
Low potential for T cell cross-reactivity with human sequences	JanusMatrix	Human Janus Homology <2 (9-mers with low potential for inducing regulatory T cell epitopes)	6	8
Increase in immunogenicity and reduced regulatory potential	EpiMatrix and JanusMatrix	Significant increase in immunogenic potential or decrease in regulatory potential compared to H7N9	2	7
No reported T cell activity in H7N9	Sequence alignment	Minimal or no overlap with reported H7N9 T cell epitopes	1	5
Optimal 3D structure	Molecular modeling	Molecular dynamics trajectories closest to wild type H7N9 HA. No structural concerns.	0	5

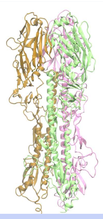
Five seasonal HA CD4+ T cell epitope 9-mers were selected from >34,000 H1-HAs and >38,700 H3-HAs isolated between 1997 and 2017 for:
- **conservation**
- **binding potential to class II supertype alleles**
- **>40% identity with corresponding 9-mer sequences in H7N9 isolates from 2013-2017**
- **T cell activity**
- **low potential for self cross reactivity**

Molecular Modeling of Second Generation H7N9 HA

Wildtype

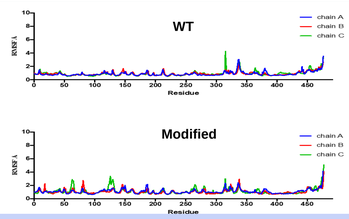


Modified



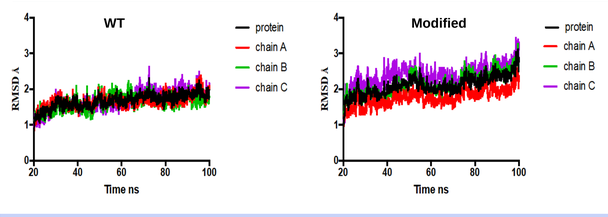
Structural modeling of HA on an H7 backbone with epitope modifications displays no structural instability

Flexibility of Modified HA



Root-mean-squared fluctuations (RMSF) reflects the backbone dynamics for each protein residue. No regions of persistently high fluctuations in Modified HA were observed.

Dynamics and Stability of Modified HA



Root-mean-squared deviation (RMSD) is a measure of the overall dynamics and stability of the system. Both WT and modified versions of HA are predicted to be stable.

Conclusions

- **Treg epitope deletion preserves H7N9 HA antigenicity and increases immunogenicity.**
- **Engineering whole antigens to remove Treg epitopes and carry memory CD4+ T cell epitopes – without perturbing native antigen structure – supports enhanced antibody development against the native antigen.**
- **Structural modeling and molecular dynamics simulation of 2nd Generation H7-HA predicts a stable structure.**

References

1. De Groot AS, Ardito M, Terry F, Levitz L, Ross TM, Moise L, Martin W. Low immunogenicity predicted for emerging avian-origin H7N9: Implication for influenza vaccine design. Hum Vaccin Immunother. 2013 May;9(5):950-6.
2. Liu R, Moise L, Tassone R, Gutierrez AH, Terry FE, Sangare K, Ardito MT, Martin WD, De Groot AS. H7N9 T-cell epitopes that mimic human sequences are less immunogenic and may induce Treg-mediated tolerance. Hum Vaccin Immunother. 2015;11(9):2241-52.
3. Wada, Yamato et al. A Humanized Mouse Model Identifies Key Amino Acids for Low Immunogenicity of H7N9 Vaccines. Sci Rep. 7 (2017): 1283.

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