Cross-reactive influenza H1N1 T cell epitopes identified by immunoinformatic methods stimulate CD4+ T cell responses

Anne S. De Groot[1,2], Christine Boyle[1], Matthew Ardito[1], Frances Terry[1], Howard Latimer[1], Ryan Tassone[1], Mindy Cote[1], Leonard Moise[1,2], William D. Martin[1]

[1]EpiVax, Inc., Providence, RI, USA; [2]Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, USA.

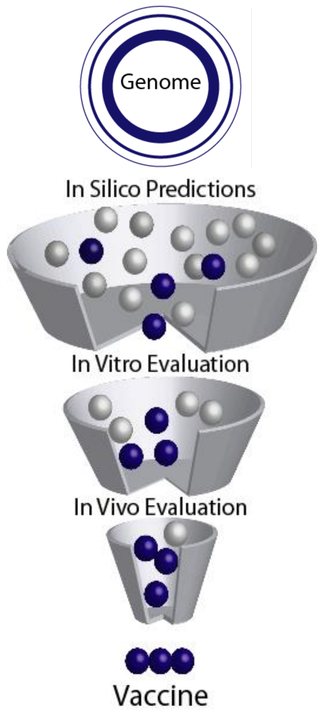
ABSTRACT

Immune responses to cross-conserved T cell epitopes in novel H1N1 influenza might explain reports of diminished influenza-like illnesses and confirmed infection among older adults, in the absence of cross-reactive humoral immunity, during the 2009 pandemic. We set out to identify and characterize cross-conserved H1N1 T cell epitopes to develop a universal H1N1 influenza vaccine.

An immunoinformatic analysis was conducted using all available pandemic and pre-pandemic HA-H1 and NA-N1 sequences dating back to 1980. From 5,738 HA-H1 and 5,396 NA-N1 sequences, 13 HA and 4 NA immunogenic consensus sequences (ICS) were selected that each cover >84% of pre-pandemic and pandemic H1N1 influenza strains, bear EpiMatrix scores ≥95th percentile and cover ≥4 HLA Class II archetypal alleles.

HLA binding assays for 6 Class II archetypal alleles showed that immunoinformatic predictions were 78% accurate. Individual ICS peptides were immunoreactive in cultured human IFNγ ELISpot assays after antigen-specific in vitro expansion. Intracellular cytokine staining showed the magnitude of IL-2-, IFNγ- and/or TNFα-expressing CD4+ T cells was boosted by 2011 seasonal trivalent influenza immunization for vaccine-matched and ICS HA peptide pools.

GENES-TO-VACCINES APPROACH



Genomes are mined using computational and experimental tools to identify genes encoding proteins with promising vaccine antigen properties such as secretion, up-regulated expression, immunogenicity and virulence.

In silico: Immunoinformatics tools are used to map protein sequences for short, linear putative T cell epitopes.

In vitro: Candidates synthesized as peptides and evaluated for MHC binding and antigenicity.

In vivo: Prototype epitope-based vaccines are evaluated for immunogenicity and protection in mice transgenic for human MHC.

IN VITRO CROSS REACTIVITY

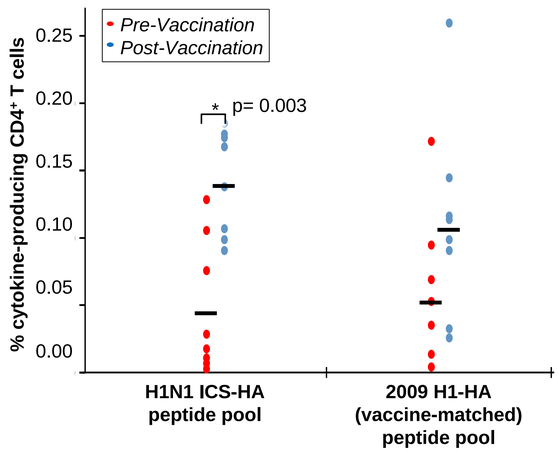
HLA binding assays confirm in silico predictions

ICS Peptide	IC50 (μM) by HLA-DRB1 allele *0101	*0301	*0401	*0701	*1101	*1501
H1-1	18.57	15.51	43.20	8.23	57.46	4.44
H1-2	0.62	0.29	2.66	3.34	10.13	0.12
H1-3	0.96	177.61	6.11	0.28	0.72	0.0016
H1-4	1.08	31.78	1.44	1.07	1.18	0.46
H1-5	7.60	27.15	18.60	0.60	0.30	1.77
H1-6	NB	14.22	NB	232.49	1.99	NB
H1-7	1.41	8.98	1.95	0.26	0.53	0.62
H1-8	0.28	NB	0.79	NB	0.18	0.000003
H1-9	36.45	NB	63.44	0.39	NB	20.08
H1-10	NB	NB	NB	NB	NB	13.80
H1-11	0.14	54.76	6.45	5.68	1.57	0.22
H1-13	68.81	96.27	96.70	4.47	2.94	0.54
H1-19	0.47	95.60	87.13	NB	NB	0.36
N1-1	0.0027	NB	3.48	0.16	12.60	6.36
N1-2	11.55	36.33	29.41	NB	41.28	0.76
N1-3	14.23	4.13	7.38	3.21	1.15	22.25
N1-4	11.56	24.17	29.70	29.07	6.68	0.11

	From:	To:
Very High Affinity	-	0.1
High Affinity	0.1	1
Moderate Affinity	1	10
Low Affinity	10	100
Very Low Affinity	100	-

- Of 108 ICS peptide-HLA binding interactions assayed, 3% bound with very high affinity, 23% with high affinity, 26% with moderate affinity, 30% with low affinity, 2% with low affinity, and 15% had no affinity for the HLA tested.
- Overall, the proportion of true positive and true negative predictions is 83%.

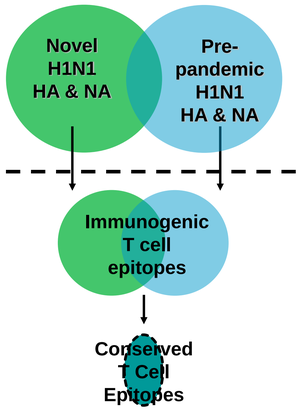
Cross-reactivity of H1N1 ICS-specific CD4+ T cells



- Total cytokine production of human CD4+ T cells following HA peptide pool stimulation of PBMCs collected before and 3 weeks after 2011 TIV vaccination.
- Vaccination boosts HA-specific CD4+ T cell frequencies.
- Vaccine mismatched H1N1 ICS HA peptides are antigenic, suggesting that they are broadly reactive sequences.

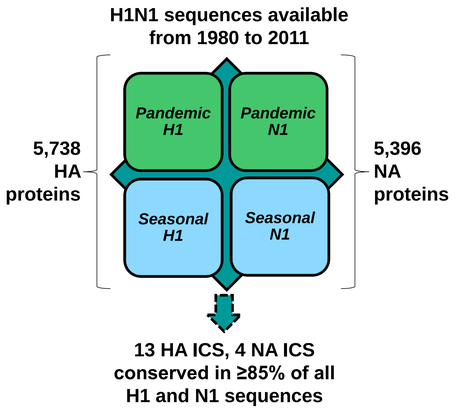
IN SILICO CROSS REACTIVITY

Epitope selection approach



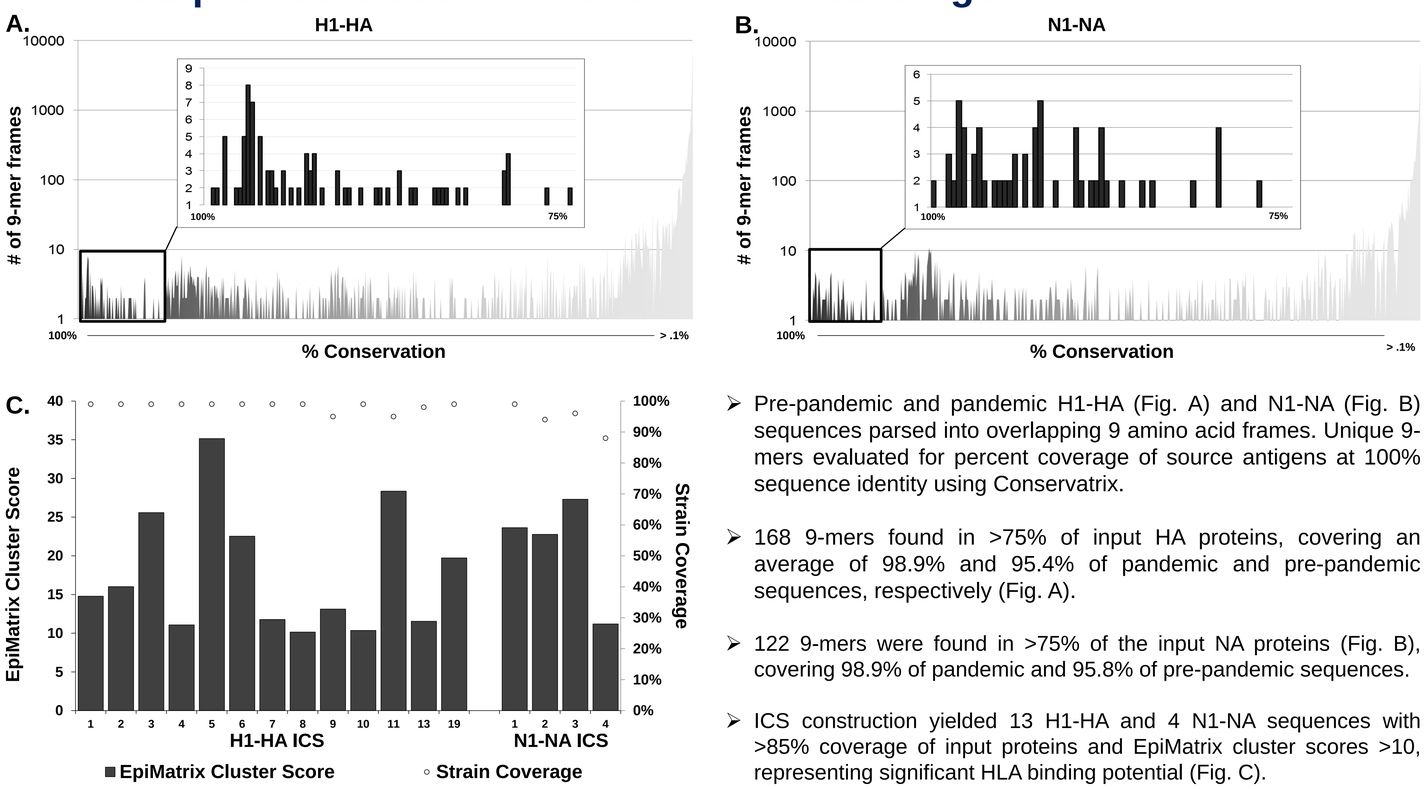
- Cross-reactive influenza H1N1 HA and NA T-cell epitopes may have contributed to low virulence observed in the 2009 pandemic.
- These sequences may contribute to universal protection across the H1N1 subtype.
- Cross-conserved H1N1 HA and NA T-cell epitopes predicted to bind to eight Class II archetypal HLA alleles were identified using EpiMatrix.

Construction of H1N1 ICS



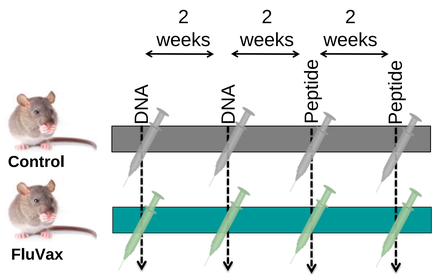
- 13 HA, 4 NA ICS with promiscuous HLA binding potential, EpiMatrix cluster scores >10, and conservation among >85% of input proteins selected for further evaluation.

H1N1 ICS provide broad H1N1 and HLA DR coverage

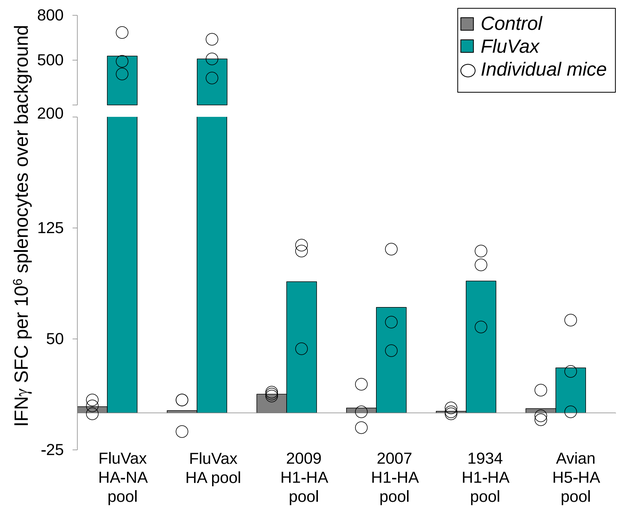


- Pre-pandemic and pandemic H1-HA (Fig. A) and N1-NA (Fig. B) sequences parsed into overlapping 9 amino acid frames. Unique 9-mers evaluated for percent coverage of source antigens at 100% sequence identity using Conservatrix.
- 168 9-mers found in >75% of input HA proteins, covering an average of 98.9% and 95.4% of pandemic and pre-pandemic sequences, respectively (Fig. A).
- 122 9-mers were found in >75% of the input NA proteins (Fig. B), covering 98.9% of pandemic and 95.8% of pre-pandemic sequences.
- ICS construction yielded 13 H1-HA and 4 N1-NA sequences with >85% coverage of input proteins and EpiMatrix cluster scores >10, representing significant HLA binding potential (Fig. C).

IN VIVO CROSS REACTIVITY



- **FluVax is immunogenic:** Significant influenza-specific IFNγ recall responses seen in vaccinated mice restimulated with H1N1 epitope pool.
- **FluVax is broadly reactive:** Vaccine-stimulated T cells react with unmatched 2009 H1-HA, 2007 H1-HA, and 1934 H1-HA influenza peptide pools.



CONCLUSIONS

- ✓ **Immunoinformatic-predicted, conserved influenza H1-HA and N1-NA sequences bind multiple HLA alleles.**
- ✓ **H1N1 ICS cross-react with CD4+ T cells from 2011 TIV donors, suggesting that they are broadly reactive antigens.**
- ✓ **FluVax is immunogenic in HLA DR3 transgenic mice and generates T cells that recognize *non-matched* H1-HA seqeunces.**
- ✓ **The conserved epitope approach promises to answer the need for prompt preparedness and delivery of a safe, efficacious, universal influenza vaccine that will protect against antigenically novel influenza viruses.**

Support for this work is funded by an R21 award AI090359 to PI De Groot from the National Institutes of Health.