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

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OVERVIEW

Immune responses to cross-conserved T cell epitopes in H1N1 influenza might explain reports of diminished influenza-like illnesses among older adults during the 2009 flu pandemic. Thus we set out to identify and characterize cross-conserved H1N1 T cell epitopes to develop a universal H1N1 flu vaccine.

An immunoinformatic analysis was conducted using all available pandemic and pre-pandemic HA-H1 and NA-N1 sequences dating back to 1980 to construct immunogenic consensus sequences (ICS).

Binding of the synthesized ICS to HLA Class II alleles showed that our in silico predictions were highly accurate.

Intracellular staining of peripheral blood mononuclear cells (PBMCs) showed that pooled ICS-HA peptides elicited a significant cytokine-expressing CD4+ T cell recall response after donor immunization with 2011 seasonal trivalent influenza.

H1LNA transgenic mice develop cross-reactive immune response upon immunization with ICS epitopes (FluVax), showing that our vaccine design approach is effective.

GENE-TO-VACCINE APPROACH

Genomes are mined using computational and experimental tools to identify genes encoding proteins with promising vaccine antigen properties.

In silico immunoinformatic tools are used to map protein sequences for T cell epitopes.

In vitro, candidate T cell epitopes are 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 IMMUNOREACTIVITY

HLA binding assays to test predicted epitope breadth

<table>
<thead>
<tr>
<th>ICS Peptide</th>
<th>IC50 (µM) by HLA-DRB1 allele</th>
<th>Affinity</th>
<th>% Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1-1</td>
<td>4.85</td>
<td>Very High Affinity</td>
<td>100%</td>
</tr>
<tr>
<td>H1-2</td>
<td>5.65</td>
<td>High Affinity</td>
<td>95%</td>
</tr>
<tr>
<td>H1-3</td>
<td>6.45</td>
<td>Moderate Affinity</td>
<td>85%</td>
</tr>
<tr>
<td>H1-4</td>
<td>7.25</td>
<td>Low Affinity</td>
<td>75%</td>
</tr>
<tr>
<td>H1-5</td>
<td>8.05</td>
<td>Very Low Affinity</td>
<td>65%</td>
</tr>
<tr>
<td>H1-6</td>
<td>9.85</td>
<td>Non-binding</td>
<td>55%</td>
</tr>
<tr>
<td>H1-7</td>
<td>11.65</td>
<td>Non-binding</td>
<td>45%</td>
</tr>
<tr>
<td>H1-8</td>
<td>13.45</td>
<td>Non-binding</td>
<td>35%</td>
</tr>
<tr>
<td>H1-9</td>
<td>15.25</td>
<td>Non-binding</td>
<td>25%</td>
</tr>
<tr>
<td>H1-10</td>
<td>17.05</td>
<td>Non-binding</td>
<td>15%</td>
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<tr>
<td>H1-11</td>
<td>18.85</td>
<td>Non-binding</td>
<td>5%</td>
</tr>
</tbody>
</table>

Overall, the proportion of true positive and true negative predictions is 83%, which is equal or greater accuracy than other prediction tools.

H1N1 ICS-HA peptides are antigenic for most subjects, suggesting that they are broadly reactive sequences.

H1N1 ICS-HA peptides elicit a significant T cell recall response, suggesting that they may be effective against influenza.

IMMUNOREACTIVITY OF H1N1 ICS-SPECIFIC CD4+ T CELLS

In vitro results demonstrate that our vaccine design approach is effective.

ICS bind to HLA alleles with 83% accuracy, validating our in silico predictions.

ICS-HA peptides react with CD4+ T cells from 2011 trivalent influenza vaccinated donors, suggesting that they are broadly reactive antigens.

FluVax is immunogenic in HLA DR3 transgenic mice and generates T cells that recognize non-matched ICS-HA sequences, showing cross-reactivity.

In vivo results demonstrate that our vaccine design approach is effective.

CONCLUSIONS

- ICS bind to HLA alleles with 83% accuracy, validating our in silico predictions.
- ICS-HA peptides react with CD4+ T cells from 2011 trivalent influenza vaccinated donors, suggesting that they are broadly reactive antigens.
- FluVax is immunogenic in HLA DR3 transgenic mice and generates T cells that recognize non-matched ICS-HA sequences, showing cross-reactivity.
- In vivo results demonstrate that our vaccine design approach is effective.

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

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