T cell epitopes in Autoimmune Hepatitis Type 2: developing biomarkers and novel therapeutics

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What is Autoimmune Hepatitis Type 2?
- Chronic autoimmune disease where self-reactive T cells direct an immunological attack on the liver
- Autoantibodies against the liver protein Cytochrome P450 2D6 exacerbate liver damage leading to fibrosis, cirrhosis and liver failure
- Diagnosed by the presence of LKM-1 autoantibodies, lymphocytic infiltration, elevated ALT, lack of viral infection, concomitant autoimmune diseases, high IgG
- Lack of specific treatment: patients prescribed broad range immunosuppressant drugs (azathioprine, corticosteroids) - high incidence of drug toxicity or intolerance

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HLA A*</th>
<th>HLA B*</th>
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<tbody>
<tr>
<td>Autoantibody production</td>
<td>anti-mitochondrial antibody (AMA), anti-smooth muscle antibody (ASMA)</td>
<td>Liver-kidney microsomal type 1 (LKM-1), smooth muscle antibodies (AMA)</td>
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<td>Age at diagnosis</td>
<td>Middle age</td>
<td>Middle-aged or elderly patients</td>
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<td>Treatment refractory</td>
<td>Liver failure</td>
<td>High risk</td>
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<td>Prevalence</td>
<td>1.0-2.0 cases per 100,000</td>
<td>3-6 cases per 100,000</td>
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<td>HLA-DR associations</td>
<td>DR1, DQ1</td>
<td>DR4, DQ8</td>
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- CD4+ T cells are the key player in establishing an autoimmune response to CYP2D6 liver protein

CD4+ T cells

In health, T cells will only attack "outsiders" - helping to clear bacterial or viral infections

In autoimmune diseases, T cells wrongly target the "self", causing damage to the body’s tissues

The autoantigen – CYP2D6

Computational sequence analysis

Aim: To "re-educate" disease causing T cells to become non-responsive to the autoantigen - establishing immune tolerance

Methods:
- Antigen desensitisation by repeated and escalating dose – similarly to allergen desensitisation.
- Use synthetic peptides that mimic disease relevant peptides from the autoantigen - APITOPES
- Antigenic peptides are monitored by T cell receptors on T cells to generate an immune response
- APITOPES do not cause immune response but drive T cells into a non-responsive, suppressive state

Outcomes:
- Disease causing T cells are specifically "switched off"
- Immune function against pathogens and cancer is maintained
- Reduced need for immunosuppressive drugs
- Safe, well tolerated and highly effective in clinical trials treating Multiple Sclerosis and Graves Disease patients

Peptide immunotherapy – antigen specific treatment

Experimental set-up

Results

Immune responses to CYP2D6 regions in healthy donors
Highest immunogenicity:
1) CYP2D6 22-91
9) CYP2D6 225-244
6) CYP2D6 266-286
3) CYP2D6 225-114

Immune responses to CYP2D6 peptides 1, 2, 3 & 9 in a HLA-DR4 restricted system

Peptides 1, 2 & 3 generate strong immune responses when presented in the context of HLA-DR4 but peptide 9 does not

A wide variety of regions within the autoantigen CYP2D6 are immunogenic even in healthy donors

Testing immunogenicity of candidate peptide in patients

Selection of immunogenic peptides for therapeutic design

Epitope mapping to locate minimum required T cell epitopes using HLA-DR3 and HLA-DR4 restricted systems

Design of highly soluble, pan-DR binding, immunomodulatory peptides (epitopes) which can induce T cell tolerance to CYP2D6 antigen and have therapeutic potential for AIH2 patients

Development of tetramer technology for rapid identification of autoreactive T cells in AIH2 and early disease diagnosis

Conclusions and future directions