The development and validation of HLA transgenic mice
Outline

• MHC molecules. Antigen presentation and Disease association
• HLA transgenic mice. Historical views and the issues of CD4/CD8 and endogenous mouse expression
• Validating studies. Processing and Immunodominance
• Transgenic expressing different alleles. The concept of HLA Superytypes
• Experimental details. Breeding, Testing and Study Design
Disclaimer

• The following slides are an informal account of my personal experience in developing and using HLA transgenics from the 90’s to date

• My opinions are not necessarily reflective of Taconics opinions, nor do I necessarily endorse their mice
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Class I and Class II MHC genes
Class I and Class II Differences

• Class I
  – Expressed on surface of all nucleated cells and platelets
  – Present antigens to CD8+ T cells
  – Antigens derived from cytosolic proteins (“endogenous” antigens)

• Class II
  – Expressed mostly on surface of professional antigen presenting cells such as B cells, macrophages and dendritic cells
  – Present antigens to CD4+ T cells
  – Antigens derived from proteins present in endosomes or lysosomes (“exogenous” antigens)
The issue of HLA polymorphism (polygenic, polylocus and allelic)

- http://hla.alleles.org/nomenclature/index.html
The issue of HLA polymorphism (Variation is in the pockets)
## Associations of HLA Serotype with Susceptibility to Autoimmune Disease

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>HLA allele</th>
<th>Relative Risk</th>
<th>Sex Ratio (♀ : ♂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankylosing Spondylitis</td>
<td>B27</td>
<td>37.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Acute Anterior Uveitis</td>
<td>B27</td>
<td>10</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Goodpasture’s Syndrome</td>
<td>DR2</td>
<td>15.9</td>
<td>~1</td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>DR2</td>
<td>4.8</td>
<td>10</td>
</tr>
<tr>
<td>Graves’ Disease</td>
<td>DR3</td>
<td>3.7</td>
<td>4:5</td>
</tr>
<tr>
<td>Myasthenia Gravis</td>
<td>DR3</td>
<td>2.5</td>
<td>~1</td>
</tr>
<tr>
<td>Systemic Lupus Erythematosus</td>
<td>DR3</td>
<td>5.8</td>
<td>10:20</td>
</tr>
<tr>
<td>Type 1 Insulin-dependent Diabetes Mellitus</td>
<td>DR3/DR4 heterozygote</td>
<td>~25</td>
<td>~1</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>DR4</td>
<td>4.2</td>
<td>3</td>
</tr>
<tr>
<td>Pemphigus Vulgaris</td>
<td>DR4</td>
<td>14.4</td>
<td>~1</td>
</tr>
<tr>
<td>Hashimoto’s Thyroiditis</td>
<td>DR5</td>
<td>3.2</td>
<td>4:5</td>
</tr>
</tbody>
</table>
HLA molecules and disease resistance

HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. Stephen A. Migueles,† et al.

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History of HLA transgenic mice

• Some of the early stuff
  – HLA-restricted recognition of viral antigens in HLA transgenic mice 1987 H. Ploegh
  – B7 (Chamberlain), Cw3 (Hammerling), A2 (Engelhard); 1988

• But….
  – “the frequency of HLA-A2.1-restricted, influenza specific CTLp was substantially lower than the frequency of H-2b restricted CTLp, indicating a poor utilization of HLA-A2.1 as a restricting element…”
The issue of CD4 and CD8
Two solutions

- Make transgenics also for Hu CD8 or CD4
- Make mice expressing chimeric humanA1/A2 but mouse A3 domains
The issue of co-expression of murine MHC molecules

- Murine MHC is generally expressed at higher levels
- Potential for inhibiting HLA restricted responses (immundominance)
- Solution: make mice that express only the HLA transgene (no H2)
- Lemonnier and collaborators pioneered for Class I; Chella David for class II
Some additional considerations and personal viewpoints

• Mice without MHC expression have substantial alterations in thymic education
• Heterozygosity does not seem to impair responses
• In my personal experience lack of expression of murine MHC appears necessary for class II, but not at all for class I
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Validation of the mice

- Expression of HLA does not automatically make mice a good model for HLA responses
- Is processing the same?
- Is dominance the same?
Is processing the same? It depends...

• Remarkable similarity between human and murine processing apparatuses
• Similar natural processed ligands
  – Falke and coworkers showed this to be the case for HLA A2*01
• In general this is a good approximation but...
  – TAP differences exist between murine and human molecules
  – Positive charged C-termini not efficiently transported by mouse TAP
• Caution with HLA-A11 and other molecules
Is Immunodominance the same? It depends…..

• The issue was experimentally addressed by several studies

• Wentworth et al compared the repertoire of HLA transgenic mice and humans

• Additional studies for several other strains showing that in general immunogenicity is very similar

• But beware of self/nonself (important for tumor vaccines)

• However…the answer for immunodominance is a bit more complex
Immunodominance and HLA transgenic mice

• The more complex the system, the more complex the situation

• Also immunodominance in humans is highly variable

• The case of VACV
Immunodominance in VACV

Vaccinia virus-derived T cell epitopes

While transgenic HLA mouse models are suitable to model responses to complex pathogens, care should be taken in interpreting the results obtained.

*Immunome Res. 2009; 5:3*
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HLA Supertypes

• Defined as families of HLA molecules with overlapping (but distinct) repertoires
• Relevance to diagnostics and vaccines because allow to rationally address the diversity in human populations (and ethnicities)
• Originally described for HLA class I, also apply to HLA class II
Few Major Supertypes Account for the Majority of HLA A and B Types

<table>
<thead>
<tr>
<th>SPECIFICITY</th>
<th>Position 2</th>
<th>C-terminus</th>
<th>PHENOTYPIC FREQUENCY *</th>
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<tbody>
<tr>
<td></td>
<td>Supertype</td>
<td></td>
<td>Caucasian</td>
</tr>
<tr>
<td>A1</td>
<td>TILVMS</td>
<td>FWY</td>
<td>47.1</td>
</tr>
<tr>
<td>A2</td>
<td>AILMVT</td>
<td>AILMVT</td>
<td>45.8</td>
</tr>
<tr>
<td>A3</td>
<td>AILMVST</td>
<td>RK</td>
<td>37.5</td>
</tr>
<tr>
<td>A24</td>
<td>YFWIVLMT</td>
<td>FYWLM</td>
<td>23.9</td>
</tr>
<tr>
<td>B7</td>
<td>P</td>
<td>AILMVFWY</td>
<td>43.2</td>
</tr>
<tr>
<td>B44</td>
<td>E</td>
<td>FWYLIMVA</td>
<td>43.0</td>
</tr>
<tr>
<td>B27</td>
<td>RHK</td>
<td>FYLWMI</td>
<td>28.4</td>
</tr>
<tr>
<td>B62</td>
<td>QLIVMP</td>
<td>FWYMIV</td>
<td>12.6</td>
</tr>
<tr>
<td>B58</td>
<td>ATS</td>
<td>FWYLIV</td>
<td>10.0</td>
</tr>
<tr>
<td>A1, A2, A3 and A24 only</td>
<td></td>
<td></td>
<td>98.4</td>
</tr>
<tr>
<td>A1, A2, A3, A24, B7 and B44 only</td>
<td></td>
<td></td>
<td>99.5</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td>99.9</td>
</tr>
</tbody>
</table>

* For derivation of phenotypic frequencies see Sidney et al., 2001.
HLA class II supertypes

Greenbaum et al Immunogenetics 2011
Relevance Of HLA Supertypes for Diagnostics & Epitope Identification

- The frequency of each allele in a supertype may vary dramatically amongst different populations, but the frequency of each supertype is relatively constant.
# HLA transgenics and Class I Supertypes

<table>
<thead>
<tr>
<th>Supertype</th>
<th>Prototype Allele</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>A*0101</td>
<td>Taconic</td>
</tr>
<tr>
<td>A2</td>
<td>A*0201</td>
<td>Taconic</td>
</tr>
<tr>
<td>A3</td>
<td>A*1101</td>
<td>Taconic</td>
</tr>
<tr>
<td>A24</td>
<td>A*2401</td>
<td>Taconic</td>
</tr>
<tr>
<td>B7</td>
<td>B*0702</td>
<td>Taconic</td>
</tr>
<tr>
<td>B27</td>
<td>B*2701</td>
<td>C.David; Chamberlain</td>
</tr>
<tr>
<td>B44</td>
<td>B*4002</td>
<td>Taconic</td>
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It would be nice to have either a B57/58 or a B62 supertype representative (B*1501)
## HLA transgenics and Class II Supertypes

<table>
<thead>
<tr>
<th>Supertype</th>
<th>Prototype Allele</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR1</td>
<td>B1*0101</td>
<td>Altmann</td>
</tr>
<tr>
<td>DR4</td>
<td>B1*0401</td>
<td>Taconics, Sonderstrup, David</td>
</tr>
<tr>
<td>DR3</td>
<td>B1*0301</td>
<td>Altmann</td>
</tr>
<tr>
<td>DPmain</td>
<td>?</td>
<td>Tsuji K (?)</td>
</tr>
<tr>
<td>DP2</td>
<td>?</td>
<td>Tsuji K (?)</td>
</tr>
<tr>
<td>DQmain</td>
<td>DQ2 and DQ8</td>
<td>David</td>
</tr>
<tr>
<td>DQ7</td>
<td>DQ6</td>
<td>David</td>
</tr>
</tbody>
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Experimental details. Breeding, Testing and Study Design

- Strain maintenance:
  - We prefer keeping the breeders on the H2b (C57BL/6) background (either with or without murine expression)
  - But for experimental animals we prefer to use BDF1 (BALB/c x C57BL/6 F1) as they breed better and have better responses
Experimental details. Breeding, Testing and Study Design

• Testing Empiricals
  – Either PCR or FACS work
  – PCR for Class II, FACS for class I
  – But it is best to use FACS for breeders as expression tend to drift lower
Example of testing DRB1*0101 expression by PCR
Experimental details. Breeding, Testing and Study Design

• Study Design Tips
  – In general in the case of mice expressing murine *domains* tetramers do not work well (remember…CD4/8 issues)
  – In the case of mice co-expressing murine *molecules* need to establish restriction
    • Best show that T cells recognize an APC expressing the transgene AND do not recognize one not expressing the transgene
    • Cases of dual restriction exist…. 
Some specific examples of HLA transgenic mice use

• Vaccine testing
  – Use in testing and releasing human vaccines for which an epitope based response is expected

• Animal models
  – In vivo study of responses against specific human pathogens
HLA transgenic mice can be used to develop and test vaccine constructs

- CTL responses important for vaccines targeting diseases such as hepatitis B and C, HIV and cancer
- Typical vaccines based on killed or inactivated pathogens or recomb. or purified proteins are ineffective at inducing CTL responses
- Potential solution is small multiepitope peptides which can induce CTL and HTL responses

<table>
<thead>
<tr>
<th>Epitope</th>
<th>Sequence</th>
<th>MHC Restriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBVpol149</td>
<td>HTLWKAGILYK</td>
<td>A11</td>
</tr>
<tr>
<td>PADRE</td>
<td>AKFVAAWTLKAAA</td>
<td>IA²</td>
</tr>
<tr>
<td>HBVcore18</td>
<td>FLPSDLFFPSV</td>
<td>A2.1</td>
</tr>
<tr>
<td>HIVenv120</td>
<td>KLTPLCVTL</td>
<td>A2.1</td>
</tr>
<tr>
<td>HBVpol551-V</td>
<td>YMDDVVLGV</td>
<td>A2.1</td>
</tr>
<tr>
<td>HBVpol455</td>
<td>GLSRYVARL</td>
<td>A2.1</td>
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<tr>
<td>HIVpol476</td>
<td>ILKEPVHG</td>
<td>A2.1</td>
</tr>
<tr>
<td>HBVcore141</td>
<td>STLPETTVVR</td>
<td>A2.1</td>
</tr>
<tr>
<td>HIVenv49</td>
<td>TVYYGVPVK</td>
<td>A11</td>
</tr>
<tr>
<td>HBVenv335</td>
<td>WLSLLVPFV</td>
<td>A2.1</td>
</tr>
</tbody>
</table>

Experimental Setup

- HLA transgenic mice immunized
- Splenocytes harvested 11-14 days later and cultured in vitro
- Splenocytes stimulated once with the appropriate epitope (A2.1- or A11-restricted)
- IFN-γ production or lysis of peptide-coated cells measured
Multiepitope peptides are more immunogenic in HLA transgenic mice compared to individual epitopes. Splenocytes from immunized HLA transgenic mice are cytotoxic.

HLA transgenic mice can be used to identify vaccine development strategies.

Immunogenicity is a function of both dose and immunogen size.

HLA transgenic mice for use in epitope identification

• HLA transgenic mice can be used to identify epitopes and study protective capacity of human epitopes from lethal challenges

• HLA transgenic mice can be used in combination with epitope predictions and in vitro HLA binding assays for epitope identification

• Choose relevant HLA transgenic mice based on the human ethnic/regional populations of interest
Arenavirus epitope identification using HLA transgenic mice

- Arenavirus protein sequences first screened in silico to identify potential peptides with HLA-A02 affinity of $\leq 100$ nM: 481 peptides identified

- HLA-A2.1 transgenic mice were infected with recombinant vaccinia viruses (rVACV) expressing individual arenavirus proteins or a control wt vaccinia virus

- Purified splenic CD8+ T cells later isolated from these mice and exposed to a human cell line expressing HLA-A2.1, which had been pulsed with one of the 481 identified peptides

- 12 peptides identified as antigenic
Arenavirus epitope identification using HLA transgenic mice

- HLA-A2.1 mice then immunized with the 12 identified peptides (individually)
- Splenic CD8+ T cells isolated and assayed for recognition of the human target cells expressing the appropriate arenavirus protein antigen
- 7 of the 12 peptides displayed robust response and thus are processed naturally in human APCs
Challenge assays in HLA transgenic mice can confirm utility of identified epitopes

• HLA-A2.1 mice primed with pool of HLA-A2-restricted arenavirus peptides
• Mice then challenged with rVACV expressing arenavirus proteins and viral titer determined

Acknowledgements

- Daisy John
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