Immunogenetics in Vaccine Design and Pharmacogenetics

M. Sue Leffell, Ph.D.
Johns Hopkins University
School of Medicine

Vaccine Achievements

- Vaccination is one of greatest public health achievements
- Almost compete eradication of some diseases
- U.S. Lifespan increased by 30 years from 1900 -1999
  - This was due largely to vaccines
- Why do we need new vaccines?
Reasons for Vaccine R&D

- Global health concerns
  - Malaria
  - HIV
  - Tuberculosis
- Antibiotic resistance
  - MRSA
- Cancer vaccines
- Bioterrorism
- Emergent pathogens
  - HIV, SARS
  - H1N1

Genetic Variation

Within populations
- High and low responders to MMR, HBV
- High immune response heritability to some viruses, e.g. Measles

Between populations
- American Indians and Native Alaskans – poor responders to Haemophilus influenzae type b, pneumococcal vaccines
- Amazon Natives – high responders to MMR
Immunogenetic Polymorphism Affecting Vaccine Responses

Components of Innate and Adaptive Immunity:
- Cell surface receptors
- Activation/signaling molecules
- Cytokines and cytokine receptors
- HLA and KIR molecules

HLA Associations – Vaccines
Early Studies

- Seronegativity or low Aby response to HBV
  - DR7, DQ3 alleles
- Non-responsiveness to trivalent influenza vaccine
  - DR7 alleles
- Strong lymphoproliferative response to Rubella
  - DR1,11; DQ5 (negative association with DR7, DQ2)
### HLA Associations: Response to Measles Vaccine

<table>
<thead>
<tr>
<th>HLA Gene(s)</th>
<th>Associations</th>
<th>Effect on IgG Aby¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alleles</td>
<td>B8, B13, B44</td>
<td>Decreased</td>
</tr>
<tr>
<td></td>
<td>B7</td>
<td>Increased</td>
</tr>
<tr>
<td></td>
<td>DRB1<em>03, DQA1</em>0201</td>
<td>Decreased</td>
</tr>
<tr>
<td></td>
<td>DRB1<em>08, DQA1</em>0104</td>
<td>Increased</td>
</tr>
<tr>
<td>Haplotypes</td>
<td>A24; Cw3; B15</td>
<td>Decreased</td>
</tr>
<tr>
<td></td>
<td>A26; Cw12; B38</td>
<td>Increased</td>
</tr>
<tr>
<td></td>
<td>DR7; DQ2/3; DP2/4</td>
<td>Decreased</td>
</tr>
<tr>
<td></td>
<td>DR15/16; DQ6; DP4</td>
<td>Increased</td>
</tr>
</tbody>
</table>

¹ Significant associations, P<0.05

Adapted from Poland, et al. Vaccine. 2008; 26:6183

### HLA Heterozygosity

- HLA heterozygosity should theoretically increase antigen presenting repertoire.
- Significant association between homozygosity at class I loci and HIV progression ([Carrington, et al, 1999](#)).
- Minimal data on HLA homozygosity and vaccine responses ([Poland, et al, 2007](#)).
  - HLA class Ib and DQA1 – lower Aby responses to measles (single dose)
Obstacles to Vaccine Development

- Pathogen escape mechanisms
  - Alterations in antigenicity
  - Mutations affecting HLA and TCR binding
    - Cytotoxic T cell (CTL) response
    - NK response

Altered Peptide Ligand Antagonism

- Some viruses and parasites vary their antigens during the course of an infection as a means of immune evasion. (mechanisms - gene rearrangement; alternative RNA splicing)
- New antigenic strains may also suppress responses to original virus strain(s).
  - Peptides from variants of HIV, Hep B suppress CTL response to original strain.
Altered Peptide Ligands
Malaria - Gilbert, Hill, et al.'98

- B35 restricted CTLs recognize polymorphic epitope of the circumsporozoite protein.
- cp26, cp29 are B35 restricted octamers that differ at 1 position - but are mutually inhibitory of CTLs.
- Co-infection with more than 1 strain in 41% of patients. cp26 and cp29 co-infect > 2x more frequently than expected.
- Mutual antagonism favors ↑ frequency of these strains in population.

HIV/AIDS

- Prime model for difficulties in vaccine development
- Complex interactions between polymorphic virus and polymorphic immune system (HLA and KIR)
- Both HLA and KIR genotypes may need to be considered in HIV vaccine designs
HLA Influence: HIV-1 Adaptation
Moore et al., 2002

• Critical part of HIV pathogenesis is ability to mutate → escape CTL.
• Examined HLA associations with reverse transcriptase sequences.
• Mutations most often at sites of least functional/structural constraint.
• These mutations were increased in association with certain class I alleles.

• B*57 restricts 3-4 immunodominant GAG epitopes in conserved regions
• Viral escape mutants often observed in these regions, but have reduced viral fitness
• B*27 restricted response to 1 conserved GAG epitope (also protective, but less so than B*57)
HIV-1 Gag Protein Substitutions: CD8+ T Cell Epitope


- Positions 1, 2, 4, 9 invariant
- Escape mutations occur at positions 3, 6, 8 – affecting TCR binding.

Optimizing Vaccine Design

1. Identify protective, immunogenic pathogen epitopes
2. Maximize MHC binding of pathogen epitopes
3. Optimize antigen processing of multi-epitope constructs
4. Ensure adequate population coverage

1. B53 associated with resistance to cerebral malaria; B53 eluted peptides – proline at P2
2. Candidate nonapeptides with P2 proline found in Plasmodium falciparum sequences; Tested for MHC binding
3. Strong MHC binding peptides tested for stimulation of T cell proliferation.

Immune Epitope Mapping

- Cells from immunized donors tested for response to viral peptides:
  - Peptides eluted from MHC of infected cells
  - Peptides predicted by HLA binding motifs
- Recent studies with:
  - HCV - from individuals with resolved infection
  - CMV - from seropositive donors
  - VACV - from smallpox vaccinated donors

Computerized predictions of subunit or peptide vaccines

- Computational approach depends upon growing databases of peptide/allele motifs.
  - MHCPEP (www.ncbi.nlm.nih.gov/gv/mhc/)
  - SYFPEITHI (www.syfpeithi.de/)
- Algorithms based on:
  - Binding motifs & allele pockets
  - Affinity of different amino acids for binding pockets.

Optimization: Antigen Processing

- Peptide epitopes generated by proteolytic cleavage
- Directed by residues before and after the peptide epitope sequence
  - Class I – residue just after C terminus (C+1) is most influential
- For multi-epitope constructs, “spacers” may be needed for response to all epitopes

Class I Processing:

Optimal Response when C+1 = Pos charge residue – lys, arg
Amides – gln, asn
Poor response when C+1 = Neg charge – asp, glu

Class II Processing:

Simple “string of beads” construct may result in response to only epitopes 1 and 3.

Introduction of GPGPG spacers → response to all 3 epitopes.

What about HLA Polymorphism?

- Many vaccines are under development using peptides restricted to common HLA alleles.
  - Eg., A*0201, B*0702
- What about racial/ethnic differences in HLA allele distribution?
Racial Differences in HLA-A Distribution

HLA-A Locus Alleles

9 Supertypes ~ >90% of known HLA alleles

- HLA-A1, 2, 3, 24
- HLA-B7, 27, 44, 58, 62

80-90% coverage in major ethnicities with:
- HLA-A1, 2, 3, 24; B7

Algorithm to predict population coverage based on MHC binding, T cell restriction data, and HLA genotype frequencies.

Single Chain MHC Class I Molecules as DNA Vaccines

- SCTs – single chain trimers are constructs of a single polypeptide chain consisting of antigenic peptide, β2m, cl I heavy chain.
- SCTs offer exciting new prospects for effective vaccines
- Autologous APC could be “engineered” for customized vaccines

Requirements for Effective T Cell Responses

- Processing by APC and presentation on cell surface in sufficient quantity to bind TCRs
- Expression on cell surface must be stable long enough to permit TCR binding
- Affinity of TCR binding to MHC: peptide must be sufficient for activation
SCT Constructs

Adapted from; Hansen, TH, Gillanders W, Connolly JM. ASHI Quarterly. 2010;34:12

Constructs with Improved Efficacy

Adapted from: Li L, et al. Vaccine. 2010;28:1911-18

Disulfide bond between peptide C terminus and HC “anchors” peptide

CD8+ T cell interaction ↑ by substitutions at HC site in α2 domain

Co-expression of “universal” DR epitope, PADRE, → CD4+ T Cell activation
SCT Features for Better DNA Vaccines

- Antigen delivery into class I processing is limiting factor of DNA vaccines
- Concern for CA vaccines, as many tumor antigens are not processed or presented effectively
- MHC class I SCTs bypass antigen processing
- Enhance the response to low affinity antigens
- Universal CD4 epitope ensures effective response
- SCTs engineered to interact with CD8 generate more effective CTL

Hansen TH, Yu YY, Fremont DH. Cur Protoc Immunol 2009; 17.5

SCT Applications

- Cancer Vaccines
  - Breast CA – epitope of mammaglobin-A
  - Ovarian CA – epitope of mesothelin
  - Cervical CA – epitope from papillomavirus
- Infectious Disease
  - Construct of HLA-A2 + HepB epitope
- Xenografts
  - Transfection of HLA-E + certain peptides into pig cells prevents lysis by human NK
Pharmacogenomics of Adverse Drug Reactions

- Serious adverse drug reactions (ADRs) are major cause of morbidity and mortality worldwide
- Idiosyncratic, immune mediated
- Identification of predisposing genotypes
  - Safer patient management
  - ADR pathogenesis

Immunologic Mechanisms of Drug Reactions

- Adverse reactions with HLA associations:
  - T cell mediated (Type IV -delayed hypersensitivity)
  - MHC restricted response to drug
- IgE immediate hypersensitivity
  - Stimulated by metabolite or drug-derived hapten (Type I)
Types of Delayed Drug Hypersensitivity Reactions

- Drug-induced hypersensitivity syndrome (DIHS)
- Drug reaction with eosinophilia and systemic symptoms (DRESS)
- Single organ drug induced diseases:
  - Drug induced liver disease (DILI)
  - Stevens-Johnson Syndrome (SJS)
  - Toxic epidermal necrolysis (TEN)

<table>
<thead>
<tr>
<th>Syndrome / Drug</th>
<th>HLA- Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxic epidermal necrolysis / Stevens-Johnson syndrome</td>
<td>B*58:01</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>B*58:01</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>B*15:02 (East Asians)</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>B38</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>B*15:02</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>B38</td>
</tr>
<tr>
<td>Drug Hypersensitivity/DIHS/DRESS</td>
<td></td>
</tr>
<tr>
<td>Abacavir</td>
<td>B*57:01</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>B*58:01</td>
</tr>
<tr>
<td>Nevirapine</td>
<td></td>
</tr>
<tr>
<td>- rash associated hepatitis</td>
<td>DRB1*01:01</td>
</tr>
<tr>
<td>- DIHS/DRESS in Italian population</td>
<td>Cw8;B14 haplotype</td>
</tr>
<tr>
<td>- DIHS/DRESS in Japanese population</td>
<td>Cw8</td>
</tr>
<tr>
<td>Carbamazepine (Caucasians)</td>
<td>DR3;DQ3;TNF2; A*31:01</td>
</tr>
<tr>
<td>Drug induced liver disease</td>
<td></td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td>B*57:01</td>
</tr>
</tbody>
</table>

Adapted from: Philips EJ and Mallal SA. Pharmacogenomics. 2010;11:973
Strength of HLA Associations

<table>
<thead>
<tr>
<th>HLA Allele</th>
<th>ADR</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>B*57:01</td>
<td>Abacavir hypersensitivity</td>
<td>&gt;500</td>
</tr>
<tr>
<td>B*15:02</td>
<td>Carbamazepine induced SJS, TEN</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>B*58:01</td>
<td>Allopurinol hypersensitivity</td>
<td>&gt;800</td>
</tr>
</tbody>
</table>

Early Study: Susceptibility to Abacavir Hypersensitivity

- Strongly associated with HLA-B*57:01;Hsp70-Hom M493T haplotype
- Found in 94.4% of hypersensitivity cases
  - Odds ratio = 3.89, P<0.00001
- Polymorphic substitution in heat shock protein, Hsp70 in residue M493T, in peptide binding region

Martin AM, et al. PNAS.2004;101:4180
Susceptibility to Abacavir Hypersensitivity

- Occurs in approximately 5% of patients treated with abacavir
- Symptoms appear within 6 weeks of receiving drug
- Symptoms resolve if drug is stopped, but re-treatment increased severity of reaction or death
- Positive predictive value of B*57:01+ for hypersensitivity and 100% negative predictive value for B*57:01- phenotypes, led to FDA recommendation for B57 typing prior to abacavir treatment

Strongly associated with HLA-B*57:01;Hsp70-Hom M493T haplotype
- Found in 94.4% of hypersensitivity cases
  - Odds ratio = 3.89, P<0.00001
- Polymorphic substitution in heat shock protein, Hsp70 in residue M493T, in peptide binding region
- More recent work has NOT confirmed the association with Hsp70 (Bharadwaj, et al. 2012)
Initial Model for induction of Abacavir hypersensitivity

- Abacavir is metabolized in APC.
  - Sequential phosphorylation to carbovir monophosphate and carbovir triphosphate
  - CBV-TP inhibits HIV reverse transcriptase
- Hsp70-Hom molecules facilitate loading of abacavir peptide onto HLA-B*57:01
- APC:B*57:01:abacavir peptides stimulate CD8 delayed hypersensitivity

Mechanism of Abacavir Hypersensitivity

- Specificity mapped to residues 114 & 116 in F pocket of B*57:01
- Recognition of abacavir by CD8+ T cells requires TAP and Tapasin - indicating creation of unique peptide restricted to B*57:01

The F pocket is more electronegative in HLA-B*5701 compared to HLA-B*5703.

Model for Abacavir Hypersensitivity

![Diagram of Abacavir Hypersensitivity]

Martin AM, et al. PNAS.2004;101:4180

---

a) Hapten/pro-hapten model
1. Abacavir or metabolite modifies cellular protein
2. Modified protein $\rightarrow$ proteasome derived peptides
3. TAP dependent loading only to B*57:01

b) Anchor site modification
1. Abacavir metabolite binds to B*57:01 during folding in ER
2. Alters anchor specificity
3. Presents new repertoire of self-peptides
4. Results in auto-immune response

Abacavir Induced Loading of Novel Self Peptides

- In vitro epitope binding assay tested peptides secreted from B*57:01 producing B cell line after treatment with abacavir
- Isolated peptides mapped by mass spectroscopy
- Drug induced peptides lacked typical B*57:01 motif at C-terminus; substituted with isoleucine or leucine
- Supports model of drug-induced autoimmunity by abacavir binding altering peptide binding groove

ADRs to Carbamazepine and other Antiepileptic drugs (AEDs)

- ADRs common feature of AEDs
  - 1st generation AEDs – carabamazepine, phenytoin, phenobarbital
  - 2nd generation AEDs – lamotrigine, topiramate, oxcarbazepine
- Reactions typically occur within 1st 8 weeks of treatment
  - Triad of fever, rash, organ involvement
  - Stevens-Johnson syndrome, toxic epidermal necrolysis, drug induced liver injury
**Model of AED Toxicity**

AED $\rightarrow$ Lymphocyte cytotoxicity when exposed to drug

- **TLRs, DAMPS from damaged cells:** fibronectin, apoptotic fragments, heparan sulfate
- **ROS**
- **TNFα, Granzyme, Perforin, IL-6**
- **Effector CTL**
- **TLRs, DAMPS from damaged cells**
- **Lymphocyte Activation**

**DILI (Drug induced liver injury)**

**Pathogenesis of AED Toxicity**

- *In vitro* lymphocyte toxicity assay $\rightarrow$ lymphocyte cytotoxicity when exposed to drug
- Type IV delayed hypersensitivity
- Reactive drug $\rightarrow$ oxidative cell damage $\rightarrow$ DAMPs $\rightarrow$ CTL activation
- Th1 cytokines released (IL-1, IL-6, TNFα)
- Results in apoptosis of cells in liver and skin
Susceptibility to Carbamezepine

- Strong association of B*15:02 among Han Chinese
  - Oxcarbazepine, phenytoin, phenobarbital
- Screening of East Asians before receiving carbamazepine is recommended by FDA

Susceptibility to Carbamazepine Among Other Populations

- Recent genome-wide mapping found no association with A*31:01 in Europeans for adverse reactions to phenytoin and lamotrigine (McCormack, et al. Pharmacogenomics.2012;13:399)
- 16th IHIWS Project - Registry for HLA associations of adverse reactions among other populations