HLA 101
(aka Human Leukocyte Antigen 101)

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Inland Northwest Blood Center
Objectives:
1) Describe the genetics, immune functions, and cell distribution of the human leukocyte antigens
   - HLA Class I antigens
     HLA-A, HLA-B, HLA-C
   - HLA Class II antigens
     HLA-DR, HLA-DQ, HLA-DP
Objectives:
2) Discuss the clinical applications for identifying or matching HLA antigens
• Vaccine trials, disease associations, adverse drug reactions
• Platelet transfusion
• Solid organ and hematopoietic stem cell transplantation
Objectives:
3) Understand the purpose and methods used for the 3 major tests performed in the HLA Laboratory:
• Typing
• Antibody screening and identification
• Crossmatching
HLA

H = Human
L = Leukocyte
A = Antigen

- Inherited characteristics that vary between persons, like blood types, but more variations
- Proteins found on surface of virtually all cells, first discovered on leukocytes (aka tissue antigens)
- Used by the body’s immune system for self vs. non-self discrimination
HLA Genetics

• HLA proteins are encoded by genes of the Major Histocompatibility Complex (MHC)
• Genes are located at 6 closely linked loci:
  – A, B, C, DR, DQ, DP
• Genes and encoded proteins categorized by structure, cell distribution and function:
  – Class I: A, B, C
  – Class II: DR, DP, DQ
HLA Genetics and Nomenclature:

• ~1000 possible alleles at each locus are identified by numbers in the order discovered:
  – A*01
  – A*02
  – A*03
  – A*11
  – etc

• Antigens: Cell surface proteins encoded by alleles (A*02 allele -> A2 antigen)
HLA Genetics: Inheritance

Each person inherits one complete set of HLA alleles (haplotype) from each parent

<table>
<thead>
<tr>
<th>A3</th>
<th>A11</th>
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<tbody>
<tr>
<td>C9</td>
<td>C4</td>
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<td>B7</td>
<td>B52</td>
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<tr>
<td>DR15</td>
<td>DR1</td>
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<tr>
<td>DQ5</td>
<td>DQ5</td>
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<tr>
<td>DP4</td>
<td>DP1</td>
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<tr>
<th>A23</th>
<th>A31</th>
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<tr>
<td>C7</td>
<td>C8</td>
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<td>B44</td>
<td>B7</td>
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<tr>
<td>DR7</td>
<td>DR4</td>
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<td>DQ2</td>
<td>DQ7</td>
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<tr>
<td>DP3</td>
<td>DP2</td>
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Together, this constellation of encoded proteins constitutes a person’s HLA type (12 different antigens): A23, 31; B7,44; C7,8; DR4,7; DQ2,7; DP2,3
HLA Genetics: Polymorphism

- Most polymorphic genetic system in the human genome
- More than 50,000 known HLA types
- Most common is present in 1:100 unrelated persons
- Rarest is present in less than 1:10 million unrelated persons
HLA Cell Distribution and Immune Function

Antigen presentation to patrolling T cells

<table>
<thead>
<tr>
<th>HLA Class I</th>
<th>HLA Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>All nucleated cells</td>
<td>Antigen presenting Cells</td>
</tr>
<tr>
<td>Intracellular Peptides</td>
<td>Extracellular Peptides</td>
</tr>
<tr>
<td>CD8+ cytotoxic T cells</td>
<td>CD4+ helper T cells</td>
</tr>
</tbody>
</table>
HLA Immune Function

T cells are programmed to recognize HLA + peptide

- Self HLA + normal peptides
  -> no response

- Self HLA + foreign peptides
  -> immune response
  - Cellular (vs intracellular derived peptides, eg virus)
  - Humoral (vs extracellular derived peptides, eg bacteria)

http://highered.mcgraw-hill.com/sites/0072495855/student_view0/chapter24/animation_cytotoxic_t-cell_activity_against_target_cells__quiz_1_.html
HLA Polymorphism and Structure

- Amino acid differences between HLA molecules are clustered in the distal part of the molecule
- Beta sheet and alpha helices form the peptide binding groove
- External surfaces of the groove are binding sites for the T cell receptor

![Diagram showing MHC class I and II variability](Image)
HLA Polymorphism: Peptide and TCR binding

Amino acid differences between inherited HLA determine which peptides can be bound and provide self-recognition sequences for the person’s own T cell receptors.
HLA Polymorphism and Immune Function

HLA polymorphism enables binding a universe of peptides from potential pathogens

• Individual level
  – 12 different HLA antigens
  – Multiple alleles -> high probability of heterozygosity

• Population level
  – Multiple HLA types increase probability some individuals will survive emerging pathogens
Time out for questions!
Objectives:
2) Discuss the clinical applications for identifying or matching HLA antigens
   • Vaccine trials, disease associations, adverse drug reactions
   • Platelet transfusion
   • Solid organ and hematopoietic stem cell transplantation
HLA Vaccine Trials: Melanoma

- Patients are vaccinated with a peptide cocktail derived from tumor cells (+/- cytokines to boost their immune response)

- Only patients with HLA that can bind one or more of the peptides in the cocktail are eligible for the vaccine trial
  - e.g. HLA-A2 and A24 bind melanoma specific peptides

HLA Typing identifies candidates for the trial
Clinical Aspects of HLA: Cases of Mistaken Identity!

T cell receptor obsession with self HLA + foreign peptide has unfortunate consequences:

• Autoimmunity: Self HLA + “self” peptide -> autoimmune disease
• Alloimmunity: Foreign HLA + any peptide -> immune destruction of transfused or transplanted cells
  – As many as 10% of T cells are alloreactive (vs foreign HLA)
**HLA Disease Associations**

- Some autoimmune diseases are associated with particular HLA because they bind “self”-peptides and are recognized by auto-reactive T cells (HLA is guilty!)

- Other diseases are associated with the inheritance of a disease susceptibility gene in the MHC that is in close proximity with a particular HLA allele (Guilt by association!)

- Relative risk (RR) = how much more frequently a disease occurs in individuals with a specific HLA allele compared to individuals not having that allele.
  - $RR > 1$ means the HLA allele is associated with disease susceptibility.

**HLA Typing may aid in diagnosis**
## HLA Disease Associations

<table>
<thead>
<tr>
<th>Disease</th>
<th>HLA Type</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankylosing Spondylitis</td>
<td>HLA-B27</td>
<td>100</td>
</tr>
<tr>
<td>Reiter’s Syndrome</td>
<td>HLA-B27</td>
<td>35</td>
</tr>
<tr>
<td>Post-infectious arthritides</td>
<td>HLA-B27</td>
<td>15</td>
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<tr>
<td>Celiac Disease</td>
<td>HLA-DQ2</td>
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<tr>
<td>Birdshot Chorioretinopathy</td>
<td>HLA-A29</td>
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<tr>
<td>Autoimmune chronic hepatitis</td>
<td>HLA-DR3</td>
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<td>Addison’s Disease</td>
<td>HLA-DR3</td>
<td>9</td>
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<tr>
<td>Graves Disease</td>
<td>HLA-DR3</td>
<td>4</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>HLA-DR4,1</td>
<td>7</td>
</tr>
<tr>
<td>Psoriasis vulgaris</td>
<td>HLA-C6</td>
<td>5</td>
</tr>
<tr>
<td>Narcolepsy</td>
<td>HLA-DR15, DQ6</td>
<td>100</td>
</tr>
</tbody>
</table>

Modified from Thorsby (1995) and Rammensee (1997)
HLA and Adverse Drug Reactions

• Abacavir is an effective drug to treat HIV
• 4% of patients develop abacavir hypersensitivity syndrome that can be life-threatening (anaphylaxis; organ failure)
• Only 4% of patients are HLA-B57+, but 90% of HLA-B57+ patients develop abacavir hypersensitivity syndrome (RR>1000)
• Mechanism:
  – Drug binds bottom of HLA-B57 groove
  – Distorts self-peptide presentation
  – Self-peptides now seen as foreign
  – Auto-immune-like hypersensitivity

HLA Typing identifies patients at risk of adverse reaction
Individuals may become sensitized to the foreign HLA of others through pregnancy, transfusion or transplantation

– HLA specific alloreactive T cells
– HLA specific alloantibodies
Alloreactive T cell destruction

- Recipient’s alloreactive T cells recognize and bind to foreign donor HLA antigens
- Bound T cells secrete toxic molecules that destroy the foreign cells
- Called cell mediated or cellular rejection
**Alloreactive B cell destruction**

- Recipient’s alloreactive B produce antibodies against the foreign donor HLA antigens
- Recipient antibodies bind to the allograft and label it for destruction
- Called antibody mediated immune destruction or antibody mediated rejection
In an ideal world….

...all recipients would have a perfect HLA matched donor!
HLA Matching in Platelet Transfusion

- Platelets express Class I HLA
- Patients who require repeated platelet transfusions (e.g. oncology patients) are exposed to many different foreign HLA and may build antibodies against them.
- HLA antibodies cause complement-mediated immune destruction of donor platelets, a condition that renders the recipient refractory to random donor platelet transfusions
HLA Matching in Platelet Transfusion

• Sensitization to foreign HLA -- and immune destruction of transfused platelets -- may be avoided or limited by providing HLA matched platelets

• Requires a **large** registry of volunteer HLA typed platelet donors to provide matched products for all recipients
• Surgical technique perfected in early 1900’s

• Repeated failures from graft rejection
  – Animal donors
  – Deceased human donors
  – Living non-identical twin donors

• First successful kidney transplant
  – 1954 identical twins
  – 1959 non-identical living donor
  – 1962 non-identical deceased donor

Joseph Murray, MD
1919-2012
Nobel Laureate

A Life of Curiosity, Humanism and Persistence
HLA Matching in Solid Organ Transplantation

Early failed transplants led to the discovery of HLA as the major barrier to transplantation

Organ-specific and endothelial cells express HLA Class I Class II expression is induced during inflammation
HLA Matching in Solid Organ Transplantation

Better matches (fewer mismatches) are associated with fewer rejection episodes and longer graft survival.
The Growing Gap

Organ allocation policies attempt to balance utility and justice: Less than 15% of all transplants are from HLA identical donors.
HLA Matching in Solid Organ Transplantation

• Non-HLA identical transplants are possible only because immunosuppressive drugs limit the recipient’s immune response to the mismatched donor HLA

  – Most effective versus T cells; antibody responses not well controlled
  – Mismatched allografts eventually fail due to antibody mediated chronic rejection
  – T ½ of deceased donor kidney is ~10-15 years

• Non-compliance with immunosuppressive drug regimens is a common cause of graft rejection and failure
HLA Matching in Hematopoietic Stem Cell (HSC) Transplantation (aka bone marrow transplantation)

First HSC transplants:
- 1950’s Identical twins
- 1969 HLA matched sibling
- 1979 HLA matched unrelated donor

In 2012:
- 60,000 HSC transplants worldwide
- 1 million HSC transplants since the beginning

E. Donnall Thomas, MD
1920-2012
Nobel Laureate
Hematopoietic Stem Cell Properties:

Self Renewal
multiply to repopulate the marrow

Multipotent
differentiate into progenitor and mature blood cells
What is HSC Transplant?

Following a conditioning regimen to destroy the patient’s blood stem cells, healthy donor blood stem cells are given to reconstitute their hematopoietic system:

- Replace cancerous blood cells
  Leukemias, Lymphomas
- Replace absent or defective blood cells
  Inherited disorders
- Replace normal blood cells
  Destroyed by chemotherapy for other cancers
Matching for HSC Transplant

**Graft Rejection:**
Recipient’s immune system attacks donor stem cells

**Graft vs Host Disease:**
Donor’s cells attack recipient’s tissues

**Graft vs Tumor Effect:**
Donor’s cells attack residual recipient tumor cells
Matching for HSC Transplant

The better the match, the better the outcome

8 of 8 Match

7 of 8 Match
Potential Matched Donors

• 30% of patients have an HLA identical sibling
• 70% search for an unrelated donor on the National Marrow Donor Program’s Be The Match registry of 12 million HLA typed adult potential donors
• 90% of Caucasians, but fewer than 60% of African and Asian Americans find a suitable match
Matching versus Disease Stage

Patients without a suitably matched donor may be transplanted:

- Partially matched cord blood unit
- Haploidentical (1/2 matched) related donor
Time out for questions!
Objectives:
3) Understand the purpose and methods used for the 3 major tests performed in the HLA Laboratory:
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HLA Compatibility Testing

• **HLA type** donor and recipient cells to identify potential targets of recipient immune response

• **HLA antibody screen** recipient’s serum to detect and identify pre-existing antibodies against HLA

• **Crossmatch** recipient’s serum with donor’s cells as a final compatibility check
HLA Typing (DNA based methods)

HLA alleles are distinguished by detecting differences in their DNA sequences:

- **SSP**: sequence specific primers bind to and amplify polymorphic stretches of DNA. Accumulated amplicons are detected on gels.
- **SSO**: sequence specific oligonucleotide probes bind to polymorphic stretches of DNA. Bound labeled probes are detected by luminex.
- **SBT**: sequence based typing identifies the entire DNA sequence of informative exons.
HLA Typing by SSP

- Different SSP primers that recognize stretches of DNA specific for particular HLA alleles are present in each well of the typing tray.

- DNA is added to every well, and is amplified only if it contains the corresponding sequence.

- HLA type determined by analyzing the DNA amplification pattern.
HLA Antibody Testing

Anti-donor HLA antibodies -> acute rejection
- If transplanted with allograft of corresponding HLA type, pre-existing antibodies target the graft for destruction

- Goal of HLA antibody testing is to detect HLA antibodies, identify their specificity and avoid donors with corresponding HLA (unacceptable donor antigens)
HLA Antibody Testing

HLA antibody if present in patient serum

Purified Antigen Coated Beads

Screening Beads

Labeled anti-IgG

OR

Single Antigen Beads
HLA Antibody Testing

- Flow Cytometry Detection System
Flow PRA Class I/II Antibody Detection

Flow PRA I = 80%

Flow PRA II = 0%
HLA Antibody Identification: Single antigen coated beads
HLA (Lymphocyte) Crossmatch

- **Goal:** To determine if the recipient’s serum has pre-existing antibodies directed against donor HLA

- The presence of anti-donor HLA antibodies is a contraindication to transplantation

- Crossmatches test patient serum vs donor:
  - T lymphocytes (express Class I HLA)
  - B lymphocytes (express Class I and Class II HLA)
Flow Cytometry Lymphocyte Crossmatch

HLA antibody (if present in patient serum)

Fluorescent labeled anti-human-IgG

HLA antigens

Donor Lymphocyte
Flow Cytometry Lymphocyte Crossmatch

Negative Control Serum

Positive Control Serum

Patient Serum
HLA Testing Goal: Identify best match for best outcome!

HLA Typing
- Identify mismatched donor antigens

HLA Antibody Testing
- Avoid unacceptable donor antigens

HLA Crossmatch
- Verify compatibility

Incompatible
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Time out for questions!
Thanks to the INBC HLA Lab...

...and especially our patients and donors