A primer in HLA:
The who, what, how and why

What?
Historical context...

- First recognized in mice during 1930’s and 1940’s. Mouse (murine) experiments with tumors.
- Independent observations were made in humans with leukoagglutinating antibodies in patients with leukopenia. In 1950’s Jean Dausset, of France, determined the leukoagglutinins were alloantibodies. This system of antibodies was analogous to the system discovered in mice.
- Jean Dausset received the Nobel Prize in 1980 for his work in histocompatibility.

Terminology...

- **HLA**: 1. Human Leukocyte Antigen  
  2. Class I Typing  
  3. General heading for both class I and class II testing (typing and antibodies)
- **DR**: 1. Class II (typing and antibodies)  
  2. Specific locus in class II
- **CLASS I**: A; B; C loci
- **CLASS II**: DRB1; DRB3,4,5; DQ; DP loci
- **CLASS III**: Complement, etc
- **HLA ANTIGEN**: Glycoprotein located on the surface of cells
- **HLA ANTIBODY**: Antibody to HLA antigens (class I or class II)
- **DSA**: Donor Specific Antibody
- **MHC**: Major Histocompatibility Complex. Often HLA, Tissue Typing and MHC are used interchangeably
Major Histocompatibility Complex

- A set of molecules on cell surfaces that are responsible for:
  - lymphocyte recognition and antigen presentation and
  - control self vs non-self immune response
- Inherited system
- Highly polymorphic – many antigens possible at each loci
- It is the most polymorphic genetic system, more than 1000 alleles

Chromosome 6
Class I: A, B, C; Present on many, but not all nucleated cells and platelets
Class II: DR, DQ, DP; Restricted to a few types of immune related cells: B lymphocytes, dendritic cells, macrophages and some endothelial cells (LMP, TAP)
Class III: Complement, etc

- MHC molecules trap peptide fragments, antigens, in a cleft to present to T cells
- Each HLA class binds to different antigens and different T cells
  - Class I antigens bind viruses and cytotoxic T cells (CD8) which in turn kill the infected cells preventing viral replication and the spread of the infection. All nucleated cells are targets for viruses.
  - Class II antigens bind pathogens in extracellular spaces and Helper and Inflammatory T cells (CD4). CD4 T cells activate macrophages, which act as garbage collectors and B cells which make antibodies.

**Why?**
Class II – CD4 T Cell
Helper and Inflammatory T Cell

Class I – CD8 T Cell
Cytotoxic T Cell

Class I and Class II HLA antigens bound to cell membrane
each contains more than 300 amino acids with public and private epitopes
Class I HLA molecule with antigen presenting cleft

Class II HLA molecule with antigen presenting cleft
Clinical implications...

- Recognition and reaction of MHC to proteins that are “not self”
- Infections
- Transplants
- Transfusions
- Pregnancies

HLA Typing

1. Disease Association
2. Organ Transplantation
3. HLA matched platelets or compatible platelets for people with HLA antibodies
4. Paternity Testing

Clinical indications for HLA testing...
HLA Antibody Screening and Identification

1. Pre Transplant testing
2. Post Transplant monitoring
3. Refractory platelets

Clinical indications for HLA testing...

- Disease Association
  - Typing, class I and class II
- Organ Transplantation
  - Kidney and Pancreas
    - Active program with living and deceased organ donors
    - Typing, Antibody Screen and ID, Cross Matching
  - Bone Marrow
    - Typing (r/o family members for FAHC physicians)
    - FAHC does not perform allogeneic stem cell transplants
- HLA Antibody Screens and Identification
  - Patients that are refractory to platelets
  - Antibodies to potential donor’s antigens
  - Donor Specific HLA antibodies (rejection)

HLA Testing at FAHC...
• **Testing Methodologies**

  • **Complement Dependent Cytotoxicity Technique**
    CDC or Serologic Technique
    • End point is cell death – determining percentage of live vs dead cells
    Typing (class I and II)
    Antibody Screens
    Crossmatching
  • **Luminex**
    Antibody Screen and Identification
    DNA HLA Typing (rSSOP)

  **Testing Methodologies... How?**

  • **Flow Cytometry**
    • Crossmatching
    • B27s
  • **ELISA**
    • Antibody Screens and Identifications
  • **DNA**
    • PCR and then different methods. SSP, SSOP, SBT
    • Low, intermediate and high resolution
    • Types to allele level

  **Testing Methodologies... How?**
Luminex... how?

Luminex for HLA antibody screening how?
Luminex LABScan 100

- Isolate T and B lymphocytes
  - Immunomagnetic methodology
    - Dynabeads
    - Magnetic (metal) core plastic beads coated with either:
      - Anti-CD8 for T cells
      - Antibody specific for a HLA Class II β chain monomorphic epitope for B cells
  - Differential sedimentation/adherence
    - Nylon wool, plastic beads, etc
  - Discontinuous Density Gradient Centrifugation
    - Ficoll-hypaque, Percoll

Cell separation... how?
Immunomagnetic Separation Technique... how?

When coated with a specific poly or monoclonal antibody, lectin or other bioactive molecule DYNABEADS® can be used to make a target "magnetic." The target can then easily be removed with a magnet. Once the magnet is removed, the DYNABEADS® resorbed target resuspend easily since the beads have no magnetic remanence.

Lymphocyte bound to dynabeads

Immunomagnetic Separation Technique...
Cells on Ice

Immunomagnetic cell separation...

- Cool the sample to prevent the monocytes from phagocytizing the beads
- Add beads

Antigen / Antibody Binding

Immonomagnetic Cell Separation...

- Provide gentle rotation to enhance the potential of contact between the antibodies on the beads and the antigens on the cells
Magnetic Cell Separation

- The beads (with the cells attached) move to the side of the glass tube in contact with the magnet
- The remainder of the blood/buffer mix can be decanted or aspirated off.
- Wash cell/bead mixture three times with buffer

Immunomagnetic Cell Separation...

Isolated Cells

- Isolated cells after washing
- (Visible due to the beads)
Determine Cell Viability and Concentration

- Use a Neubauer hemocytometer to determine cell viability and appropriate concentration for testing
- Adjust cell concentration
  - Class I = 2.5x10⁶/ml
  - Class II = 1 – 1.5x10⁶/ml
- Isolated T and/or B cell preparations are ready for testing.

Immunomagnetic cell separation...

- Typing Trays
  - Commercially or in-house prepared
  - Wells contain known anti-sera to HLA antigens
- Frozen Cell Trays
  - Commercially or in-house prepared
  - Wells contain known HLA typed cells
- Blank Trays
  - Techs add serum and cells
  - Used for crossmatches and projects

Microtiter trays...
Adding Cells to Microtiter Trays

Complement Dependent Cytotoxicity (CDC) Testing...

- Add 1 µl cell suspension to each well with a microsyringe pipette
- Mix
- Incubate to allow antigen antibody complex to form

Complement Step

Complement Dependent Cytotoxicity (CDC) Testing...

- Excess complement is added to each well and incubated
- Complement:
  - recognizes and binds to the ag/ab complex
  - Starting the complement cascade which affects the integrity of the cell membrane and makes holes in it
  - Ultimately this causes cell death
  - If there aren’t any ag/ab complexes, complement does not cause any damage to the cell
Can you see the bead/cell mixture?  A multichannel pipette is used to add the fluoroquench

Magnet is used for mixing and settling cells to the bottom of the wells

Fluoroquench, a fluorescent staining reagent, is added to each well

Fluoroquench…

• Fluoroquench stains and fixes lymphocytes
  • Active ingredients:
    • EDTA stops the complement dependent cell lysis
    • Ethidium Bromide stains dead cells (reddish orange)
    • Acridine Orange stains live cells (green)

Fluoroquench…
**“Reading” the trays...**

- Trays are read with an inverted fluorescent microscope – (read from the bottom of the tray)
- Scoring system – Determine the percentage of dead cells to total cells in each well and score on a worksheet
  - 0 = QNS, (not enough cells to score = Invalid)
  - 1 = 0 - 10% dead = Negative
  - 2 = 11 – 20% dead = Negative (?)
  - 4 = 21 – 50% dead = Weak Positive
  - 6 = 51 – 80% dead = Positive
  - 8 = 81 – 100% dead = strong Positive

**Quality control...**

- Each tray has positive and negative wells
- Some trays have T and B cell control wells
- Panel members
- New lots and shipment of trays qc’ed with five known panel members
- Fluoroquench and Dynabeads are qc’ed with each new shipment
- Techs perform a blind typing every month
- Twelve CAP surveys each year
- ASHI proficiency testing surveys
Just for fun…

solving the puzzle…

- Father: A24, A32, B27, B35

**HLA phenotyping/genotyping**
Father: A24, A32, B27, B35
Mother: A2, A31, B62, B60

HLA phenotyping/genotyping
• Father: A24, A32, B27, B35  
  A24 | A32  
  B35 | B27

• Mother: A2, A31, B60, B62  
  A2 | A31  
  B62 | B60

• Patient: A2, A32, B27, B62  
  A2 | A32  
  B62 | B27

**HLA phenotyping/genotyping**
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**HLA phenotyping/genotyping**