Clinical Relevance of the HLA System in Blood Transfusion

Dr Colin J Brown PhD FRCPath.

October 2017
Outline of talk

• HLA genes, structure and function
• HLA and immune complications of transfusion
  – TA-GVHD
  – Platelet Refractoriness
  – TRALI
• The relevance of high resolution typing and antibody definition technologies
Major Histocompatibility Complex

- many species have an MHC
- in mouse = H2, in chickens = B complex
- in humans MHC includes the Human Leucocyte Antigens (HLA)
- Chromosome 6p21.3
- HLA complex spans about ~4Mb
- >200 identified loci
- 40% of expressed loci have immune function
# HLA Polymorphism

<table>
<thead>
<tr>
<th>Allele</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-A</td>
<td>3,968</td>
</tr>
<tr>
<td>HLA-B</td>
<td>4,828</td>
</tr>
<tr>
<td>HLA-C</td>
<td>3,579</td>
</tr>
<tr>
<td>HLA-DRB1</td>
<td>2,103</td>
</tr>
<tr>
<td>HLA-DQB1</td>
<td>1,142</td>
</tr>
<tr>
<td>HLA-DPB1</td>
<td>894</td>
</tr>
</tbody>
</table>

June 2017
HLA-A+B+DR Mismatches
Deceased Donor, First Kidney Transplants 1990-2015

Graft survival (%)

Post-transplant time (years)

0 MM n=12,126
1 MM n=15,572
2 MM n=38,747
3 MM n=57,218
4 MM n=46,315
5 MM n=24,473
6 MM n= 7,722

CTS Collaborative Transplant Study
HLA matching in HSCT


Figure 1. Survival of patients with early, intermediate, and advanced disease depending on degree of HLA matching (8/8, 7/8, and 6/8) for HLA-A, -B, -C, and -DRB1. (A) Early-stage disease for 8/8, 7/8, and 6/8, respectively: 1-year survival 63%, 52%, and 39%; 5-year survival 50%, 39%, and 28%. (B) Intermediate-stage disease for 8/8, 7/8, and 6/8, respectively: 1-year survival 48%, 40%, and 32%; 5-year survival 32%, 27%, and 22%. (C) Advanced-stage disease for 8/8, 7/8, and 6/8, respectively: 1-year survival 31%, 29%, and 24%; 5-year survival 17%, 15%, and 10%.
HLA and Transfusion

Human Leucocyte Antigens
HLA

Human Platelet Antigens
HPA (+HLA)

Human Neutrophil Antigens
HNA (+HLA)

LYMPHOCYTES
HLA

PLATELETS
HPA (+HLA)

NEUTROPHILS
HNA (+HLA)
HLA and Immune Complications of Transfusion

- Platelet Refractoriness
- TRALI
- TA-GVHD
Transfusion - Associated Graft versus Host Disease (TA-GvHD)

- TA-GvHD is a generally fatal immunological complication of transfusion practice.
- Involves the engraftment and clonal expansion of immunocompetent donor lymphocytes in a susceptible host.
- Most frequently involves an immunocompromised patient sharing one haplotype with a HLA homozygous donor.
<table>
<thead>
<tr>
<th>Manifestation</th>
<th>HSCT-GvHD</th>
<th>TA-GvHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time sequence</td>
<td>35-70 days</td>
<td>2-30 days</td>
</tr>
<tr>
<td>Skin Rash</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pancytopaenia</td>
<td>Rare to minimal</td>
<td>Almost always</td>
</tr>
<tr>
<td>Liver enzyme elevation</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bone marrow hypoplasia</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Occurrence of GvHD</td>
<td>70%</td>
<td>0.1 - 1.0%</td>
</tr>
<tr>
<td>Response to therapy</td>
<td>80-90%</td>
<td>None</td>
</tr>
<tr>
<td>Mortality</td>
<td>10-15%</td>
<td>90-100%</td>
</tr>
</tbody>
</table>
TA-GVHD reported to SHOT
Prevention of TA-GvHD

At risk patient groups receive $\gamma$-irradiated blood products to inactivate immunologically active lymphocytes.
Platelet refractoriness

- Poor increment ($<10 \times 10^9/L$ or $CCI < 7.5$) after at least two consecutive transfusions of random donor platelets

- Platelet count are taken 1hr post transfusion
Immunological causes of platelet refractoriness

- HLA - class I specific antibodies
- HPA - antibodies
- ABO - antibodies
Immune Mechanism

patient antibodies

transfused platelets

SPLEEN

phagocytosis

removal from circulation

complement FcR

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Management of alloimmunised platelet refractory patients

• Provide HLA/HPA compatible donors from an HLA/HPA typed apheresis donor panel
  – Patient: HLA-A*01, A*03; B*07, B*08
  – Donor: HLA-A*01, A*03; B*07, B*08

• Define HLA/HPA antibody specificity and select antigen compatible apheresis donors
  – Patient: HLA-A*01, A*03; B*07, B*08 + anti-HLA-A2,68,69.
  – Donor: HLA-A*03, A*11; B*07, B*35

• Cross-match random apheresis platelets to select compatible donors
Transfusion Related Acute Lung Injury
TRALI

SHOT definition:

“Acute dyspnoea with hypoxia and bilateral pulmonary infiltrates during or within 6 hours of transfusion, not due to circulatory overload or other likely causes”.
How does the TRALI occur?

1. HLA/HNA abs
   - HLA
   - HNA

2. C’ activation
   - C
   - C5
   - Chemotactic factor

inactive → active

- Adherence of neutrophils to pulmonary endothelium or epithelium
- Cell membrane permeabilisation
- Lung oedema
- Secretion of IL-1β, TNFα, IL-8 may amplify the reaction
Histology

Normal

Acute Lung Injury
TRALI cases reported to SHOT

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Case study

• A 43 year old patient
• Readmitted 2 days post hysterectomy
• Hb 5.5 g/dl
• Received 6 RBCs, 3FFP
• Became hypoxaemic
  Pulse oxymetry → reduced $O_2$ saturation (76%)
  Breathlessness/hypotension
• Chest X-ray bilateral hilar infiltrates
Laboratory Findings
Patient received 3 FFP and 6 RBC

Donor Antibody Screen
Granulocyte specific antibodies identified in 2 RBC donors
HLA-class I, anti HLA-A2 identified in 1 FFP donor

Cross Match Studies
Positive with all 3 donors
Donors’ serum against Patient’s leucocytes
Patient’s HLA typing result A*01,A*02; B*08,B*44

Conclusion
TRALI due to anti-HLA-A2/Anti granulocyte antibodies
Fig 2. First TRALI episode. Clearance of chest x-ray: 48 hours after ventilation. Hb 9g/dl
Relevance of HLA
High Resolution Technology

• Next Generation Sequencing
  – Allele level HLA typing
    – HLA-B*44      HLA-B*44:02:01:01

• Single Antigen Luminex Beads
  – Allele specific antibodies
    – HLA-B44      HLA-B*44:03 specific antibody
**Donor HLA Type**

**Pre-NGS HLA Type**

- HLA-
  - A*24:02/15/20/21/25/26/27/30/32/34/35/37/38;
  - A*32:01/03/05

- B*08:01/10/15/18; B*56:01

- C*01:02/07; C*03:03/12

- DRB1*01:01/03/04/05;
  - DRB1*14:01/54

- DQB1*05:03;
  - DQB1*02:01/02

- DPB1*04:01

**NGS HLA Type**

- HLA-
  - A*24:02:01:01
  - A*32:01:01
  - B*08:01:01:
  - B*56:01:01:03
  - C*01:02:01
  - C*03:03:01
  - DRB1*01:01:01
  - DRB1*14:01:01
  - DQB1*05:03:01:01
  - DQB1*02:01:01
  - DPB1*04:01:01
  - DPB1*13:01:01
| ANTIBODIES | A*02:01 | +
| A*02:02 | +
| A*02:03 | +
| A*02:04 | +
| A*02:05 | +

| T CELLS     | A*02:01 | +
| A*02:02 | -
| A*02:03 | -
| A*02:04 | -
| A*02:05 | -

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HLA Matching

HLA Locus

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>DRB1</th>
<th>DQB1</th>
<th>DPB1</th>
</tr>
</thead>
</table>

PLATELETS

KIDNEY

HAEM STEM CELL

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A match = No mismatch

The donor and patient are not serologically mismatched for the four antigens of the A and B loci.

<table>
<thead>
<tr>
<th></th>
<th>Donor</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>donor</td>
<td>A<em>01-A</em>02 / B<em>08-B</em>44</td>
<td>A<em>01-A</em>02 / B<em>08-B</em>44</td>
</tr>
<tr>
<td>patient</td>
<td>A<em>01-A</em>02 / B<em>08-B</em>44</td>
<td>A<em>01-A</em>02 / B<em>08-B</em>44</td>
</tr>
<tr>
<td>donor*</td>
<td>A<em>01-A</em>01 / B<em>08-B</em>08</td>
<td>A<em>01-A</em>02 / B<em>08-B</em>44</td>
</tr>
<tr>
<td>patient</td>
<td>A<em>01-A</em>02 / B<em>08-B</em>44</td>
<td>A<em>01-A</em>02 / B<em>08-B</em>44</td>
</tr>
</tbody>
</table>

* homozygous donor
B match (B₁-B₄) = Mismatched

The donor and patient are mismatched

\[
\begin{align*}
\text{B1 donor:} & \quad A^*01-A^*02 / B^*08-B^*27 \\
\text{patient:} & \quad A^*01-A^*68 / B^*08-B^*27 \\
\text{B2 donor:} & \quad A^*01-A^*02 / B^*08-B^*07
\end{align*}
\]
HLA Epitopes

From: Kostyu et al. Human Immunology 57, 1-18, 1997

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Antigen vs. Epitope matching

Consider a platelet patient with HLA type A2, A30; B42, B53 and two potential donors D1 and D2 with types as listed

<table>
<thead>
<tr>
<th>HLA Type</th>
<th>MM</th>
<th>Epitopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A<em>02, A</em>30; B<em>42, B</em>53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td></td>
<td>B2</td>
</tr>
<tr>
<td>A<em>02, A</em>29; B<em>07, B</em>53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td></td>
<td>B3</td>
</tr>
<tr>
<td>A<em>30, A</em>69; B<em>08, B</em>35</td>
<td></td>
<td></td>
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</tbody>
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Summary

• The clinical relevance of HLA in transfusion is well established.

• Platelet refractoriness, TRALI and TA-GVHD are all hazards of transfusion caused by HLA incompatibility.

• New approaches such as Next Generation Sequencing and epitope matching will allow the provision of more immunologically relevant HLA matched transfusions.
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