Clinical Relevance of the HLA System in Blood Transfusion

Dr Colin J Brown PhD FRCPath.
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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 GENES & MOLECULES

Chromosome 6

HLA

Class II
Class III
Class I

Chromosome 15

β2-microglobulin

HLA GENES & MOLECULES

HLA Polymorphism

June 2017

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Count</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>
HLA matching in HSCT

Lee et al 2007, Blood, 15 vol 110 (13): 4576 - 4583

Figures 1. Survival of patients with early, intermediate, and advanced disease depending on degree of HLA matching (0, 1, 2, and 3) for HLA-A, -B, -C, and -DR. All 8 evaluable cases for HLA-A, -B, and -DR were matched in disease stage. Disease stage for HLA-A, -B, and -DR was: 0-year survival 67%, 0% and 0%; 3-year survival 77%, 69%, and 52%; 0-year survival 89%, 89%, and 57%; 3-year survival 73%, 67%, and 51% for disease stage for HLA-A, -B, and -DR, respectively. Disease stage for HLA-A, -B, and -DR was: 0-year survival 85%, 89%, and 62%; 3-year survival 77%, 78%, and 82%; 0-year survival 85%, 87%, and 90%; 3-year survival 77%, 76%, and 81% for disease stage for HLA-A, -B, and -DR, respectively.

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HLA and Transfusion

Human Leucocyte Antigens HLA
Human Platelet Antigens HPA
Human Neutrophil Antigens HNA
LYMPHOCYTES HLA
PLATELETS HPA (HLA)
NEUTROPHILS HNA (HLA)

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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.

Comparison of GvHD associated with stem cell transplantation (HSCT-GvHD) and transfusion (TA-GvHD)

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>HSCT-GvHD</th>
<th>TA-GvHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time sequence</td>
<td>35-70days</td>
<td>2-30 days</td>
</tr>
<tr>
<td>Skin Rash</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pancytopenia</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 1 hr post transfusion
Immunological causes of platelet refractoriness

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

Immune Mechanism

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. Adherence of neutrophils to pulmonary endothelium or epithelium
2. Cell membrane permeabilisation
3. Lung oedema
4. Secretion of IL-1β, TNF-α, IL-8 may amplify the reaction
Case study

- A 43 year old patient
- Readmitted 2 days post hysterectomy
- Hb 5.5 g/dl
- Received 6 RBCs, 3 FFP
- Became hypoxaemic
  - Pulse oxymetry → reduced O₂ saturation (76%)
  - Breathlessness/hypotension
- Chest X-ray bilateral hilar infiltrates
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
Relevance of HLA High Resolution Technology

- Next Generation Sequencing
  - Allele level HLA typing
    - HLA-B*44:02:01:01
- Single Antigen Luminex Beads
  - Allele specific antibodies
    - HLA-B*44:03 specific antibody

Donor HLA Type

<table>
<thead>
<tr>
<th>Pre-NGS HLA Type</th>
<th>NGS HLA Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA- A<em>24:02:15/20/21/23/26/27/30/32/34/35/37/38; A</em>32:01/03/05</td>
<td>HLA- A*24:02:01:01</td>
</tr>
<tr>
<td>B<em>08:01/15/18; B</em>56:01</td>
<td>B<em>08:01:01; B</em>56:01:01:03</td>
</tr>
<tr>
<td>C<em>01:02/07; C</em>03/03/12</td>
<td>C*01:02:01</td>
</tr>
<tr>
<td>DRB1<em>01:03/04/05; DRB1</em>14:01</td>
<td>DRB1*01:01:01</td>
</tr>
<tr>
<td>DQB1<em>05:03; DQB1</em>02:01/02</td>
<td>DQB1*05:03:01:01</td>
</tr>
</tbody>
</table>
| DPB1*04:01 | DPB1*04:01:
| DPB1*13:01 | DPB1*13:01:01 |

ANTIBODIES

- A*02:02
- A*02:03
- A*02:04
- A*02:05

T CELLS

- A*02:01 Specific
- A*02:01:01
- A*02:02
- A*02:03
- A*02:04
- A*02:05
A match = No mismatch

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

- donor: A*01-A*02 / B*08-B*44
- patient: A*01-A*02 / B*08-B*44

B match (B₁-B₄) = Mismatched

The donor and patient are mismatched.

- B1 donor: A*01-A*02 / B*08-B*27
- patient: A*01-A*02 / B*08-B*27
- B2 donor: A*01-A*02 / B*08-B*07
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>Epitope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>A<em>02, A</em>30, B<em>42, B</em>53</td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>A<em>02, A</em>29, B<em>07, B</em>53</td>
<td>S2</td>
</tr>
<tr>
<td>D2</td>
<td>A<em>30, A</em>69, B<em>08, B</em>35</td>
<td>S3</td>
</tr>
</tbody>
</table>

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.
Acknowledgements

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