Providing HLA Compatible Platelets for Refractory Patients

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Overview

- Platelet antigens and reasons for platelet support
- HLA epitopes - application
- Donor selection process
- Platelet support for haploidentical transplant patients
Platelet Transfusion Refractoriness (PTR)

- Many causes:
  - not always immune, can be due to sepsis, fever, bleeding
  - important to determine if antibody mediated

- Patients who are requiring regular platelet support – high risk of PTR
  - Generally platelet transfusions are using pooled platelet products
  - Pooled product consists of platelets from 4 donors
  - Duration can be weeks - months - years
  - Lots of different antibodies can be produced
  - Almost always due to HLA antibodies – but can be due to HPA
The Search Process

- Patient request and samples sent to the lab
- Patient HLA typing and HLA antibody identification
- HLA Matchmaker to identify epitopes (triplets)
- Generate donor list in Platelet Panel Database
- Patient progress review
Patient request and samples

- Request is received by Medical Services from clinical unit for HLA compatible single donor platelet support (SDP)

- Patient samples required for testing (if not already performed):
  - HLA typing (HLA-A, B, C)
  - HLA antibody specificity (HLA-A, B, C)

- Transfusion nurses supply a request for SDP to the lab outlining transfusion requirements:
  - Urgent / immediate unit required (platelets on site)
  - Plan for ongoing support (full donor call up list required)
  - Number of transfusions per week, and timeframe to allow SDP scientists to determine the number of donors required for the patient
Antigens on Platelets

- Can be recognised by patient immune system and mount response (platelet destruction)
- The more HLA seen by a patient the greater the response
- HLA expression on platelets is ~10x more than that on lymphocytes
HLA – human leucocyte antigens

- Major Histocompatibility Complex has a central role in regulating immune responses

- Main set of genes in the human MHC: Human Leukocyte Antigens (HLA)
  - expressed on the surface of cells
  - control the immune response through continually presenting "self" and "non-self" peptides to T lymphocytes
    - assists in the immune elimination of foreign material from the body
    - immunological dialogue regarding what belongs and what doesn’t! (eg bacteria, virus, cancer)
HLA genes

- Polygenic:
  - 2 main groups - clinical significance in the transplantation environment:
    - **Class I:** HLA-A, HLA-B, HLA-C
    - **Class II:** HLA-DRB1, HLA-DQB1, HLA-DPB1

- HLA genes are inherited in groups, the set of alleles found on one chromosome is known as a **haplotype**

- The antigens are expressed co-dominantly:
  - individual expresses both the maternal and paternal inherited alleles (2xA, B, C)

- Highly polymorphic:
  - the presence of many variants or different forms of a gene
  - ethnically restricted (different HLA antigens present in different populations)
HLA (Tissue) Typing Methods

Molecular typing

- Extract DNA to use in tests
- Analyse the genetic sequence coding for the HLA antigen
- Determine the specificity of the HLA genes
- Use peripheral blood (ACD anticoagulant)
- Can use buccal swabs for patients with low cell counts,
HLA antibody testing (Luminex)

- 100+ beads used per sample
- Each bead has a unique HLA marker bound to the bead surface
- Beads are incubated with patient DNA or sera
- Beads are then run through the flow cytometer to determine patient’s results

Ref: Serologicals Corporation UK website

https://wwwfom.sk.med.ic.ac.uk/resources/6DB4DBCF-7A48-4655-85ED-21C3E2C63932/
Aim in platelet searches

- Identify acceptable mismatches for sensitised patients with pre-formed anti-HLA antibodies:
  - Determine the patient’s antibody profile
  - Review patient’s HLA antibodies with patient’s HLA type
  - Identify permissible mismatches that won’t elicit anti-HLA antibody responses after transfusion

- Our approach is not risk free, and depends on donor pool available.
Antibodies are directed towards epitopes

- Epitopes are polymorphic residues on the HLA molecule

- Antibodies recognise these epitopes

- Epitopes can be allele specific but more often are:
  - Shared within an antigen family
    - (eg. HLA-A*02, A*68)
  - Shared across other antigens
    - (eg. HLA-A*02, B*57)
HLAMatchmaker

A computer algorithm used for identifying acceptable and unacceptable HLA mismatches:
• Based on exposed triplets in the peptide binding groove of the HLA Class I molecule
• Enables determination of which epitopes (triplets) are acceptable, or not

Can use patient HLA typing and antibody result to:
• provide platelets to which the patient should achieve satisfactory increments
• reduce the chances of immunising the patient further
Luminex Single Antigen Results

Positive - Avoid

Negative

All antibody testing performed using One Lambda products on the Luminex platform
HLA Matchmaker:
Review HLA antibody profile to determine causative triplets

<table>
<thead>
<tr>
<th>Triplets</th>
<th>Antigens containing the triplet</th>
</tr>
</thead>
<tbody>
<tr>
<td>A02, A03, A01</td>
<td></td>
</tr>
<tr>
<td>A03, A01, A02</td>
<td></td>
</tr>
<tr>
<td>A01, A02, A03</td>
<td></td>
</tr>
</tbody>
</table>

**Select destination and press ENTER or choose Paste**
Case scenario: Patient LS

- **Clinical background:**
  - 57yo female with anaplastic large cell lymphoma
  - Referred January 2017 for compatible platelet support
  - Platelet count: 6
  - Not incrementing to apheresed or pooled platelets

- **HLA typing:**
  - A*01, A*23; B*08, B*44; C*02, C*07

- **HLA antibodies:**
  - Tested 31/1/2017
  - 97% cPRA
Patient LS:  HLA antibody results

Self HLA antigens
Patient LS:
Review HLA antibody reactivity and determine all triplets (epitopes)

The following HLA triplets were identified in the HLA antibody profile for patient LS:

<table>
<thead>
<tr>
<th>Triplet name</th>
<th>Antigens with this triplet</th>
</tr>
</thead>
<tbody>
<tr>
<td>246S a246S</td>
<td>A25 A26 A29 A31 A32 A33 A34 A43 A66 A74</td>
</tr>
<tr>
<td>207S a207S</td>
<td>A02 A25 A26 A29 A31 A32 A33 A34 A43 A66 A68 A69 A74 A80</td>
</tr>
<tr>
<td>151aHe a151aHe</td>
<td>A03 A25 A26 A34 A43 A66</td>
</tr>
<tr>
<td>45Ma b45Ma</td>
<td>B13 B46 B57 B62 B63 B75 B76 B77</td>
</tr>
<tr>
<td>70aSa b70aSa</td>
<td>B57 B58 B63</td>
</tr>
</tbody>
</table>
Patient LS: Enter HLA typing and triplets into HLA Matchmaker to determine antigen scores
Enter HLA Matchmaker **antigen** scores into Platelet Panel Database (PPD)

- Specific for each individual patient

The HLA score is detrimental to transfused platelet survival

<table>
<thead>
<tr>
<th>DONOR ANTIGEN</th>
<th>SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A* 01</td>
<td>0</td>
</tr>
<tr>
<td>A* 02</td>
<td>98</td>
</tr>
<tr>
<td>A* 03</td>
<td>98</td>
</tr>
<tr>
<td>A* 11</td>
<td>6</td>
</tr>
<tr>
<td>A* 23</td>
<td>0</td>
</tr>
<tr>
<td>A* 24</td>
<td>98</td>
</tr>
<tr>
<td>A* 25</td>
<td>98</td>
</tr>
<tr>
<td>A* 26</td>
<td>98</td>
</tr>
<tr>
<td>A* 29</td>
<td>98</td>
</tr>
<tr>
<td>A* 30</td>
<td>98</td>
</tr>
<tr>
<td>A* 31</td>
<td>98</td>
</tr>
<tr>
<td>A* 32</td>
<td>98</td>
</tr>
<tr>
<td>A* 33</td>
<td>98</td>
</tr>
<tr>
<td>A* 34</td>
<td>98</td>
</tr>
</tbody>
</table>

Scores edited by user Hudson, Fiona (VIC)
Patient LS: Donor list generated from Platelet Panel Database (PPD)

The HLA score is detrimental to transfused platelet survival
Haploidentical transplant patients and platelet support

Some very good questions!

- What is a haploidentical transplant?

- What does this mean, and what are the impacts for searching purposes?
Family Search

Parents

A*01
B*08
DRB1*03

A*02
B*44
DRB1*04

A*03
B*07
DRB1*15

A*24
B*35
DRB1*11

Patient

Sib 1

Sib 2

Sib 3

1:4 = Probability of any sibling matching
Family Search – Haplo-identical Match

Parents

A*01
B*08
Cw*07
DRB1*03

A*02
B*44
Cw*05
DRB1*04

A*03
B*07
Cw*07
DRB1*15

A*24
B*35
Cw*04
DRB1*11

Parents

A*01
B*08
Cw*07
DRB1*03

A*03
B*07
Cw*07
DRB1*15

Sib 1

Patient

A*01
B*08
Cw*07
DRB1*03

A*03
B*07
Cw*07
DRB1*15

Sib 2

A*02
B*44
Cw*05
DRB1*04

A*03
B*07
Cw*07
DRB1*15

Sib 3

A*02
B*44
Cw*05
DRB1*04

A*24
B*35
Cw*04
DRB1*11

b
Platelet support for haplo patients

- **Important to know:**
  - WHICH donor is being used for transplant?
  - WHAT is the proposed transplant date?
  - WHAT is the actual transplant date and donor? (ie Day 0)
  - WHEN is platelet support needed: pre and/or post transplant?

- **Requirements to give platelet support to the patient:**
  - Avoid jeopardising the graft with platelet transfusions – may cause an immune response to the mismatched donor antigens
  - The degree of engraftment will differ depending on the time from transplant
Platelet Panel Database (PPD)

- Currently 63 patients nationally require specialised platelet support:
  - Most due to platelet transfusion refractoriness
  - Some also in preparation for HLA mismatched stem cell transplant

- Patient support usually medium to long term range:
  - 6 months to 7 years
  - Post transfusion platelet increments are vital to assist with patients with non-zero donor scores
  - **need a process to get these after every HLA compatible platelet transfusion**

- Database of approximately 12,300 apheresis platelet donors nationally
  - Interfaces directly with blood donor system (NBMS)
  - Interfaces with lab system for HLA/HPA result transfer
Conclusion

- HLA typing and HLA antibody test results of an individual can be used to find causative HLA epitopes

- Epitopes can be used to determine donor HLA antigens to avoid

- Application of HLA epitopes has been proven as a useful tool in compatibility matching for platelet transfusion