Cases from ARC Reference Laboratory

Now for something a little different...

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American Red Cross
Immunohematology Reference Laboratory
Case 1 - JH

• 41 y/o African American female
• Admitted for abdominal pain
• Sample submitted to IRL for ABO discrepancy resolution
  – “forward types as AB with liquid reagents in tubes and reverses as B”
  – No discrepancy seen with solid phase testing
IRL Initial ABO results (tube testing)

<table>
<thead>
<tr>
<th>Anti-A</th>
<th>Anti-B</th>
<th>$A_1$ cells</th>
<th>B cells</th>
<th>Auto cells</th>
<th>$A_2$ cells</th>
<th>O cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>3+</td>
<td>0</td>
</tr>
</tbody>
</table>
What is causing the hospital’s discrepancy?

- Recent transfusion or BMT
  - None
- Sample mix-up
  - Unlikely
- Reagent issues
  - Hospital reported that forward typing done with Bio-Rad reagents
### Testing other sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Pt cells + anti-A</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ortho BioClone #1</td>
<td>1+</td>
<td>MH04 and A3D3 MM Blend</td>
</tr>
<tr>
<td>Immucor Series 1</td>
<td>0</td>
<td>Birma-1 Murine monoclonal</td>
</tr>
<tr>
<td>Ortho BioClone #2</td>
<td>+w</td>
<td>MH04 and A3D3 MM Blend</td>
</tr>
<tr>
<td>Immucor Polyclonal (human)</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>Immucor Gamma-clone (Initial ABO)</td>
<td>0</td>
<td>Birma-1 Murine monoclonal</td>
</tr>
</tbody>
</table>

- From BioClone® insert (paraphrased): The anti-A reagent may detect A antigen in a small number of group B individuals now identified as B(A) cells. The agglutination is usually weaker than expected and is easily dispersed. Testing the rbcs with monoclonal anti-A derived from a cell line other than MH04 may be useful in discrepancy resolution.
The B(A) phenotype

- First seen with monoclonal anti-A reagents that were formulated to agglutinate cells of the weak A subgroup, $A_x$.
- Yates, et al. found that A and B gene-specified transferases have overlapping specificities, so that the B gene-specified transferase not only adds D-galactose to H structures to form B antigen, but has some ability to add N-acetyl-galactosamine to other H structures (A antigen).
- Beck et al. showed that the B(A) individuals had high levels of galactosyltransferase and their rbcs are agglutinated by selected, potent monoclonal anti-A reagents.
Formation of ABO Antigens

Gene ➞ Transferase ➞ Sugar

- H gene ➞ Fucose
- A gene ➞ N-acetylgalactosamine (GalNAc)
- B gene ➞ D-galactose
- O gene ➞ No functional enzyme
GalNAc

Galactose

FUCOSE

Galactose

N-Acetylglucosamine

Galactose

Glucose

Ceramide

A Antigen
### B(A) vs $A_{\text{sub}B}$

<table>
<thead>
<tr>
<th></th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>$A_1$ cells</th>
<th>B cells</th>
<th>Auto cells</th>
<th>$A_2$ cells</th>
<th>O cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>B(A)</td>
<td>+w</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>POS</td>
<td>0</td>
</tr>
<tr>
<td>$A_{\text{sub}B}$ with anti-$A_1$</td>
<td>+w</td>
<td>4+</td>
<td>1+ to 4+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- It is important to differentiate B(A) phenotype from $A_{\text{sub}B}$ with anti-$A_1$.
- B(A) individuals have anti-A in their plasma, like other Group B persons (reacts with $A_1$ and $A_2$ rbcs).
Bio-Rad Anti-A

• Bio-Rad Seraclone® Anti-A is a murine monoclonal IgM antibody derived from cell clone line A003

• Seraclone® Anti-A is formulated to react 2-3+ with A_x and A_xB red cells
Conclusions

- Results from ABO testing for JH are most consistent with B(A) phenotype
  - Reactivity noted with anti-A from MH04 clone
  - Plasma demonstrated anti-A in reverse typing
- Patient should be treated as group B for all transfusion purposes
Questions?
Case 2 - SF

• 82 y/o Caucasian female referred for antibody identification and 2 unit crossmatch (April 2011)
• Diagnosis: lung cancer
• Transfusion history: Received 4 PC and platelets in the preceding 2 months
• Antibody screen: All cells positive by Gel-IAT
Initial IRL results

• Group O, Rh positive
• DAT negative
• Antibody screen:

<table>
<thead>
<tr>
<th></th>
<th>IS</th>
<th>PeG IAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>SII</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>SIII</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>AC</td>
<td>0</td>
<td>0 ✓</td>
</tr>
</tbody>
</table>
RBC Phenotype

- Patient had been recently transfused
  - used microhematocrit cell separation method to obtain autologous reticulocytes for testing
- Rh phenotype: C+E+c+e+
- K-; Fy(a+b-); Jk(a-b+); S+s+M+; P_{1}+; Le(a-b+)
- Other: H+
Selected Cells

- DAT/AC negative - antibody is most likely alloimmune
- Phen sim cells tested to differentiate between antibody to HIA and multiple antibodies to common antigens

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<thead>
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<tr>
<td>Phen sim</td>
<td>1+</td>
<td>2+</td>
</tr>
<tr>
<td>Phen sim</td>
<td>2+</td>
<td>2+</td>
</tr>
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</table>
Selected Cells

- To help characterize the antibody, tested same cells after treatment with 1% papain and 0.2M DTT.
- Papain inconclusive (AC pos); Antibody appeared to be reactive with a DTT-sensitive antigen.

<table>
<thead>
<tr>
<th></th>
<th>IS</th>
<th>PeG</th>
<th>Papain PeG</th>
<th>DTT PeG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phen sim</td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
<td>0/mi+</td>
</tr>
<tr>
<td>Phen sim</td>
<td>2+</td>
<td>2+</td>
<td>3+</td>
<td>0/mi+</td>
</tr>
<tr>
<td>Auto</td>
<td></td>
<td></td>
<td>3+</td>
<td>0/0 ✓</td>
</tr>
</tbody>
</table>
Antigens destroyed/weakened by DTT treatment

- Kell; LW; Scianna; Yt\textsuperscript{a}; Indian; JMH

- Cromer; Knops; Lutheran; Dombrock; AnWj; MER2
Testing Rare Cells
(Neat plasma or eluate from adsorption/elution)

<table>
<thead>
<tr>
<th>Phenotype</th>
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</tr>
</thead>
<tbody>
<tr>
<td>$K_0$</td>
<td>0/mi+</td>
<td>Cr(a-)</td>
<td>2+</td>
</tr>
<tr>
<td>Lu(a-b-)</td>
<td>2+</td>
<td>p (PP$^1$P$^k$-)</td>
<td>2+</td>
</tr>
<tr>
<td>LW(a-)</td>
<td>2+</td>
<td>Vel-</td>
<td>2+</td>
</tr>
<tr>
<td>Tc(a-)</td>
<td>2+</td>
<td>I-</td>
<td>2+</td>
</tr>
<tr>
<td>Yt(a-)</td>
<td>2+</td>
<td>Gy(a-)</td>
<td>2+</td>
</tr>
</tbody>
</table>
# Testing Rare Cells
(Neat plasma or eluate from adsorption/elution)

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</tr>
</thead>
<tbody>
<tr>
<td>K₀ #2</td>
<td>0/mi+</td>
<td>Kp(b-)</td>
<td>2+</td>
</tr>
<tr>
<td>Js(b-)</td>
<td>2+</td>
<td>McLeod</td>
<td>0/mi+</td>
</tr>
<tr>
<td>K: -14</td>
<td>2+</td>
<td>K: -11</td>
<td>2+</td>
</tr>
<tr>
<td>K₀ #3</td>
<td>0/mi+</td>
<td>TOU- (K: -26)</td>
<td>2+</td>
</tr>
<tr>
<td>K: -22</td>
<td>2+</td>
<td>EGA-treated</td>
<td>1+</td>
</tr>
</tbody>
</table>
What do we have so far?

• Antibody appears to be directed at antigen in Kell system
  – Weak, but not compatible with $K_o$ cells
  – Weakened but not destroyed by DTT-treatment of red cells

• Could this patient be $K_o$ or $K_{mod}$ with anti-Ku-like antibody?
Typings for Kell system antigens

- Anti-k: Neg
- Anti-Js^b: Neg
- Anti-Kp^b: Neg

Patient’s cells do not appear to have detectable Kell system antigens...
Putting together the pieces...

- Samples sent to 2 other IRL laboratories for confirmation of our results
  - 1 lab found 1 $K_{\text{mod}}$ cell to be compatible
  - 2nd lab tested 2 addn’l sources of $K_o$ cells: one was compatible, one was microscopically incompatible
Putting together the pieces...

- Sample sent for Monocyte Monolayer Assay (MMA) to help determine clinical significance of antibody
- MMA results showed 46.6% reactive monocytes against random donor cells
  - “Normal” range is 0-3% reactive monocytes. Values above 3% suggest antibody will cause accelerated clearance of antigen-positive rbc's.
Putting together the pieces...

- Sample sent for genotyping by HEA BeadChip by ARC National Molecular Lab.
- Patient appeared to have normal Kell system genes - predicted to be antigen-positive for k, Kpb, Jsb. No other unusual genotypes noted.
- Sample was referred for sequencing of the *KEL* gene to identify possible Kell variants.
The *KEL* gene has 19 exons that produce the Kell glycoprotein
- Sequencing is time consuming due to the high number of exons. For comparison, *RhD* gene has 10 exons, *FY* has 2 exons, *DO* has 3 exons.

4 months later...sequencing confirmed the *KEL* genotypes from the HEA BeadChip, but revealed several nucleotide changes that have not been previously reported.
**KEL sequencing**

- Mutations present on exon 5, exon 12, and exon 14.
- Sequencing results, with absence of Kell antigens on the rbcs and the presence of an antibody to a high incidence Kell antigen, suggest that one of more of these mutations may represent a new high incidence antigen in the Kell system.
- Next step: cDNA sequencing to identify phasing of the changes (whether they are *in cis* or *in trans* with each other).
Managing this patient

- Red cells of the rare $K_o$ or $K_{mod}$ phenotypes are likely the best source for transfusion. No compatible units are currently available.
- Siblings would be a good source of potential donors, but patient does not have any siblings.
- Pt’s son was tested; appears to have normal K antigens, cells were incompatible with patient’s plasma (3+).
- Patient’s anemia is currently being managed with erythropoietin. Prognosis from 4/2011 was 1 to 1 1/2 year survival (due to cancer).
Summary

• 36 antigens currently in Kell System
• SF’s antibody may define a new HIA in Kell system
• R. Persa from Oklahoma Blood Institute presented similar case in 2011 of a possible novel \textit{KEL} silencing allele in an Apache Native American with anti-Ku (not published)
  – Pt. appeared to be K_{o}, but had normal \textit{KEL} genotype
  – Sequencing showed deletion in exon 18 of 1 allele, but revealed a 2nd normal allele
• We wait to see what these findings represent for the Kell system!
The End