Rare blood groups – characterisation and clinical significance

Jill R. Storry, Ph.D.,FIBMS
Clinical Immunology and Transfusion Medicine
University and Regional Laboratories
Lund, Sweden
Overview

- Blood group antigen classification
- ISBT antigen nomenclature
  - Case study - how do numbers get assigned?
- Characterisation of rare blood
- Significance of rare blood phenotypes
Blood group antigens are carried on functional molecules on the RBC

Figure courtesy of ES Wester
Lund University
Blood group antigen classification

☀ Three levels:
- Blood group systems – 33
- Collections – 7
- Series – 2

- Total = 339 antigens (2012-07-07)
Blood group antigen classification

- Assigned numbers by the ISBT working party on Red Cell Immunogenetics and Blood Group Terminology
- Based on different degrees of confidence
  - Serological evidence e.g. specific alloantibody; discrimination from other blood group systems
  - Genetic evidence
  - Molecular characterisation
297 antigens are assigned to 33 blood group systems.
18 antigens are assigned to blood group collections

<table>
<thead>
<tr>
<th>Number</th>
<th>Name</th>
<th># antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>205</td>
<td>COST</td>
<td>2</td>
</tr>
<tr>
<td>207</td>
<td>li</td>
<td>1</td>
</tr>
<tr>
<td>208</td>
<td>ER</td>
<td>3</td>
</tr>
<tr>
<td>209</td>
<td>GLOB</td>
<td>2</td>
</tr>
<tr>
<td>210</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>212</td>
<td>VEL</td>
<td>2</td>
</tr>
<tr>
<td>213</td>
<td>MN CHO</td>
<td>6</td>
</tr>
</tbody>
</table>

When collections are elevated to blood groups, the collection number becomes obsolete.
24 antigens are assigned to “Highs and Lows” series

- **901 series** – high incidence antigens
  - 6 antigens
- **700 series** – low incidence antigens
  - 18 antigens

Defined by specific antibodies but cellular localisation or genetic background unknown
Highlights of WP meeting at the International ISBT meeting, Cancun

- 3 new blood group systems (FORS, JR, LAN)
- 10 new antigens added to existing blood groups
- Many different groups trying to resolve orphan antigens by different methods
Finding evidence for a blood group antigen

- Antibody that defines an unknown antigen
- Serologic characteristics e.g. enzymes; "null" RBCs
- Family studies
- Candidate gene sequencing
What is Rare Blood?

A rare Blood type is any Blood type that is difficult to find.
What is Rare Blood?

About one person in 1,000 will inherit a rare Blood type:

- Unusual combinations of common antigens
- Negative for a high incidence antigen
  - Homozygosity for a recessive gene e.g. Kp(b-), Rh\textsubscript{null}
  - Inheritance of an "Inhibitor" gene e.g. Lu(a-b-)
- Absence of a whole protein
  - Lan, Jra, Jk3
# Rare blood types that are challenging to find

<table>
<thead>
<tr>
<th>Country</th>
<th>Challenging type to obtain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>Rare Rh Phenotypes with antibodies to high prevalence antigens and other common antibodies</td>
</tr>
<tr>
<td>Brazil</td>
<td>McLeod, Ko, Lan–, U–, RH29–, RH17–</td>
</tr>
<tr>
<td>Finland</td>
<td>Vel–, Ge:–2</td>
</tr>
<tr>
<td>France</td>
<td>U– D–, HrS–, HrB–, Js(b–), RN/RN, Rhnull, Jr(a–), Co(a–b–)</td>
</tr>
<tr>
<td>Germany</td>
<td>Fy(a–b–), In(b–), Ge:–2,–3,</td>
</tr>
<tr>
<td>China (Hong Kong)</td>
<td>Di(b–), Fy(a–b–), Jk:–3</td>
</tr>
<tr>
<td>Oman and India</td>
<td>D– –, In(b–), Co(a–b–)</td>
</tr>
<tr>
<td>Israel</td>
<td>p, Jr(a–), Oh, Ko, U–, Vel– Lan–</td>
</tr>
<tr>
<td>Italy</td>
<td>Sc:–1, Lw(a–), Ko, Jk:–3, U–, Di(b–)</td>
</tr>
<tr>
<td>Japan</td>
<td>Ge–, En(a–), MkMk, Lan–</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>U– D–, Fy(a–b–), Lu(a–b–) D–, At(a–), Cr(a–)</td>
</tr>
<tr>
<td>New Zealand</td>
<td>D– –, Ko, McLeod, p, Ge:–2, Js(b–),</td>
</tr>
<tr>
<td>Spain</td>
<td>Yt(a–), Co(a–), Js(b–), Lan–, Ge–, I–, Jr(a–)</td>
</tr>
<tr>
<td>Switzerland</td>
<td>Kp(b–), Vel–, Pk, Jk:–3, D– –, Ko, Lan–</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>Rhnull, Sc:–1, P1k, Ge:–2,–3, D– –, McLeod, U</td>
</tr>
<tr>
<td>United States</td>
<td>At(a–), En(a–), Hy–, Ko, Cr(a–), Ge:–2, In(b–), Lan–, Di(b–), Ge:–2, Jk:–3, Lu(a–b–), hrS– E–, Gy–, K:–2 Vel–, Wes(b–)</td>
</tr>
</tbody>
</table>

Rare Blood and Ethnicity

- Founder effect?
  - PP1Pk– phenotype in the Amish people, Northern Sweden
  - Jr(a–) in Roma people in Slovakia, Japan

- Spontaneous mutations
  - e.g. PP1Pk– phenotype in Europe

- Genetic selection based on pathogens
  - E.g. Fy(a–b–) RBCs are resistant to infection by *Plasmodium vivax*
  - Ok(a–)? Basigin receptor for P Falciparum (Crosnier Nature 2011)
World Conflicts 2000-2009
Impact of migration

- Genetic blood disorders that require transfusion
  - SCD, thalassemia

- Different blood group antigen profiles

- Common W. African RBC profile:
  - D+ C-E-c + e+, K-k+, S-s+, Fy(a-b-), Jk(a+b-)
  - 30-40% of African American donors
  - 1:1000 Caucasian donors?
  - Screen D- units?
Rare blood – multiple alloantibodies

- 51 year old man - Swedish
- Transfusion-dependent, terminally ill
- Plasma contained anti-c, -E, -K, -Jk^a, -s
- Incidence of the phenotype:
  - \(0.15 \times 0.98 \times 0.24 \times 0.13 = 0.00459\)
  - That is 4.59 donors/1000
- To find one unit, need to screen 218 donors (ABO-compatible!!)
Rare blood – multiple alloantibodies

- Supported by blood transfusions every week from all over Sweden
- Enormous stress on screening resources
  - Very expensive
  - Very time-consuming
Characterisation of rare blood groups – Serological methods

- Often dependent on limited supplies of antisera from patients
  - No QA
  - Limited infectious-disease testing
- Monoclonal antibodies available for some antigens
## Screening at the Japanese RC

### Table 2: Screening for rare blood by monoclonal antibodies (1987–2006)*

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Number of tested</th>
<th>Rare blood</th>
<th>Number of detected</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-U+N</td>
<td>2 062 873</td>
<td>U−</td>
<td>3</td>
<td>000015%</td>
</tr>
<tr>
<td>anti-En3</td>
<td>1 369 681</td>
<td>En(a−)</td>
<td>2</td>
<td>000002%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M^MM^k</td>
<td>1</td>
<td>000001%</td>
</tr>
<tr>
<td>anti-Hro</td>
<td>2 285 766</td>
<td>−D−</td>
<td>183</td>
<td>000082%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhnull</td>
<td>10</td>
<td>000005%</td>
</tr>
<tr>
<td>anti-CD44</td>
<td>6 730 998</td>
<td>Lu(a−b−)</td>
<td>607</td>
<td>000902%</td>
</tr>
<tr>
<td>anti-K2,</td>
<td>16 290 609</td>
<td>Ko</td>
<td>286</td>
<td>000176%</td>
</tr>
<tr>
<td>anti-K5,</td>
<td></td>
<td>McLeod or Kmod</td>
<td>83</td>
<td>000112%</td>
</tr>
<tr>
<td>anti-K14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anti-Fy3</td>
<td>795 239</td>
<td>Fy(a−b−)</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>anti-Ge</td>
<td>6 648 374</td>
<td>Ge−</td>
<td>17</td>
<td>000027%</td>
</tr>
<tr>
<td>anti-IFC</td>
<td>237 459</td>
<td>IFC−</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>anti-i</td>
<td>1 086 228</td>
<td>i−</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>anti-Jr3</td>
<td>10 708 646</td>
<td>Jr(a−)</td>
<td>4454</td>
<td>004159%</td>
</tr>
<tr>
<td>anti-Lan</td>
<td>713 523</td>
<td>Lan−</td>
<td>14</td>
<td>000196%</td>
</tr>
</tbody>
</table>

*Including repeat donors.

Characterisation of rare blood groups – Genotyping methods

Genotyping programs

- Molecular basis for (almost) all rare blood group phenotypes known
  - Majority are single nucleotide polymorphisms
- Various commercial kits and platforms available for genotyping
# Genotype-derived phenotypes by BioArray

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Predicted phenotype</th>
<th>Donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPB S-s-</td>
<td>S-s-U-</td>
<td>2</td>
</tr>
<tr>
<td>FY265 AB</td>
<td>Fy(a+b\text{weak})</td>
<td>1</td>
</tr>
<tr>
<td>FYB265 BB</td>
<td>Fy(a-b\text{weak})</td>
<td>1</td>
</tr>
<tr>
<td>LU AA</td>
<td>Lu(a+b-)</td>
<td>3</td>
</tr>
<tr>
<td>LU AB</td>
<td>Lu(a+b+)</td>
<td>5</td>
</tr>
<tr>
<td>SC AB</td>
<td>Sc 1,2</td>
<td>2</td>
</tr>
<tr>
<td>DO323</td>
<td>Hy-</td>
<td>1</td>
</tr>
<tr>
<td>DO350</td>
<td>Jo(a-)</td>
<td>2</td>
</tr>
<tr>
<td>DI AA</td>
<td>Di(a+b-)</td>
<td>1</td>
</tr>
<tr>
<td>DI AB</td>
<td>Di(a+b+)</td>
<td>3</td>
</tr>
<tr>
<td>KEL AB</td>
<td>K1/K2</td>
<td>2</td>
</tr>
</tbody>
</table>

Data generously shared by Lilian Castilho, Hemo-Centro
Medium throughput screening

Wagner FF et al. Transfusion 2008;48:1169-75

- Identified high incidence antigens with an antigen-negative frequency of ~1:500
  - Yt\textsuperscript{a}, Co\textsuperscript{a}, Lu\textsuperscript{b}, Kp\textsuperscript{b}
- Designed a multiplex (4 amplicons) to detect the negative phenotype
  - Different amplicon sizes
- Used a ”quick and dirty” DNA prep method: Extract-N-Amp
  - PCR directly on blood
In normal samples, PCR showed 4 bands. Where a specific band was missing, confirmatory serology performed on the sample to confirm absence of the antigen. Tested 3422 group O, RhD-negative donors:
- 1 Kp(b-); 6 Co(a-); 10 Yt(a-); 5 Lu(b-)
Medium throughput screening

- Time and cost analysis:
  - Hands-on time – 91 tests/102 minutes
  - Cost €1.52/test

- Effective method for medium throughput screening

- Can add SNPs of interest, e.g. HPA-1A
Medium throughput screening


- 35 blood group antigen SNPs in 6 multiplex reactions/sample

- Screened 6000 donors:
  - Lu(b-) 9
  - k- 5
  - Kp(b-) 1
  - Yt(a-) 24
  - Co(a-) 11
Medium throughput screening


- 35 blood group antigen SNPs in 6 multiplex reactions/sample
- Costs:
  - Serology – 35-39€
  - Genotyping – 15€
- Repeat donors selected
Challenges finding rare blood

- How can Rare Donors be identified?
- High incidence antigen-negative patient?
- Always test relatives where possible
  - Siblings 1:4 chance of inheriting a rare blood group
  - Small communities have a higher incidence of rare types
SCARF

- Serum Cells And Rare Fluids
- Exchange program
  - Started in the USA
  - Worldwide exchange of rare RBCs and sera (inhibition substances)
  - Requires sending out one sample/year
  - What’s common in your lab could be rare
  - On-line request system
Rare Donor Panels

✦ Several large Rare Donor Panels:
  – DGTI
  – WHO (Bristol, UK)
  – ARDP (USA)
  – CBS
  – Regional and National

✦ ISBT Working Party lists 118 facilities working with the various Rare Donor programs
Rare blood listed on the IDP

Categories of rare phenotypes currently listed on the IDP
(from the 2004 Working Party report)

- Oh
- CDE/CDE, CdE/CdE, CwD-/CwD-, -D/-D-, Rh_null, Rh:-51
- LW(a-b+), LW(a-b-)
- S-s-U-, S-s-U(+), En(a-)
- Pp, Pk
- Lu(a+b-), Lu(a-b-)
- Kp(a+b-), Kp(a-b-), Js(a+b-), K_0, K:-11
- Jo(a-), Gy(a-), Hy-
- Fy(a-b-), Jk(a-b-), Di(b-), Yt(a-), Sc:-1, Co(a-), McLeod, Vel-, Ge, Lan-, At(a-), Jr(a-), ln(b-), Cr(a-), Er(a-), Ok(a-), JMH-
How can clinically important antibodies be distinguished from insignificant antibodies?

What has history taught us........
Blood Groups That Cause Destruction

ABO
Rh
Kell
Kidd
S, s, U
Duffy
Vel
Colton

Relatively simple to identify
Rarely significant

Lewis
M/N
P1
Knops
Cs^a
Ch/Rg
JMH
Sd^a
Xg^a

Easy to work around

More difficult to define
Sometimes significant

$Y^a$

Lutheran

LW

Cromer

Dombrock

Gerbich

Lan

The most difficult group

How can these antibodies be handled?
Serum/Plasma Testing

- Look for variation in antigen strength between panel RBCs
  - Characteristic of Knops system, Lan, Vel
- Test cord RBCs (if available)
  - Weak reactivity characteristic of Knops, I, AnWj
- If antibody remains unidentified
  - absorb plasma with phenotype-matched RBCs and look for underlying alloantibodies
- Observation: antibodies to papain-sensitive antigens, e.g. Yt\textsuperscript{a}, Ch/Rg, JMH are not usually significant
How good are in vitro tests of survival?

<table>
<thead>
<tr>
<th>MMA</th>
<th>1hr</th>
<th>24hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>%R</td>
<td>&gt;70%</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>0-5</td>
<td>100%</td>
<td>33%</td>
</tr>
<tr>
<td>5.1-20</td>
<td>100%</td>
<td>67%</td>
</tr>
<tr>
<td>&gt;20</td>
<td>60%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Ardnt & Garratty Transfusion. 2004;44:1273-81.
## Correlation of MMA with Transfusion Reactions

<table>
<thead>
<tr>
<th>MMA % reactive</th>
<th>Transfusion Reaction*</th>
<th>Clin signs</th>
<th>Lab signs only</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5%</td>
<td>0%</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>5.1 – 20%</td>
<td>33%</td>
<td>67%</td>
<td></td>
</tr>
<tr>
<td>&gt;20%</td>
<td>64%</td>
<td>75%</td>
<td></td>
</tr>
</tbody>
</table>

* Based on definition of "significance"

Ardnt & Garratty Transfusion. 2004;44:1273-81.