Uncommon Blood Groups: The Diego System

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Objectives:
1. Discuss case studies detailing the history of Diego system antigens and antibodies.
2. Describe the characteristics of antigens and antibodies of the Diego System.

Recognized blood group systems
- The International Society of Blood Transfusion (ISBT) Working Party for Red Cell Immunogenetics and Blood Group Terminology
- Currently 43 recognized blood group systems
- Containing 345 red blood cell antigens

Red blood cell antigens
- Systems of one or more antigens governed by a single gene or complex of two or more closely linked genes

Discovery of red cell antigens
- Historically due to antibody presence/detection or involvement in transfusion reaction or hemolytic disease of the fetus and newborn (HDFN)
- New antigens discovered today due to advances in genomic testing
- Important that laboratorians are aware of “uncommon” antigens/antibodies

Common versus uncommon
- Common: antigens to which antibodies are commonly encountered
- FDA requirements for antigen makeup of commercial red blood cells for antibody detection and identification
- Uncommon groups have high and/or low prevalence antigens, so the corresponding antibodies are not commonly encountered
Antigen frequency/prevalence

- **High prevalence**: antigens carried by 98 – 99% of the population
  - Will not be stimulated to produce the corresponding antibody
- **Low prevalence**: antigens carried by <1 – 2% of the population
  - Very few donor units would contain the antigen

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**Discovery of Di\(^a\)**

- 1953 Venezuela, South America
- Miguel Layrisse and co-workers are studying the serum of an infant who died from severe jaundice
- Blood specimens from the mother and the infant were sent to Philip Levine in New York
- The infant’s RBC were strongly coated with antibody
- No antibody was demonstrable in the mother’s serum

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**Discovery of Di\(^a\)**

- 1955 Venezuela
- Mrs. Diego consults Layrisse about a new pregnancy
- Di\(^a\) antigen was found in 4 generations of this family
- Evidence of family mixture with South American Indians
- Further study showed the Di\(^a\) antigen in groups of Native Americans in the US and in people of Chinese and Japanese ancestry

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**Diego blood group antigens**

- Di\(^a\)
- Di\(^b\)
- Wr\(^a\)
- Wr\(^b\)
- 17 others, Low incidence antigens

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**Discovery of Di\(^a\)**

- ABO and Rh incompatibility were excluded
- In October 1953, the father visits Levine in New York
- The father’s RBC were tested against the maternal serum, and a strong agglutination reaction occurred
- Levine and the father agreed to name this mysterious blood factor “Diego” after their family name

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**Layrisse, Levine, and Diego**

- Miguel Layrisse
- Diego Blood System
- Philip Levine
Discovery of Di<sub>b</sub>

- Studied in 1964-65; published in 1967
- Mrs. Luebano, group B, Rh-positive, Mexican-Indian heritage
  - Experienced jaundice after 3-unit transfusion during elective surgery
  - Has delivered 5 infants with no evidence of HDFN
  - Previous transfusion in 1943
- Mrs. Ramirez, group A, Rh-positive, Mexican-Indian heritage
  - Pre-surgery crossmatches reacted with all donors at AHG
  - Has delivered 9 normal infants
  - No prior transfusion history

Discovery of Wr antigens

- First described in 1953
- Implicated in a case of HDFN
- Named after the family in which the antibody was found
- Assigned to the Diego blood group in 1995
- Antithetical Wright antigens, Wr<sup>a</sup> and Wr<sup>b</sup>
  - Wr<sup>a</sup> is low-prevalence in all ethnic groups <0.01%
  - Wr<sup>b</sup> is high-prevalence with universal expression in all groups

Frequency of Diego antigens

- Diego blood group is very interesting, especially to anthropologists
- 21 known antigens in the Diego system
- Di<sup>a</sup>, Di<sup>b</sup>, Wr<sup>a</sup> are the most significant
  - Di<sup>a</sup> found mainly in populations of Mongolian descent but is rare in Whites and Blacks
  - Di<sup>b</sup> found universally in most populations

Frequency of Diego phenotypes

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Whites</th>
<th>Blacks</th>
<th>Asians</th>
</tr>
</thead>
<tbody>
<tr>
<td>Di(a=b+)</td>
<td>&gt;99%</td>
<td>&gt;99%</td>
<td>&gt;90%</td>
</tr>
<tr>
<td>Di(a+b+)</td>
<td>&lt;0.1%</td>
<td>&lt;0.1%</td>
<td>10%</td>
</tr>
<tr>
<td>Di(a+b=)</td>
<td>&lt;0.1%</td>
<td>&lt;0.1%</td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>Di(a=b=)</td>
<td></td>
<td></td>
<td>Extremely rare among all races</td>
</tr>
</tbody>
</table>

Di<sup>a</sup> antigen is low prevalence among Whites and Blacks
Di<sup>b</sup> antigen is high prevalence among all races

Linking people of Asia to the Americas

- Di<sup>a</sup> antigen has been used as an anthropologic population migration marker

Where are the Diego antigens?

- On the RBC membrane
  - Associated with Band 3 and Glycophorin A

Phenotype

- Di(a=b+)
- Di(a+b+)
- Di(a+b=)
- Di(a=b=)
Where are the Diego antigens?

- In the kidneys
  - On alpha-intercalated cells lining the distal tubules and collecting ducts of nephron

Molecular basis of Diego antigens

- SLC4A1 gene on chromosome 17
- Anion exchanger 1 proteins
- Chloride/bicarbonate exchange
- 2 versions of AE1 protein:
  - Long – on the RBC
  - Short – in the kidney

Long AE1 proteins (RBC)

- Band 3 protein on the RBC
- Major anion exchanger
- Structural framework (cytoskeleton) of the RBC
- Interacts with Glycophorin A

Band 3 on the RBC membrane

- Most abundant membrane protein in human RBC
- Physically linked to ankyrin
- Facilitates exchange of CO₂ and HCO₃⁻
- Associated with Glycophorin A
- ABO, H/h, I/i antigens

Anion exchange across the RBC

- Exchange one Cl⁻ for one HCO₃⁻
- CO₂ diffuses across and is converted into HCO₃⁻
- Direction of exchange depends on the concentration of ions on either side of the RBC

Diego proteins of Band 3

- Involved in the transport of ions across the RBC membrane
What’s up with Glycophorin A?

- Major intrinsic membrane protein of RBC
- Required for high activity of Band 3 anion exchange

Glycophorin A structure

- Site of M and N antigens
- Associates with Band 3 to form the Wrb antigen

Wrb proteins of Band 3

- Evidence suggests the Wrb antigen is formed by linkage between Band 3 and Glycophorin A
- Both are required for expression of the Wrb antigen
- Wrb will be absent from RBC that lack GYPA

Hematological disease associations

- Mutations in the SLC4A1 gene can cause several blood disorders
- Autosomal dominant inheritance
- Leads to reduction of AE1 proteins or development of abnormal AE1 proteins
  - Hereditary spherocytosis
  - Hereditary stomatocytosis
  - Southeast Asian ovalocytosis

Hereditary spherocytosis

- Mutation to SLC4A1 gene
- Overly rigid, mishapen cells
- Spherical instead of disc shaped
- Cells cannot flex and change shape to travel through blood vessels
- Spherocytes removed from circulation and broken down in the spleen

Hereditary spherocytosis

- Anemia
- Jaundice
- Splenomegaly
- High risk for developing gallstones
Hereditary stomatocytosis

- Abnormal AE1 proteins
- Allow sodium and potassium to leak out of cell
- RBC are unstable and are broken down more quickly than usual

Southeast Asian ovalocytosis

- Most common in regions where malaria is endemic
- Deletion of 9 amino acids in AE1 protein
- Reduction of AE1 on surface of RBC
- Cells are unusually rigid and oval shaped
- May be lethal if mutation is homozygous

Short AE1 proteins (kidney cells)

- Specialized kidney cells
- Alpha-intercalated cells
- Distal tubules and collecting ducts
- Resorb substances that are needed
- Eliminate wastes

Diego proteins on kidney cells

- Exchange of bicarbonate through AE1 protein allows acid to be released from cell into urine for excretion
- Renal tubules return useful substances to blood circulation

Kidney disease associations

**SLC4A1-associated distal renal tubular acidosis**

- At least 18 different mutations to SLC4A1 gene
- Leads to buildup of acid in the blood (metabolic acidosis)
- Autosomal dominant and autosomal recessive forms

Distal renal tubular acidosis

- Altered AE1 proteins either stuck inside the cell or trafficked to the wrong side of the cell
- Disrupts bicarbonate exchange = acid buildup in blood
- Soft, weak bones, calcium deposits, kidney stones
Clinical significance of Diego antibodies
- May be IgM or IgG
- Capable of causing Hemolytic Disease of the Fetus and Newborn (HDFN)
- Capable of causing immediate and delayed hemolytic transfusion reactions

Anti-Di\textsuperscript{a} and Anti-Di\textsuperscript{b}
- Clinically significant for transfusion practice and pre-natal testing
- Will cause mild to severe HDFN
- Few cases of hemolytic transfusion reaction
- Selected units should be Diego antigen negative and crossmatch compatible

Anti-Wr\textsuperscript{a}
- Clinically significant for transfusion practice and pre-natal testing
- Occurs in up to 2% of blood donors
- Frequently found in patients with autoimmune hemolytic anemia
- Often found in association with other antibodies

Anti-Wr\textsuperscript{a}
- Will cause mild to severe HDFN
- Known to cause acute and delayed hemolytic transfusion reactions, sometimes severe
- Selected units should be Wr\textsuperscript{a} antigen negative and crossmatch compatible
Transfusion practice

• FDA mandates that commercial cell panels include RBCs with D, C, E, c, e, M, N, S, s, P1, Lea, Leb, K, k, Fya, Fyb, Jka and Jkb antigens
• Currently no requirement for Diego system antigens
• Routine donor antigen typing may not include the Diego system antigens

Applications to patient management

• Rare donor files
• Select cell panels
• Consider Diego system when antibody to high incidence antigen found in Japanese and other non-White populations

Thank you!

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