IMMUNOHEMATOLOGY

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Rh BLOOD GROUP SYSTEM

- discovered in 1940 by Landsteiner & Wiener
- most complex erythrocyte antigen system; located on chromosome 1
- found exclusively on surface of rbc → integral part of red cell membrane
- primary antigen → if present, consider Rh (+)
- lack corresponding naturally-occurring antibodies in serum

Rh BLOOD GROUP SYSTEM

CLASSIFICATION/NOMENCLATURE SYSTEM

Wiener

- **Multiple allele hypothesis**
- **5 antigens: Rh_o, rh', rh", hr', hr"**
- **Single locus inheritance system with 8 alternate common alleles coding for agglutinogens → 1 individual produces 2 agglutinogens inherited from both parents**

Rh BLOOD GROUP SYSTEM

CLASSIFICATION/NOMENCLATURE SYSTEM

Fischer & Race

- Three alleles: D/d, C/c and E/e
- Five antigens: D, C, E, c, e
- d → no D locus → no antigenic products

Rosenfeld

- Numerical system
- Rh1 to Rh5

Rh BLOOD GROUP SYSTEM

Rh Antigens

- **with three integral membrane proteins**
  1. **RhD**
  2. **RhCcEe**
  3. **Rh-associated glycoprotein (Rh50, RhAG)**
- **D antigen → resides in RhD protein → most immunogenic followed by c, E, C and e**

Rh BLOOD GROUP SYSTEM

Weak D Antigen (D^u)

- **Rho variant**
- **weak or absent red cell agglutination by anti-D → detected only with use of anti-human globulin reagent → use bovine anti-D**
- **weakened form caused by 1 of 3 situations:**
  1. **a piece of the D antigen is missing**
  2. **D gene is on a chromosome opposite a C gene → (+) steric hindrance**
  3. **Inheritance of a gene coding for less D antigen**

Rh BLOOD GROUP SYSTEM

- **Presence of D = presence of Rh_o factor → Rh (+)**
- **Absence of D → Rh (-)**

Rh BLOOD GROUP SYSTEM

Testing for Rh_o (D) Antigen:

- **use antisera originating from human source**

- **antisera with different constituents → use of high protein media necessary to produce agglutination since antigens are an integral part of the red cell membrane → less numerous than ABO antigens**

Rh BLOOD GROUP SYSTEM

Testing for D^u Variant:

- use bovine or albumin-suspended anti-D reagent
- incubate at 37°C for 15-60 minutes to facilitate formation of Ag-Ab complex
- interpretation: (+) D^u → consider Rh (+)
- women who appear to be Rh (-) should be proven to be D^u (-) before they are considered to be eligible to receive transfusion

Rh BLOOD GROUP SYSTEM

Rh Antibodies

- not naturally-occurring → immune antibodies → produced upon sensitization → IgG isotype
- reactive at 37°C → enhanced with enzyme-treated red cells
- can cross the placenta
- associated with hemolytic transfusion reaction and hemolytic disease of the newborn (HDN)

Rh BLOOD GROUP SYSTEM

Rh Typing – slide or test tube method

- **False (+) results:**
  1. **Drying**
  2. **Roleaux formation**
  3. **Auto-agglutination**
  4. **Patient's red cells heavily coated with Ab's**
  5. **Presence of cold agglutinins**

Rh BLOOD GROUP SYSTEM

Rh Typing

- **False (-) results:**
  1. **Use of old cells**
  2. **Wrong cell concentration**
  3. **Hemolysis**
  4. **Inadequate mixing of cells**
  5. **Inactive typing sera**
  6. **Incorrent temperature**
  7. **Existence of D^u variant**
  8. **High concentration of blocking antibodies**

MINOR BLOOD GROUP SYSTEMS

Significance:

1. **For medico-legal parenthood studies**
2. **May cause transfusion reaction or HDN**

MINOR BLOOD GROUP SYSTEMS

Systems with cold-reacting antibodies

- Antibodies formed react at temperatures 25^0C or colder
- Not considered clinically significant since any reaction seen in the test tube will not be seen in the warmer temperatures of the body
- Not likely to cause a transfusion-related accident

MINOR BLOOD GROUP SYSTEMS

Systems with cold-reacting antibodies

1. ***Lewis (Le) System***
   - **Antigens: Le^a and Le^b → formed in secretions & absorbed onto surface of rbc later**
   - **Antibodies – often encountered in individuals with no antigens; may be present at certain times (e.g. pregnancy) and then disappear**
2. ***MNS System***
   - **Antigens are weakly antigenic**
   - **Antibodies: naturally-occurring or stimulated by direct exposure**

MINOR BLOOD GROUP SYSTEMS

Systems with cold-reacting antibodies

3. *P-p System*

- **P1 antigen most antigenic → present on cells of 79% of whites & 94% of African-Americans**

4. *Ii system*

- **Antigens: I and i → both present in all individuals**
- **I antigen – present in large quantities in adults**
- **i antigen – present in large quantities on cells taken from the umbilical cord**
- **Anti-I → freq. seen in serum of patient's with recent infectious mononucleosis**

MINOR BLOOD GROUP SYSTEMS

Systems with warm-reacting antibodies

- reactive at 37^0C in anti-human globulin medium
- Clinically significant → most likely to cause HDN and HTR

1. ***Kell (K) – Cellano (k) System***
   - **k Ag present in 98% of the white population**
   - **antibodies primarily IgG**
2. ***Kidd System***
   - **Antigens: Jk^a & Jk^b – not very antigenic**
   - **Antibodies stimulated by direct exposure via either pregnancy or transfusion**

MINOR BLOOD GROUP SYSTEMS

Systems with warm-reacting antibodies

3. Duffy System

- **Antigens: Fy^a & Fy^b**
- **Antibodies stimulated through direct exposure → capable of causing HDN and HTR**

HEMOLYTIC DISEASE OF THE NEWBORN

- involves hemolysis of red cells in the fetus and neonate
- antibody is present in the mother that corresponds to an antigen on the surface of the red cells of the fetus → Ab crosses placenta → attaches to fetal Ag → hemolyze red cells of fetus
- Differential diagnosis: physiologic jaundice, septicemia, CID, toxoplasmosis, congenital syphilis

HEMOLYTIC DISEASE OF THE NEWBORN

ABO Disease

- **Most common type**
- **Most cases are mild & do not require exchange transfusion**
- **Most common scenario: *mother is group O and infant is group A***
- ***Even first baby is affected***

HEMOLYTIC DISEASE OF THE NEWBORN

ABO Disease

Features:

1. **Spherocytosis**
2. **Increased reticulocyte count**
3. **Increased indirect bilirubin in 1st 72 hours of life**
4. **Jaundice appearing during first 24 hrs of life**

Good evidence for ABO disease is detection of immune anti-A or anti-B in the cord blood of the newborn.

HEMOLYTIC DISEASE OF THE NEWBORN

Rh Disease

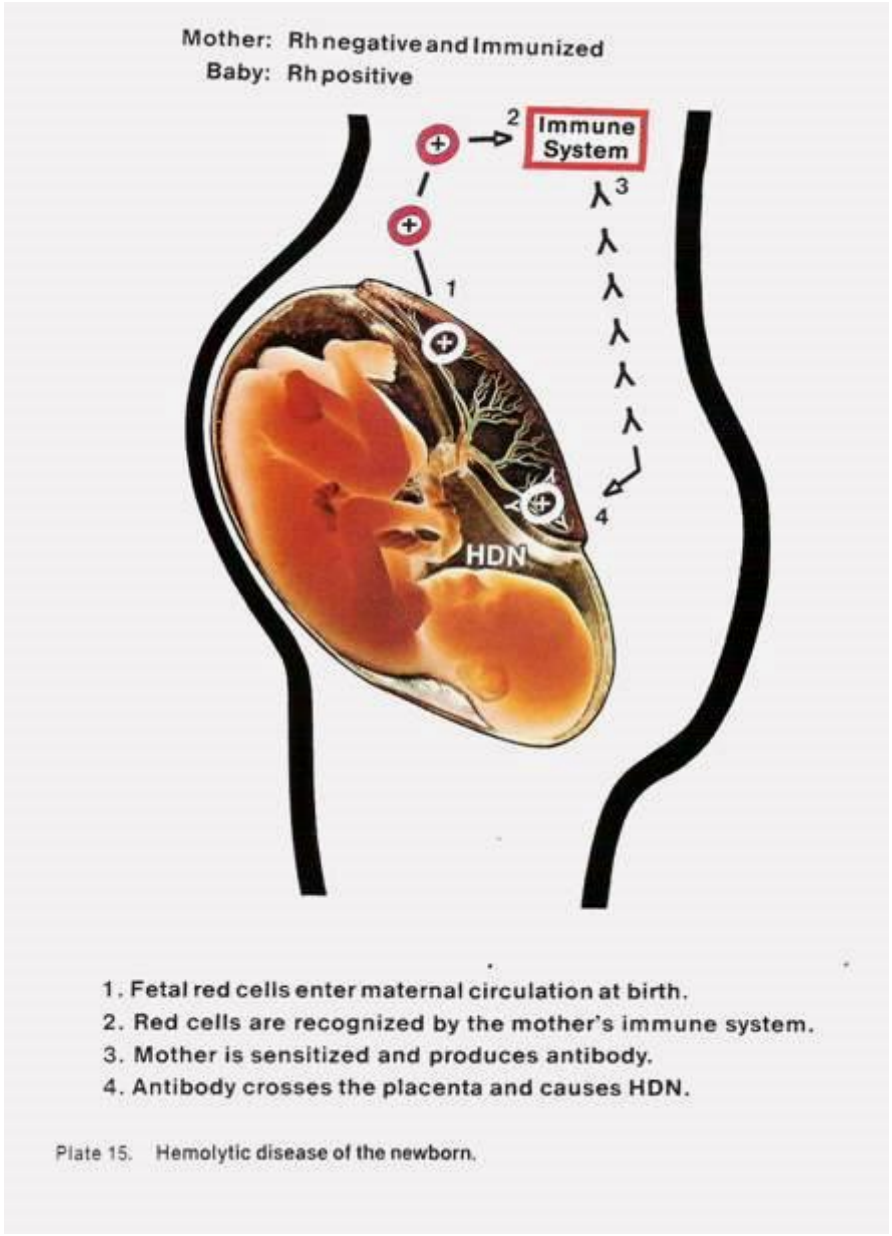
- most severe; Rh (+) fetus & Rh (-) mother
- **FIRST PREGNANCY**

  Rh (+) baby → Ag enters maternal circulation → sensitize Rh (-) mother → anti-Rh production (IgG) → cross placenta → enter fetal circulation → baby not affected

- **SUBSEQUENT PREGNANCIES**

  Ab already present in mother → enter fetal circulation → (+) intravascular hemolysis →accumulation of rbc destruction products → jaundice or kernicterus (**erythroblastosis fetalis**)

HEMOLYTIC DISEASE OF THE NEWBORN



1. Fetal red cells enter maternal circulation at birth.
2. Red cells are recognized by the mother's immune system.
3. Mother is sensitized and produces antibody.
4. Antibody crosses the placenta and causes HDN.

Plate 15. Hemolytic disease of the newborn.

HEMOLYTIC DISEASE OF THE NEWBORN

Rh Disease

- first baby usually unaffected since it is the first time the mother is exposed to the antigen
- occasionally, firstborns are affected either because of:
  1. previous maternal exposure (e.g. previous aborted pregnancy)
  2. unusually great maternal susceptibility to Rh stimulus during normal pregnancy

HEMOLYTIC DISEASE OF THE NEWBORN

Rh Disease

Characteristics of Erythroblastosis Fetalis:

1. **Increased number of circulating nucleated red cells**
2. **Increased osmotic fragility of cells**
3. **Increased amount of indirect/unconjugated bilirubin**

Main Clinical Findings:

1. **Anemia - < 15 gm/100 ml or 150 gm/L**
2. **Rapidly developing jaundice**

HEMOLYTIC DISEASE OF THE NEWBORN

Rh Disease

Management:

For the mother

- **RhoGam (Rh Immune Globulin)**
  - ✓ **concentrated anti-D**
  - ✓ **coats Rh (+) fetal cells in maternal circulation → recognized by mother's system as abnormal & removed from circulation → prevents maternal immune system from processing the Ag on surface of fetal cells → no antibody formed**

HEMOLYTIC DISEASE OF THE NEWBORN

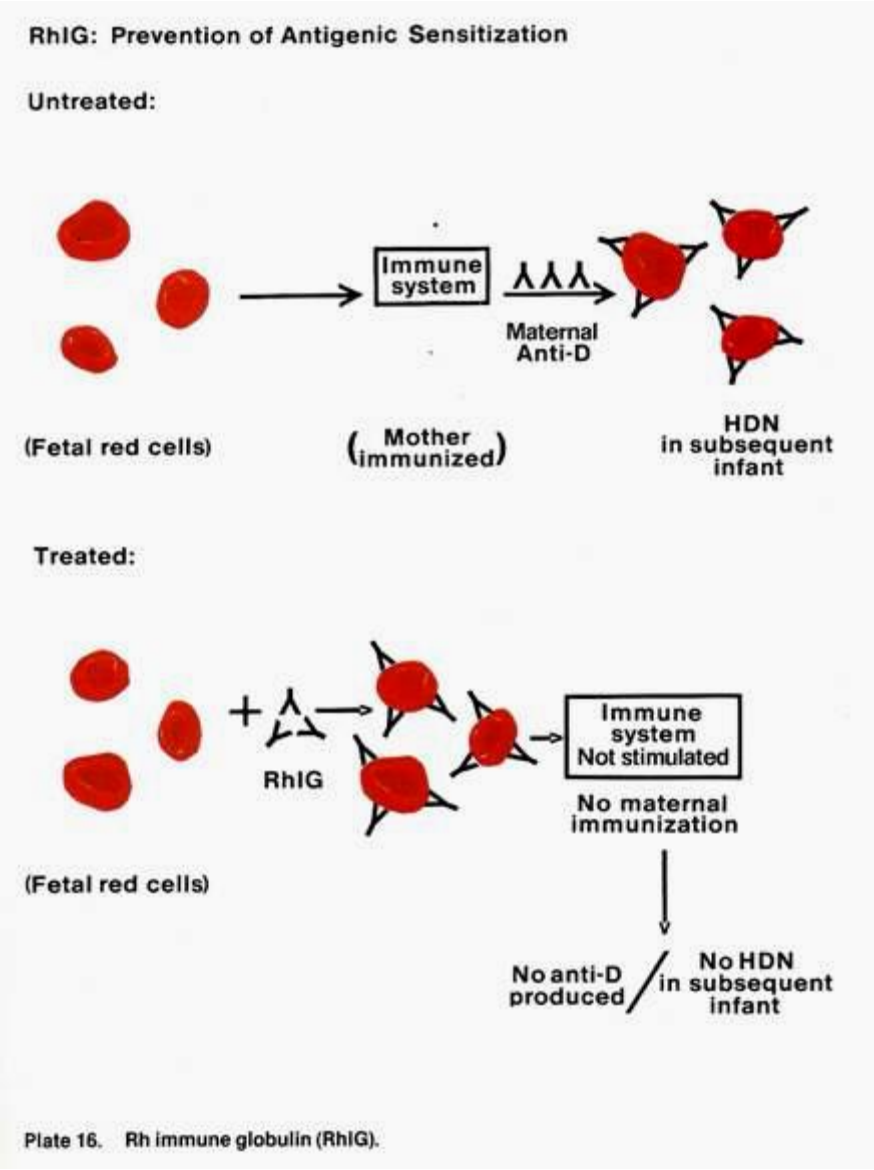


Plate 16. Rh immune globulin (RhIG).

HEMOLYTIC DISEASE OF THE NEWBORN

For the mother

- **RhoGam (Rh Immune Globulin)**
  - ✓ **Dose: routinely administered 2x – at 28 wks AOG & within 72 hrs after birth of an Rh (+) infant**
  - ✓ **Also administered following termination of any pregnancy, after amniocentesis in an Rh (-) mother & following accidental transfusion with Rh (+) red cells**

HEMOLYTIC DISEASE OF THE NEWBORN

For the baby: EXCHANGE TRANSFUSION

- **Indications:**
  1. **Infant serum indirect bilirubin > 20mg/100 ml (342 μmol/L) for fullterm infants OR >15mg/100 ml (257 μmol/L) for premature infants**
  2. **Cord blood indirect bilirubin > 3 mg/100 ml (51 μmol/L)**
  3. **Cord blood hemoglobin < 13 gm/dL (130 g/L)**
  4. **Maternal Rh antibody titer of 1:64 or more**

HEMOLYTIC DISEASE OF THE NEWBORN

Beneficial Effects of Exchange Transfusion:

1. **Removal of bilirubin**
2. **Removal of sensitized RBCs**
3. **Removal of incompatible antibody**
4. **Replacement of incompatible RBCs with compatible RBCs**
5. **Suppression of erythropoeisis (reduced production of incompatible RBCs**

HEMOLYTIC DISEASE OF THE NEWBORN

Comparison of ABO versus Rh HDN

Characteristic	ABO	HDN
First pregnancy	Yes	Rare
Disease predicted by titers	No	Yes
Antibody IgG	Yes (anti-A,B)	Yes (anti-D)
Bilirubin at birth	Normal range	Elevated
Anemia at birth	No	Yes
Phototherapy	Yes	Yes
Exchange transfusion	Rare	Common
Intrauterine transfusion	None	Sometimes
Spherocytosis	Yes	Rare


ThankYou!