IN SUMMARY
ISO-IMMUNIZATION

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History Of Rh Isoimmunization

- The Rh story is one of multiple foci of independent investigations
  - –Occurring at different sites
  - –Different times
- High level of competition that can develop
  - –Laboratory scientists
  - –Clinical scientists
  - –Pharmaceutical industry

Ortho Pharmaceutical Company

- Trade name RhoGAM, three decades ago
- Rh Isoimmunization affected approximately 1 percent of the pregnancies in the U.S. at the beginning of this century

Clinical findings

- Hemolytic anemia
- Edema of the fetal tissues known as **hydrops (eryhtroblastosis) fetalis**
- Autopsy evidence of proliferation of red blood cells in multiple sites
- Large of number of immature red cells

- 1930s - recognized as one clinical entity were
  - Hydrops fetalis
  - Icterus gravis neonatorum
  - Congenital anemia
  - Erythroblastosis fetalis
- World War II lead to discovery of antigenic blood factors, which might result in immunization and cause transfusion reactions.
- Major contributors
  - Alexander Wiener
  - Philip Levine,
  - Karl Landsteiner

Causes of fetal hydrops

- Lymphatic Abnormalities
  - Lymphangiectasia
  - Cystic hygroma
  - Turner's syndrome (XO)
  - Noonan's syndrome
  - Multiple ptergium syndrome
  - Pulmonary Malformations
  - Lymphangiectasia
  - Chylothorax
  - Cystic adenomatoid malformation
  - Hypoplasia
  - Other

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- Hematologic
  - Fetal hemolytic anemia
  - α-Thalassemia
  - Fetomaternal or twin-to-twin transfusion
- Congenital Infections
  - Viruses
  - Cytomegalovirus
  - Parvovirus B19
  - Toxoplasmosis
  - Syphilis
  - Chagas Disease
- Cardiovascular
  - Arrhythmias
  - Cardiomyopathy
  - Structural anomalies: lesions that result in increased right atrial pressure and volume
  - primarily with atrioventricular regurgitation
  - left sided obstructive lesions
  - Ebstein's anomaly
  - Premature closure of the foramen ovale
  - Intracardiac tumors (tuberous sclerosis)
  - Vascular malformations
  - Chorangioma of the placenta, chorionic or umbilical vessels
  - Hemangiomas (Hepatic, Klippel-Trenaunaysyndrome)

- Other Causes
  - Obstructive uropathy
  - Congenital nephrosis
  - Chromosomal abnormalities
  - Trisomy 15, 18, 21
  - XX/XY
  - Neoplasms
  - Storage diseases
  - Bone diseases
  - Placental abnormalities
  - Neurologic abnormalities
  - Idiopathic

Genetics and Biochemistry of the Rh Antigen

Nomenclature

•1940, Landsteiner and Wiener - rabbit immune sera to rhesus monkey erythrocytes
•Agglutinated the majority (85 percent) of human erythrocytes
•Named this the Rh factor.
•Agglutinated cells were called *Rh positive*
•Disease caused by antibody directed against an erythrocyte surface antigen of the rhesus blood group system.
•High degree of polymorphism

Five major antigens can be identified

- Many variant antigens
- Three Systems of Categorization
- Fisher-Race
- Wiener system
- HLA-like system of Rosenfield

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Fisher-Race System

- Nomenclature is best known in Obstetrics
- Presence of three genetic loci
- Each with two major alleles
- C, c, D, E, and e
- *No antiserum specific for a "d" antigen*
- *"d" indicates the absence of a discernible allelic product*
- Anti-C, anti-c, anti-D, anti-E, and anti-e designate specific anti-sera directed against the respective antigens.
- Rh gene complex described by the three appropriate letters

Rh Antigen Complexes

Eight gene complexes (decreasing frequency in humans)

Cde
cde
Cde
cDe
Cde
cdE
CDE
CdE

Table removed due to copyright restrictions.
[Rh Gene Frequencies in 2000 Unrelated Caucasian Adults]

Nomenclature

- Written in the order C(c), D(d), E(e)
- Actual order of the genes on chromosome 1 is D, C(c), E(e).
- Vast majority of Rh Isoimmunization - incompatibility with respect to the D antigen
- *Rh positive* indicates the presence of the D antigen
- *Rh negative* indicates the absence of D antigen

Weiner System

- Assumption of only one genetic locus
- Eight genotypes are designated (in decreasing order of frequency in the white population) R^1 , r, R^2 , R^0 r, r, R^Z , and r^v.

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Variants of D Antigen

- Unique Rh antibodies have been used to identify more than 30 antigenic variants
- Two of the most common
- C^w antigen
- D^u antigen
- Heterogeneous group of clinically important D antigen variants most often found in African Americans
- D^u -positive individuals - quantitative decrease in expression of the normal D antigen,
- Some D^u variants are significantly different antigenically
- Two cellular expressions responsible for the D^u phenotype
- Reduction in the number of D antigen sites with all epitopes represented
- Expression of only some of the various D antigen epitopes with some epitopes missing

D^u Variant

- D^u -positive erythrocytes - bind anti-D typing sera
- *In some cases only by sensitive indirect antiglobulin methods*
- At least some D^u -positive patients are capable of producing anti-D, presumably by sensitization to missing D epitopes.
- Could result in a D^u -positive mother becoming sensitized to her D-positive fetus

Genetic Expression

- Genetic locus for the Rh antigen on the short arm of chromosome 1
- Within the Rh locus are two distinct structural genes adjacent to one another,
- RhCcEe and RhD.
- Likely share a single genetic ancestor,
- Identical in more than 95 percent of their coding sequences
- First gene codes for the C/c and E/e antigens
- Second gene codes for the D antigen
- D-negative lack the RhD gene on both their chromosomes.
- D-negative patients have a deletion of the D gene on both their chromosomes 1
- Expression of the Rh antigen on the erythrocyte membrane
    - Genetically controlled
    - Structure of the antigen
    - Number of specific Rh-antigen sites (e.g., D, E, C, c, or e)
    - Relatively constant amount of Rh antigen sites available
    - About 100,000 sites per cell
    - Evenly divided between C(c), D, and E(e) antigens

Allelic Interactions

- CDe/cde express less D antigen than cDE/cde.
- CDe/cDE express less C antigen than CDe/cde

Structure and location of antigens

- The Rh antigens - polypeptides
- Embedded in the lipid phase of the erythrocyte membrane
- Distributed throughout the membrane in a nonrandom fashion
- D antigen sites - spaced in a lattice-like pattern
- 92 nm in Rh(D) heterozygotes
- 64 nm in homozygotes
- Rh polypeptides are polymorphic
- MW of the D antigen 31,900 d
- C(c) and E(e) antigen MW 33,100 d

Biochemistry & Immunology

- Rh polypeptide lies within the phospholipid bilayer of the membrane

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- Spans the membrane 13 times
- Short segments extending outside the red cell
- Extrude into the cytoplasm
- D antigen appears very early in embryonic life - 38-day-old fetus
- Expressed early in the erythroid cell series – pronormoblasts
- Seven different D antigen epitopes have been identified or deduced using human monoclonal anti-D antibodies, and others may exist. One hypothesis suggests that these different epitopes are part of the same protein-lipid complex more or less expressed according to the depth of polypeptide

Clinical Issues

- Anti-D antibody titer of greater than 1:4 - considered Rh sensitized
- Consider possibility that the fetus might be Rh negative
- Fathered by another partner
- Mismatched blood transfusion
- Determining the paternal Rh-antigen status is reasonable
- DNA analysis can be used to determine his zygosity
- Father homozygous - all his children will be Rh positive
- Father heterozygous - 50 percent likelihood that each pregnancy will have an Rh-negative fetus
- Cordocentesis with analysis of fetal red blood cells
- Blood sampling for fetal Rh antigen status at 18 to 20
- Increased risks of fetal loss and fetomaternal hemorrhage

Current Technology

- *The Rh locus on chromosome 1p34-p36 has been cloned*
- Polymerase chain reaction (PCR)
- Uncultured amniocytes
- 2 ml of amniotic fluid
- 5 mg of chorionic villi.

Ultrasound and Doppler Studies

- Sonographic findings that might predict the severity of Erythroblastosis fetalis
- Avoid the need for invasive assessments
    - Pre-hydropic changes
    - Polyhydramnios
    - Placental thickness
    - Pericardial effusion
    - Dilation of the cardiac chambers
    - Chronic enlargement of the spleen and liver
    - Visualization of both sides of the fetal bowel wall,
    - Dilation of the umbilical vein

Figure removed due to copyright restrictions.

[Liley curve]

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SUMMARY OF IMPORTANT FACTS IN RH DISEASE

- To reduce the incidence of Rh sensitization, Rh-immune globulin should be given to Rh-negative unsensitized women at 28 weeks' gestational age and again after delivery if the newborn is Rh positive. Rh-immune globulin is also indicated for these patients in cases of miscarriage, ectopic pregnancy, chorionic villus sampling, amniocentesis, or fetomaternal hemorrhage.
- The gene coding for the D antigen has been cloned, and in the near future prenatal determination of fetal Rh status should be routinely available from uncultured amniocytes obtained at amniocentesis.
- Measurement of amniotic fluid bilirubin remains the standard for assessment of pregnancies at risk for significant fetal anemia. Neither ultrasound alone nor Doppler are adequately sensitive to identify anemic fetuses.
- The timing of the first amniocentesis is based on history, maternal anti-D titers, gestational age, and ultrasound findings. The timing of subsequent amniocenteses is based on the $DeltaOD_{450}$ values and trends.
- Analysis of amniotic fluid bilirubin before 26 weeks is controversial. Although most data suggest that $DeltaOD_{450}$ values and trends are accurate before the third trimester, more liberal use of cordocentesis may be appropriate.
- Fetal transfusion can be performed using either the intraperitoneal or intravascular route. For hydropic fetuses, intravascular transfusion is clearly superior. For non-hydropic fetuses, perinatal survival rates are similar with either method.
- With the reduction in Rh disease brought about by widespread use of Rh-immune globulin prophylaxis, sensitization to the minor or atypical antigens has become relatively more common. A number of these minor antigens can cause several fetal anemia.

FUNDAMENTAL QUESTIONS

1. Give a brief history of the discovery of the Rh antigen.
2. Where is this antigen located and what are its biochemical characteristics?
3. Name the antigens?
4. Which one is of major significance?
5. What are the clinical manifestations of isoimmunization?
6. What is hydrops fetalis and what are the causes?
7. What us a D^u variant?
8. What determines the severity of clinical disease in the fetus?
9. What is a Liley curve and how is it used?
10. What diagnostic modalities are useful in the detection and therapy of Rh disease?
11. What is Rho-gam and how is it obtained in the year 2004?
12. Are there any risks to giving Rho-gam?
13. How can one determine the amount of Rho-gam that is needed for any given patient?

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