ISO-IMMUNIZATION
HISTORY OF Rh Isoimmunization

The Rh story is one of multiple foci of independent investigations occurring at different sites and different times. High level of competition that can develop includes:

- 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, affecting:

- hemolytic anemia
- edema of the fetal tissues known as hydrops fetalis
- autopsy evidence of proliferation of red blood cells in multiple sites
- large of number of immature red cells

Erythroblastosis fetalis noted by:

- Louis Diamond
- Kenneth Blackfan
- Arthur Hertig
- John Ferguson
- Stewart Clifford

1930s - were recognized as one clinical entity:

- hydrops fetalis
- icterus gravis neonatorum
- congenital anemia
- Erythroblastosis fetalis

Iso-immunization
World War II discovered antigenic blood factors, which might result in immunization and cause transfusion reactions.

Major contributors

- Alexander Wiener
- Philip Levine,
- Karl Landsteiner.

1939 that Levine published his observations in a case brought to his attention by observant clinicians. Mary Sino was a woman who had delivered an erythroblastotic child after which she was given a transfusion of her husband's blood with which she was compatible by the major blood groups known at that time. She suffered a major transfusion reaction.

1 year later - Weiner and Landsteiner published their article identifying the Rh factor.

1941 - Levine demonstrates the presence of Rh antibodies in another mother of an erythroblastotic baby.

John Grant Gorman was an Australian educated in Melbourne - He had received his MB, BS at the University of Melbourne - internship in Australia.

Freda was born in the United States - undergraduate education at Columbia University - medical degree from New York University (NYU).

Freda had a great interest in the work of Weiner at NYU
- Weiner became the important scientific mentor for Freda
- John Scudder, a surgeon who founded the blood bank at Presbyterian Hospital in 1938
- Donald McKay became chair of the Department of Pathology at Columbia that Gorman received the encouragement that he deserved in pursuing his interests in blood banking
- Freda received consistent encouragement from Howard Taylor

Iso-immunization
Gorman did not receive the same level of support and interest from his department of pathology before the appointment of McKay.
Taylor placed Freda on major committee as a resident to represent his department with regard to Rh issue.
in response Scudder appointed Gorman as his resident representative from the department of pathology.

Ronald Finn was pursuing his studies for a thesis leading to his MD degree in England.
interest was in identifying fetal cells in maternal circulation using the Kleihauer technique.
Finn developed a theory similar to that developed by Freda and Gorman - suggested that the elimination of fetal cells circulating in maternal circulation by the use of an antibody might prevent Rh sensitization in pregnancy.

Theobald Smith published in established the scientific basis for the method of prevention that they eventually would prove to be successful.
Ruth Rent Darrow also pursued the concept of incompatibility between mother and fetus, but focused her interest on hemoglobin as the possible causative agent.

Kurt Stern identified the peculiar protective effect of ABO incompatibility on patients who had concomitant Rh incompatibility.
Hertig, Hellman, and others recognized the protected status of the first pregnancy and the increasing risk of fetal involvement with subsequent pregnancies.

Freda and Gorman recognized contribution of Theobald Smith as to how immunization might be prevented by the administration of antibodies.
Developed relationship with William Pollock of the Ortho Pharmaceutical.

Iso-immunization
Levine did not agree with the theory that had been proposed by Freda and Gorman.
Pollock was intrigued by the theory, and worked to develop the antibody preparation needed by Freda and Gorman.
Freda and Gorman graduated from their residency training programs.
Gorman became assistant director of the blood bank at Lenox Hill Hospital in New York City.
Columbia recruited him to be the director of the blood bank at the Columbia-Presbyterian Medical Center. Freda was made of an Rh clinic at Columbia. All patients from this large obstetric service with the potential for Rh incompatibility were referred to that clinic.
1960 - Freda and Gorman developed thesis for how Rh incompatibility could be prevented.
Submitted to Science in 1960 but was rejected.
Application to the New York City Health Research Council in February of 1961 under Gorman's name was returned unapproved.
McKay added his name to Gorman's on the proposal, and it was approved.
Freda had developed an NIH proposal, - reviewed by senior investigators working in the same general area but whose theories conflicted with those of Freda and Gorman - proposal was rejected.
May 27, 1961, the British Medical Journal published a report by Finn and his colleagues where the same theory was presented.
1961 - Through the cooperation of the physician at Sing-Sing Prison and its warden, their studies to use Pollack's immunoglobulin for protection were begun.
First trials began by injecting Rh antigen into Rh-negative male prisoners with some receiving the antibody preparation and some serving as controls.

First publication of the Sing-Sing experiment was in the Bulletin of the Sloan Hospital for Women.
Gorman later presented the gamma globulin data at the International Society for Hematology in Mexico City of 1962.
After this time that a bit of irony occurred when the Liverpool group, having visited Freda and Gorman, asked if they could be given a sample of the gamma globulin used by Freda and Gorman to try in their own facilities. Subsequently, the Liverpool team published their success in preventing Rh sensitization by injection of gamma globulin by using the material obtained from Freda and Gorman. This report upstaged Freda and Gorman's important work who received only a footnote credit that the materials were
derived from the ongoing New York experiment. This gives an indication again of the issue of premature publication and the intensity of rivalry that occurs in the scientific community.

Theobald Smith's discovery in 1908 was also proving applicable in the early 1960s whereby the passive use of injected antibodies could indeed prevent sensitization in the body when challenged by a foreign antigen.

Freda and Gorman were basing their prevention strategy on the belief that injection after delivery would be the best method.

The standard treatment widely used today—that injection should occur within 3 days of delivery—came about in a peculiar manner. The warden of the Sing-Sing prison was concerned by the proposal by Freda and Gorman. Their proposal was that they return soon after injecting prisoners with Rh-positive cells to administer the antibody. The warden perceived that the inmates might use a short interval in some mischievous way. Finally, a compromise was reached whereby a 3-day interval would be satisfactory to the warden. Freda felt that an obstetric reality for patients who would delivered on a Friday, considering the laboratory limitations of that time, was that the injection of the antibody might not be possible until Monday. Therefore, the 3-day interval satisfied the needs of both groups and became an international standard based on those arbitrary decisions.

First woman injected with RhoGAM is interesting

Gorman's sister-in-law, Kathryn, and his brother Frank, an ophthalmologist, were in England for a period of study. Kathryn became pregnant and was found to be Rh negative

John's father, a physician in Australia, became very concerned that the Rh incompatibility between Frank and Kathryn would interfere with subsequent pregnancies.

Knowing the success of John's studies at the Sing-Sing prison, he insisted that John do something to protect Kathryn from Isoimmunization.

Finally John Gorman and Vincent Freda agreed that they would violate all of the rules and agreements they had made and would ship a vial of gamma globulin to Frank in England 1 month before Kathryn's delivery date.

John personally took the vial of gamma globulin to the Kennedy Airport and had it air freighted to Heathrow Airport.
Frank and Kathryn retrieved the package at Heathrow, but surprisingly, she began premature labor as they drove to their home in West Hertfordshire.

Kathryn subsequently delivered after a difficult labor and immediately Frank presented the attending physician with a vial of material which he insisted that she receive by injection.

Her obstetrician was unwilling to do this without some additional confirmation of its safety and contacted the Liverpool research group regarding this request.

The Liverpool group recognized the importance of the event historically and also its importance to Kathryn. They advised the physician to follow Frank's request.

Therefore, on January 31, 1964, John Gorman's sister-in-law Kathryn became the first woman known to be injected with gamma globulin to prevent isoimmunization.

She did not become immunized, and in a subsequent pregnancy, became one of the first women to be protected with gamma globulin after a second pregnancy.

The formal clinical trials of injecting pregnant women opened in New York City on March 31, 1964.

Liverpool studies began in May 1964.

The first injection in the New York study was given by Freda on April 1, 1964.

At a meeting where these data were presented, Dr. Eugene Hamilton from Missouri came forward to show his experience with raw plasma he had obtained personally from highly sensitized donors known to him through his practice. More than 500 pregnant patients - outstanding record of prevention.

**Genetics and Biochemistry of the Rh Antigen**

**Nomenclature**

1940, Landsteiner and Wiener immobilized sera to rhesus monkey erythrocytes

Agglutinated the majority (85 percent) of human erythrocytes

*Rh factor.*

Agglutinated cells were called *Rh positive*

Antibody directed against an erythrocyte surface antigen of the rhesus blood group system.

High degree of polymorphism

Iso-immunization
Five major antigens can be identified

Many variant antigens

Three Systems of Categorization

- Fisher-Race
- Wiener system
- HLA-like system of Rosenfield.

Fisher-Race 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

Eight gene complexes (decreasing frequency in humans)

- Cde
- cde
- CDe
- cDe
- Cde
- cdE
- CDE
- CdE

CDe/cde and CDe/Cde are most common genotypes

55% of all whites having the CcDe or CDe phenotype

Iso-immunization
CdE has actually *never* been demonstrated.

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

**Wiener**

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 \( r^v \).

**Rosenfield**

Rh1 through Rh48.

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 blacks.

\( D^u \)-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.

**Iso-immunization**
D\textsuperscript{u}-positive erythrocytes - bind anti-D typing sera

_in some cases only by sensitive indirect antiglobulin methods_

At least some D\textsuperscript{u}-positive patients are capable of producing anti-D, presumably by sensitization to missing D epitopes.

_Could result in a D\textsuperscript{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)

Alterations in number of the number of specific Rh-antigen sites

- gene dose
- relative position of the alleles
- presence or absence of regulator genes.

Iso-immunization
Relatively constant amount of Rh antigen sites available

- about 100,000 sites per cell
- evenly divided between C(c), D, and E(e) antigens

_Gene dosage_

Example: erythrocytes of individuals homozygous for the c allele have twice as many c antigen sites expressed as are found on the erythrocytes of heterozygotes

_Allelic interaction_

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

**Biochemistry and Immunology**

- 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

Rh polypeptide lies within the phospholipid bilayer of the membrane

- spanning the membrane 13 times
- short segments extending outside the red cell

Iso-immunization
• 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

✓ Immunologic variation in the Rh blood group system (and fetal hemolytic disease) explained by the variable expression of the D antigen epitopes and the specificity of the antibodies formed against them.

Precise function of the Rh antigens are unknown

• ? role in maintaining red cell membrane integrity
• may interact with a membrane adenosine triphosphatase
• functioning as part of a proton or cation pump
• controls volume or electrolyte flux across the erythrocyte membrane

Rh\textsubscript{null} erythrocytes

• increased osmotic fragility
• abnormal shapes

The Rh-Isoimmunized Pregnancy: Assessment of the Fetus

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

Iso-immunization
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

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.

Antibody Titer

First sensitized pregnancy - the level of the anti-D antibody titer determines the need for amniocentesis

"critical titer" – FETAL DEATH DOES NOT OCCUR

1:16 or 1:32 rarely if ever resulted in fetal death but errors and lab variations cause us to use an anti-D titer of 1:8 or greater as an indication for amniocentesis

If the initial anti-D titer is less than 1:8, and if the patient does not have a history of a previously affected infant, the pregnancy may be followed with anti-D titers every 2 to 4 weeks and serial ultrasound assessment of the fetus.

maternal serum anti-D titers are not particularly useful in the management of most Rh-isoimmunized pregnancies
titers may remain stable in as many as 80 percent of the severely affected pregnancies.

(1) binding constant of anti-D varies between individuals
(2) Rh-antigen expression on the fetal erythrocyte membrane varies between individuals,
(3) ability of the fetus to replace erythrocytes without compromising liver function varies between individuals.
    ✓ Fetal hemolytic disease tends to be either as severe, or more severe, in subsequent pregnancies
    ✓ Previous intrauterine or neonatal death from hemolytic disease carries a particularly grave prognosis
Amniotic Fluid Analysis

- spectrophotometric determinations of amniotic fluid bilirubin
- correlated with the severity of fetal hemolysis
- by-product of fetal hemolysis
- excretion into fetal pulmonary and tracheal secretions
- diffusion across the fetal membranes and the umbilical cord

Semilogarithmic plot,

Optical density of normal amniotic fluid is approximately linear

Wavelengths of 525 and 375 nm

Bilirubin - spectrophotometric density - peak at a wavelength of 450 nm

The amount of shift linearity at 450 nm (the $\Delta OD_{450}$) - used to estimate the degree of fetal red cell hemolysis

Liley

Management of Rh-immunized pregnancies

Based on $\Delta OD_{450}$

Third trimester

Correlated amniotic fluid $\Delta OD_{450}$ values with newborn outcome

Three zones

- Unaffected fetuses and those with mild anemia - zone I (the lowest zone)
- Mild to severe - Fetuses with zone II values (the middle zone)
- Severely affected fetuses - zone III (the highest zone)

Fetal Blood Analysis

Direct fetal vascular access with fetoscopy

ultrasound-guided umbilical vein puncture

concern for potential fetal and maternal morbidity

Iso-immunization
successful in greater than 95 percent of cases

fetal loss rates between 0.5 and 2 percent per procedure

risk of fetomaternal bleeding

worsened maternal sensitization

Traversing an anterior placenta with the sampling needle

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

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<sub>450</sub> values and trends.

Analysis of amniotic fluid bilirubin before 26 weeks is controversial. Although most data suggest that DeltaOD<sub>450</sub> 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 severe fetal anemia.

Management of pregnancies complicated by sensitization to one of the serious minor antigens (such as Kell) is based on the experience with Rh disease. However, amniotic fluid bilirubin analysis may not be as accurate an indicator of fetal anemia with Kell sensitization as with Rh disease.

Platelets also carry distinct antigens, and, in a process similar to Rh sensitization, platelet sensitization can occur. Unlike Rh disease, firstborn fetuses can be affected, and maternal antibody titers do not predict fetal platelet count.

The optimal management scheme for pregnancies affected with platelet sensitization is unclear. A protocol involving (1) cordocentesis at 28 weeks for documentation of thrombocytopenia, (2) weekly maternal administration of intravenous immunoglobulin, and (3) cordocentesis at about 37 weeks to determine platelet count before delivery has been suggested and seems to balance risk and potential benefit.