Immunohematology

Intro

Care and detail are extremely important in blood banking.

Hemagglutination is the agglutination of red blood cells and involves cellular antigens (**agglutinogens**) and antibodies (**agglutinins**).

IgM or IgG antibodies can cause agglutination. They can also cause hemolysis if complement (C') is present.

> Ab fix C'→RBCs lyse→release HgB
>
> Therefore: look for clumping (hemagglutination) or a cherry red color.

There are non-immune causes of hemagglutination.

- Some viruses
  - RBCs contain substances that can act as cell receptors which can bind viruses
- Certain acids or bases
- High NaCl environment
  - Isotonic saline is 0.85%
- **Lectins** or **phytohemagglutinins**
  - Produced from seeds of legumes (beans and peas)
  - Seed extract can cause non-immune hemagglutination

The ABO Blood Group System

Carl Landsteiner (1901)

A small group (6 people) divided their cells and serum then recombined them in different ways and observed the results.

- Saw agglutination reactions
- Identified different groups and labeled them A, B and O
- Were actually doing the first forward and reverse typings
- Found
  - Serum from group A agglutinated cells from group B
  - Serum from group B agglutinated cells from group A
  - Serum from group O agglutinated cells of both A and B

Key Words

Hemagglutination

Agglutinogen

Agglutinins

Lectins

Phytohemagglutinins

Carl Landsteiner

Von Descatello & Sterle (1902)

Repeated the Landsteiner experiment with a larger group

Found AB group

- Serum of AB people would not agglutinate A or B cells

These discoveries were fortuitous.

The ABO system is unique (and dangerous) because all normal, healthy people have serum antibodies to the ABP antigens they lack.

"Naturally occurring" is a misnomer: Anti-A, Anti-B, and Anti-AB antibodies are not present at birth. They are too low to be detected until 3-6 months. This is why reverse typing is not usually done until 6 months old.

Titers peak at 5-10 years and then gradually decline. After 65 years, the titers are low.

ABO antibodies likely formed via normal mechanisms (stimulation with antigens from nature).

- B antigenic specificity can be found on E. coli
- A antigenic specificity can be found in plant tissue

When doing experiments with germ-free animals, you often find that they do not develop the expected "naturally occurring" antibodies

ABO Blood Group System		
Groups	**Agglutinogen (Ag)**	**Agglutinin (Ab)**
O	None	Anti-A, Anti-B, Anti-AB
A	A	Anti-B
B	B	Anti-A
AB	A + B	None

You find a wide variation in titers of these Abs. (remember serial dilutions)

- Titer of Anti-A from O person > titer of Anti-A of a B person
- Titer of Anti-A from B person > titer of Anti-B from A person

ABO Abs are usually IgM, but IgG and IgA forms can occur

- IgM: does not cross the placenta
- IgG, Immune form: can cross the placenta

More commonly develop immune ABO antibodes in group O individuals vs. A or B groups.

Key Words

Von Descatello & Sterle

ABO Blood Group System

IgM, IgG, IgA forms

GSS

In the 1950s you often tittered anti-A, anti-B, and anti-AB

O negative is the "universal donor," and the titer levels had to be less than 1200

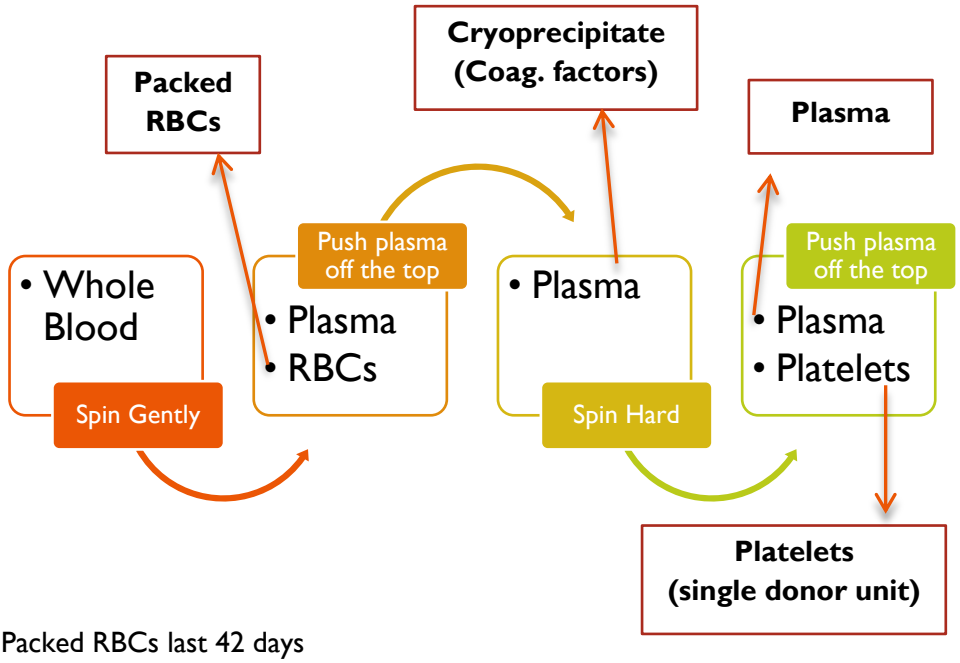
Why? They used whole blood.

Group Specific Substances (GSS) could be used for the titer.

- GSS is made from gastric mucosa of animals
  - Hog: contain Ag with the same antigenic determinant as A Ag
  - Horse: same Ag with the same antigenic determinant as B Ag
- You can add hog or horse GSS to absorb the antibodies out of plasma

Distribution of ABO Blood Groups			
Group	Caucasians	American Blacks	Orientals
O	44%	49%	43%
A_1	34%	19%	27%
A_2	10%	8%	Rare
B	9%	19%	25%
A_1B	3%	3%	5%
A_2B	1%	1%	Rare

Donated Blood



Packed RBCs last 42 days

Fresh Frozen Plasma lasts a year

Key Words

Group Specific Substance

Distribution of ABO blood groups

Inheritance of ABO Blood Groups

Described by Bernstein in 1924

- A and B are codominant
- O gene is considered an amorph
    - No detectible antigen is produced
- ABO follows simple Mendelian genetics
- Genes are on chromosome #9

Terms

- **Diploid genes**: determine heredity characteristics
- **Chromosome**: carry genes at specific locations (loci)
- **Alleles**: alternate genes at given loci
- **Genotype**: an individual's actual genetic make up
- **Phenotype**: the outward expression of genes

ABO Phenotypes and Genotypes	
A	AA or AO
B	BB or BO
O	OO
AB	AB

6 possible genotypes yield 21 possible matings

Possible matings = n(n+1)/2

Formation of Agglutinogens

ABO Antigens

ABO genes do no code for Antigens themselves, rather code for specific enzymes that add sugars to a basic precursor substance (glycoprotein or glycolipid). ABO genes interact with other genes, like the H gene which also produces a specific enzyme.

Ceramide: a lipid, part of phospholipid group on red blood cells. This component makes the antigen a glycolipid.

Paragloboside: immediate precursor for H or P_1 Ags.

Oligosaccharide chain: upon hydrolysis forms 2-10 monosaccharides.

Precursor substance: a common carbohydrate residue upon which ABO glycolipid antigens are built

Key Words

Bernstein

Diploid

Chromosome

Allele

Genotype

Phenotype

Ceramide

Paragloboside

Oligosaccharide Chain

Precursor Substance

Gene	Encoded Enzyme
H	Alpha-2-fucosyltransferase
A	Alpha-3-N-acetylgalactosaminyltransferase
B	Alpha-3-D-galactosyltransferase
O	---

There are two types of precursor chains: Type 1 and Type 2. They are differentiated by how the terminal galactose is connected to the N-acetylglucosamine.

Type 1 Precursor Chain: β 1-3 linkage

Type 2 Precursor Chain: β 1-4 linkage

RBCs only have Type 2, the first sugar is Glucose, and it is attached to a lipid in the phospholipid membrane.

Non RBC Antigens:

- Find type 1 and type 2 precursors.
- They are glycoproteins (anchored on proteins, not lipids).
- The first sugar is not glucose; it's N-acetylgaloctosamine.

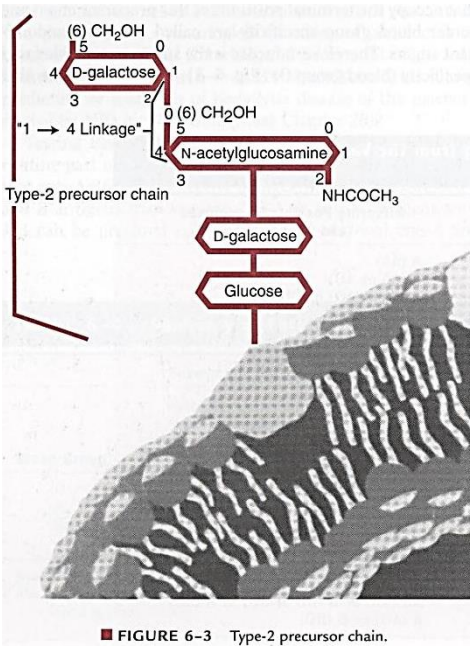


FIGURE 6–3 Type-2 precursor chain.

Studying the Structure of ABO Antigens

1. Haptene Inhibition
2. Destruction of Antigens with Enzymes
3. Absorption with GSS

For the fluid, you can use:

- Saliva (will use in secretory status lab)
- Ovarian cyst fluid (can generate huge volumes of fluid)

Researchers could break apart the ABO antigens and find various sugars (fucose, galactose, glucose, N-acetylgalactosamine, etc.).

Researchers started using degredation with enzymes

A Substance –(A enzyme from *Clostridium*)→H Substance + N-acetylgalactosamine

B Substance—(B enzyme from *Trichomonas*)→H Substance + Galactose

Key Words

Type 1 & Type 2

Precursor Chains

Now:

Anti-A and N-acetylgalactosamine and type A cells have no agglutination due to haptene inhibition.

N-acetylgalactosamine=immunodominant sugar for A antigen.

Anti-B and galactose and type B cells has no agglutination

Galactose=immunodominant sugar for B antigen.

Investigation of H Antigen

Was found that *serum from cattle and eels would agglutinate O cells*

It would also agglutinate other ABO groups in the following strengths:

$$O>A_2>B>A_2B>A_1>A_1B$$

Something on RBC surface is more exposed in O than A_2 etc.

Later: **Bombay Os** were identified. They were reported by Bhende in 1952. They found people who forward type as O. the sera contained anti-A, anti-B, anti-A,B and antibody that would agglutinate O cells. This antibody was named anti-H. It was directed against the H antigen, the precursor for A and B antigens.

H gene codes for L Fucosyl transferase which transfers L-fucose to the terminal galactose of the precursor chain.

The H gene is very common; it allows us to form Antigens. HH+Hh> 99.99% of the population, hh is called Bombay O.

With A people, almost all H sites are converted to A Ag.

- A Ag- 810.000 to 1.17 million antigenic determinant sites/ RBC
- B Ag- 600,000 to 830,000 antigenic determinant sites/RBC
- AB blood groups inherit both glycosyltransferases.
- A sites- 600,000 antigenic determinant sites/RBC
- B sites- 720,000 antigenic determinant sites/ RBC
- O blood group- no enzyme produced, no sugar added.

Secretory Gene (Se)

Secretory gene= Se and determines whether we see ABO antigens in secretions

- SeSe=homozygous, Sese=heterozygous, both are secretors.
- sese=homozygous and a non-secretor

Se controls the expression of H gene in the tissues, not on red blood cells.

Key Words

H Antigen

Bombay O

Antigenic Determinant Sites

Secretory Gene (Se)

A non-secretor will not make H substance in their tissues, therefore no L-fucosyltransferase produced and no substrate for enzymes that produce A or B antigens- no soluble A or B antigens. If you are a secretor, the soluble Ags are produces but are glycoproteins, not glycolipids. They have both types of precursor chains, the first sugar in the chains is N-acetylgalactosamine.

Subgroups of A & B

There are 2 types of A individuals:

- **A_1 (most common) = cells react strongly with Anti-A**
- **A_2 (less common) = cells react weakly with Anti-A**

There are certain A_2 people that make Anti-A_1 (~8% of A_2). This is naturally occurring, but doesn't usually react at 37C, therefor it is not usually a problem if kept warm enough.

Tests to Differentiate A_1 from A_2

1. "The Old Way"

Used absorbed serum: group B people produce Anti-A Abs (will agglutinate A_1 and A_2 cells) and Anti-A_1 Ab (will agglutinate only A_1 cells). So, if you add A_2 cells to this B person's serum, the cells will absorb the Anti-A antibodies. Centrifuge and separate cells and serum…you have now eliminated Anti-A antibody. The result is a reagent that can be used to screen (now have Anti-A_1 absorbed serum).

2. Current Technique

Type with a plant lectin (seed extract from a legume).

Dolichos biflorus: lectin specific for A_1 or A_1B = "Lectin Anti-A_1"

Ulex europaeus: "Lectin Anti-H"

Bandeiraea simplicifolia: "BS-1," Anti-B, will agglutinate only true B antigens (not acquired Bs, from *E. coli* for example).

Other Subgroups of A

A_1, A_2, A_3, A_X, A_{end}, A_M, A_Y, A_{el}

>99%

Quantitative and Qualitative Differences of A_1 & A_2

Qualitative: some A_2 people make naturally occurring anti-A_1 (1-8%); some A_2B people produce anti-A_1B (22-35%)

Quantitative: A_1 = 810,000-1.17 x10^6 sites; A_2 = 240,000-290,000 sites

B subgroups are very rare

Key Words

A_1 and A_2 Subgroups

Lectin Anti-A_1

Lectin Anti-H

BS-1

Lewis Blood Group System

Is unique→only blood antigen system (that we will discuss) *not* made by RBCs.
Lewis antigens are made by tissue cells and then absorbed onto RBCs.
Lewis A (Le^a) and Lewis B (Le^b) are not alleles, meaning there is *no* Lewis AB.

- Depends on a person's secretor status
- Secretors are generally Lewis B people
- Non-secretors are generally Lewis A

Formation of Lewis antigens depends on:

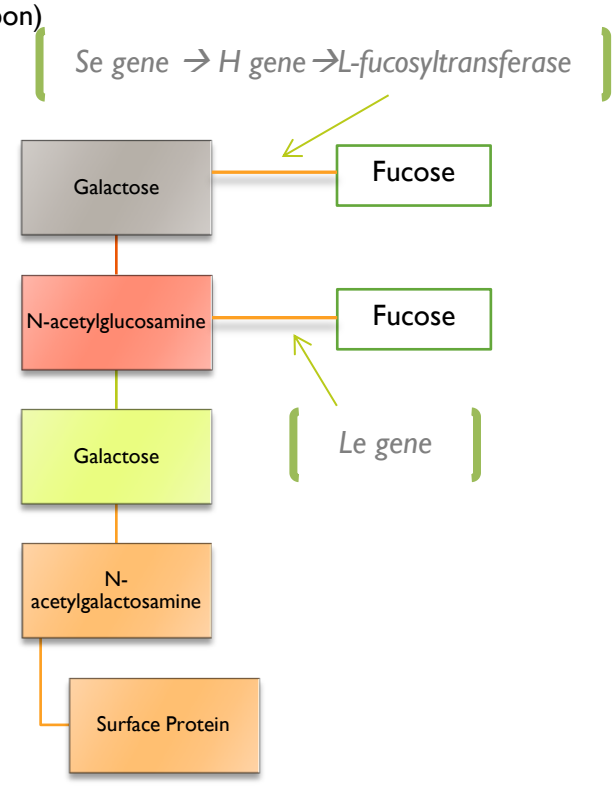
- H-gene
- Se-gene
- Le-gene

Le^B

Lewis B is produced by an interaction of these genes.

- Se activates H's L-fucosyltransferase
- Le produces another fucosyltransferase

To build Le^B, you must start with a **Type I** precursor chain (Le gene uses the #4 Carbon)



= Le a-b+ (72% of population)

Key Words

Lewis Blood Group

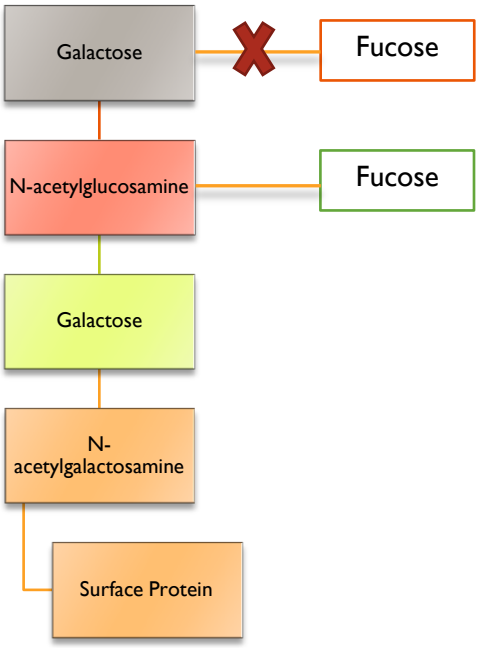
Lewis B

Le^a

Genes for Lewis A: **Le** (either LeLe or Lele); **H** (not really needed, but most people have it except Bombay Os); and **sese**

Again, need the **Type I** precursor chain.

Same mechanism as Lewis B, except there is no Se to activate the H, so the terminal Fucose does not get attached.



= **Le a+b- (22% of population)**

The Other 6%

The other 6% of the population consists of:

- Le a-b-
  - Don't have the lewis gene (lele); these are the people who usually make lewis antibody (anti-Le^a or anti-Le^b)
- Le^C
  - Are recessive for Lewis gene (lele) and are not secretors (sese)
- Le^D
  - Are recessive for Lewis gene (lele) and are secretors (Sese or SeSe)

• • •

Lewis antibodies are predominantly IgM and react across a wide thermal range. But, they're usually cold, IgM Abs and not usually involved in HTR

• • •

Key Words

Lewis A

Other Lewis Groups

Rh Blood Group

Chapter 7

There are different sets of nomenclature
This is the 2nd most important blood group

History

Discovered by Landsteiner and Wiener (1940)
Separate observations led to the discovery

1. **Levine** (1939) was an obstetrician. He transfused a woman with her husband's blood (they were both O). A transfusion reaction occurred. Further studies: her serum agglutinated 80% of all group O bloods. Levine decided that she was probably immunized during her 1st pregnancy.
2. **Landsteiner** & **Wiener** were doing phylogenetic studies with Rhesus monkeys.
    a. They took rhesus monkey erythrocytes and injected them into a guinea pig or a rabbit.
    b. Got anti-monkey erythrocyte Ab
    c. Then, test human RBCs against these Ab
    d. Found: 85% of the people's blood tested agglutinated
    e. Concluded: some antigen was on human RBCs and monkey RBCs
    f. They named it "Rh antigen" for the rhesus monkey
3. **Wiener** was investigating people who had transfusion reactions. Many of these people had antibodies to the Rh_0 antigen. They also lacked the Rh_0 antigen and produced agglutinins to it. All of these people with transfusion reactions had had previous transfusions or babies.
4. **Levine** was investigating women who had children with erythroblastosis fetalis (now known as HDN, once termed Icterusgravus neonatum)

Hemolytic Disease of the Newborn (HDN)

A disease characterized by fetal abortion, still births, or live babies with varying degrees of anemia and jaundice.

Clinical indication:

- Anemia
    - Increased number of younger RBCs (up to erythroblasts, if young enough)
    - Low hematocrit
- Jaundice
    - Increased destruction of RBCs
    - Increased bilirubin in serum or plasma associated with increased release in hemoglobin

Key Words

Rh Blood Group

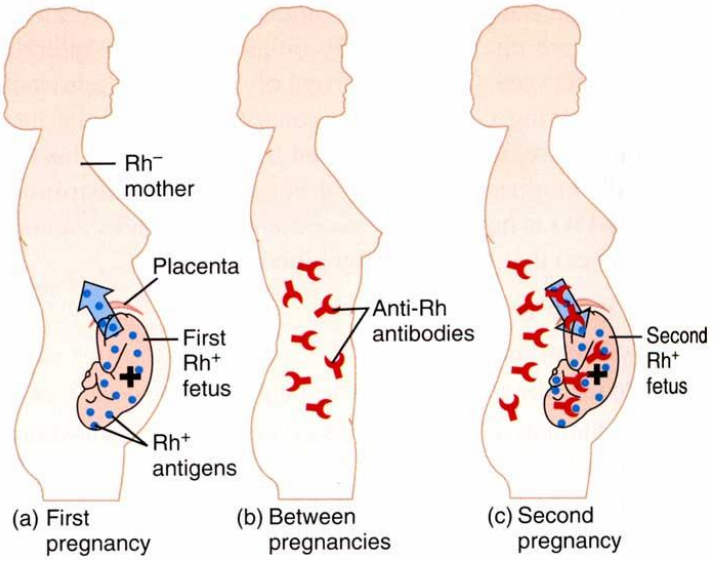
Landsteiner

Wiener

Levine

Hemolytic Disease of the Newborn (HDN)

The source of HDN is an incompatible marriage in which the mother is Rh negative, the father is Rh positive, and the child is Rh positive.



(See page 384)

In the 1950s, incidents of HDN were 1 in 200 to 1 in 400 births.

This is lower than theoretically expected, but is accounted for by heterozygous fathers, the fact that not all women exposed to Rh+ will make Abs, and that the chances of a problem increase with the number of births.

Treatment for HDN

In severe cases, an **exchange transfusion** is performed.

Incidents are decreasing: now predicted to be about 1 in 20,000 births.

Can manage with **Rhogam**: a prophylactic administration of anti-Rh_0 Ig that is given to Rh- mothers delivering Rh+ babies (within 72 hours of delivery).

Mechanism:

- You remove erythrocytes before immunization can occur
- Feedback mechanism in which the presence of Rh_0 antibodies somehow suppresses the immune response

Lab Tests Done in Investigation of HDN

- Determine the baby's bilirubin and H+H (hemoglobin + hematocrit)
- Coombs Test
    - Use Coombs reagent (AHG, anti-human globulin)
    - Can be direct or indirect

Key Words

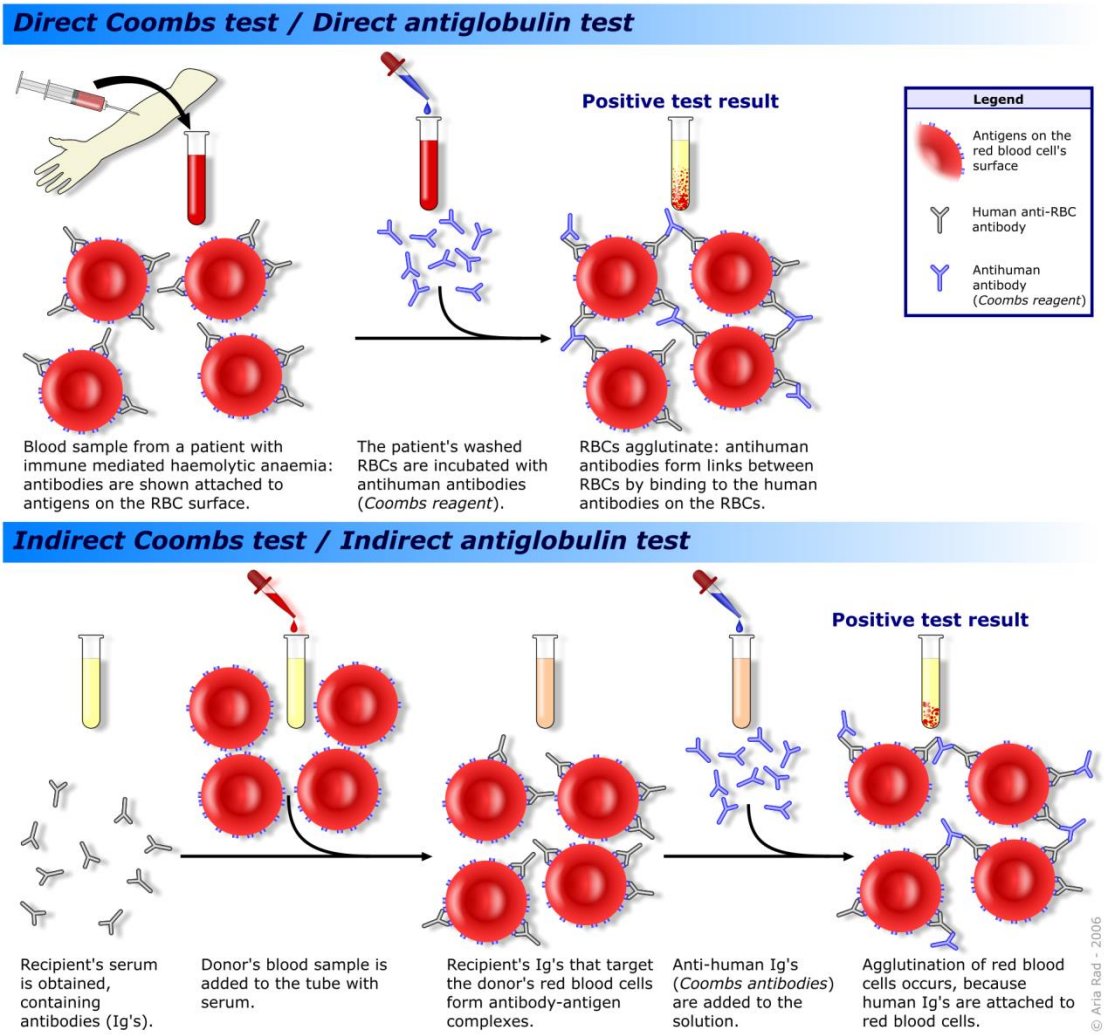
HDN

Rhogam

Coombs Test

A **direct Coombs test** shows what *has* happened

An **indirect Coombs test** shows what *could* happen



(see plate 1 before page 1 in text)

Rh Nomenclature

- Wiener
- Fisher Race
- Rosenfield, et al
- ISBT (international society of blood transfusion)

Wiener and Fisher Race are based on postulated genetic mechanisms
Rosenfield and ISBT are alphanumerical systems
Fisher Race is the most commonly used

Key Words

Direct Coombs Test

Indirect Coombs Test

Rh Nomenclature

Wiener

Fisher Race

Rosenfield

ISBT

Numerical Systems:

- If a number is listed, then the antigen is present
- If a "-" is by the number, the antigen is not present
- If the number is not listed, then the antigen was not tested for

Wiener found Rh_0 as the original Rh factor; others were later found.

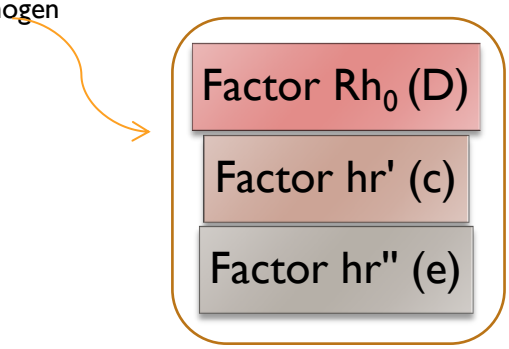
Wiener Factor	Fisher Race
Rh_0	D
rh'	C
rh''	E
hr'	c
hr''	e

Note: "d" has never been found. It's likely an amorph

Inheritance

- Weiner believed that Rh genes produced an agglutinogen which contained a series of blood factors that he termed "serological factors" and are now known as antigenic sites.
- There are at least 3 serological factors on each agglutinogen.
- The agglutinogen is produced by 1 gene
- Antibodies will recognize individual factors within the agglutinogen

R^0 gene → Rh_0 Agglutinogen



See Page 137, Table 7-3

TABLE 7–3 Rh-Hr Terminology of Wiener

Gene	Agglutinogen	Blood Factors	Shorthand Designation	Fisher-Race Antigens
Rh^0	Rh_0	$Rh_0hr'hr''$	R_0	Dce
Rh^1	Rh_1	$Rh_0rh'hr''$	R_1	DCe
Rh^2	Rh_2	$Rh_0hr'rh''$	R_2	DcE
Rh^z	Rh_z	$Rh_0rh'rh''$	R_z	DCE
rh	rh	$hr'hr''$	r	dce
rh'	rh'	$rh'hr''$	r'	dCe
rh''	rh''	$hr'rh''$	r''	dcE
rh^y	rh_y	$rh'rh''$	r^y	dCE

Key Words

Wiener Factor

Fisher Race

Rh inheritance

Fisher Race

- Postulated that Rh antigens were produced by 3 closely linked sets of alleles (too close for crossing over)
- 5 major Rh system antigens (d is a spaceholder/amorph)
    - D,d,C,c,E,e
- We have 5 Rh antisera to work with

See table 7-6 on page 139 for possible reaction patterns.

Numerical Systems

Rosenfield

In the early 1960s, the first numeric system was Rosenfield, et al.

- Assigned a number to each antigen discovered or recognized in the Rh system
- Examples
    - DCEce = 1,2,3,4,5
    - dce = -1,-2,-3,4,5
- Rosenfield is not really used clinically
- Limiting factor: there are other numerical systems (Kell, Kidd, Duffy)

ISBT

There was a need for something easier: a universal language to facilitate cooperation and data sharing. They wanted a uniform nomenclature that was both eye and machine readable and in keeping with genetic basis of blood groups.

A 6-digit number was assigned to each authenticated blood group

- The first 3 digits refer to the group
    - "004" is assigned to the Rh system
- The second 3 digits identify antigen specificity

See page 138, table 7-5

Expression of the Rh Antigens

- ➢ Production is genetically determined
- ➢ Randomly distributed on RBC membrane
- ➢ Biochemically are **proteins**
- Number of sites/RBC
    - With ABO: 200,000-1.17 million
    - Smaller range with Rh: about 10,000-33,000
        - Exception: D_ _ ("D, null, null"), with 110,000 – 200,000

Key Words

Fisher Race

Rosenfield

ISBT

Rh Ag Expression

Rh gene interaction that can alter expression:

- Cis interacting genes (genes on the same chromosome)
  - Example: Cis suppression
    DcE/dce vs. Dce/dcE (no suppression)
    When D and E are on the same chromosome, you see a weaker expression of E
- Trans suppression (genes on opposite chromosomes)
  - When C is trans to D, it weakens the expression of D
    DCe/dCe will have D suppressed
    - Results in D^u phenotype or "weak Ds"
    - Can see Weak Ds with:
      - Suppression of D expression (C trans D)
      - Inheritance of a gene for a weak D antigen
      - D mosaic

D mosaic

Partial D, see page 140.

Some people who are D+ produce an anti-D that does not react with their cells.

Wiener proposed that the D (Rh_0) was a mosaic antigen composed of 4 pieces and that these people are able to make antibodies against the piece they are missing. They are still a D+ individual.

Other Rh Possibilities

- Go^a is another Rh antigen, found on RBCs that lack part of the D mosaic
- D-deletion genes
  - People who don't code for C,c,E, or e but do make D
  - D _ _/D _ _
- Rh null (rare)
  - _ _ _ / _ _ _
- Other variant Rh antigens
  - G antigen: the genes that code for D and C can also make G
    - Transfuse a person who is dce/dce and give them DCe/DCe blood
    - This patient can make anti-C, anti-D, *and anti-G*
  - f antigen (ce antigen) results when c and e genes are in cis position
    - Dce/DCE person has f antigen
    - DcE/DCe person does not have f antigen

Key Words

Rh Gene Interaction

Cis

Trans

Weak D / D^u

D mosaic

Go^a

D-deletion genes

Rh null

G antigen

f antigen

LW Antigen

The antibody of **L**andsteine & **W**iener is *not* identical to the Ab of Levine. It's related to the Rh system, but not part of it

The Four Nomenclatures

Anti-004001	Anti-004002	Anti-004003	Anti-004004	Anti-004005	ISBT	
Anti-Rh1	Anti-Rh2	Anti-Rh3	Anti-Rh4	Anti-Rh5	Rosenfield	
Anti-Rh_0	Anti-rh'	Anti-rh"	Anti-hr'	Anti-hr"	Wiener	
Anti-D	Anti-C	Anti-E	Anti-c	Anti-e	Fisher Race	
					Agglutinogen	
					Fisher Race	Wiener
+	-	-	+	+	Dce	Rh_0
+	+	-	-	+	DCe	Rh_1
+	-	+	+	-	DcE	Rh_2
+	-	-	+	+	DCE	Rh_z
-	-	-	+	+	dce	rh
-	+	-	-	+	dCe	rh'
-	-	+	+	-	dcE	rh"
-	+	+	-	-	dCE	rh_y

The Anti-Human Globulin Test

After Landsteiner discovered the ABO group in 1901, it took until the 1940s for the next major advance.

- Introduced a technique that allowed detection of "incomplete" antibodies
- Techniques:
  - Use of albumin in cross matching
  - Use of enzymes
  - Use of anti-human globulin
- Landsteiner antibodies are mostly IgM and naturally occurring
- Rh antibodies are immune antibodies: IgG in general, they require immunization (stimulation with RBC antigens) and usually react best at 37C.

Hemagglutination with IgM & IgG

RBCs have a high sialic acid content in their membranes (sialic acid = N-acetylneurominic acid), which gives a negatively charged RBC membrane.

- With negatively charged RBCs in ionic solution, a cloud of positively charged ions will form (predominantly Na+).
- This results in a diffuse, double-layered ionic cloud formed around the RBC.
- The inner layer is firmly associated with the RBC and travels with it. The outer layer moves freely.
- The outer edge of the inner layer is the **Boundary of Shear**

Key Words

Anti-Human Globulin Test

Sialic acid

Boundary of Shear

The repulsion of RBCs from each other helps them not stick together in normal blood flow

Zeta Potential

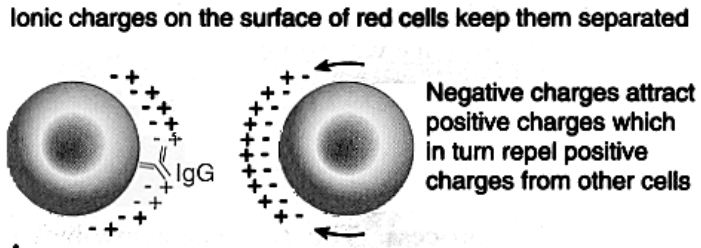
The Zeta potential is the difference in energy between the RBC surface and the boundary of shear.

- It is normally 17-18 mV
- With a decrease in Zeta potential, the red cells get closer
    - RBCs normally repulse each other
    - The degree of repulsion can be manipulated

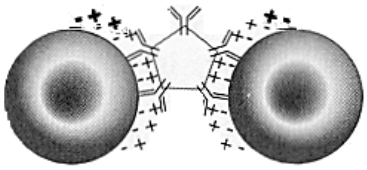
Normally, RBCs in saline are at least 25nm apart.

IgG has a maximum reach of 14nm. So, it needs some help to cause hemagglutination. This is why it was termed "incomplete."

IgM has a maximum reach of 35nm, so it can cause hemagglutination. This was referred to as the "complete" antigen, because it could bridge that distance.



A IgG cannot bridge the space between red cells because it is too small a molecule



B IgM is large enough to bridge the space between red cells

In 1944: **Wiener** and **Race** separately reported incomplete antibodies (antibodies that were expected to react, but didn't).

- Most Rh antibodies are IgG; in order to detect them, one must:
    - Use a test system that gets the RBCs closer together
      Or
    - Have a way to detect the IgG bound to the cells

Three general methods were used to facilitate detection of incompletes

1. High protein environment (in serum)
    a. Increases the dielectric constant of the medium (electrical insulating cushion)

Key Words

Zeta-potential

Incomplete and

Complete antigens

IgG, IgM

   b. Decreases the Zeta potential and allows the cells to move closer together
   c. Used:
      i. Bovine Serum Albumin (BSA)
      ii. Polyvinyl pyrolidone (PVP)
      iii. Protamine
2. Enzyme Treatments
   a. Will expose antigenic sites and decrease Zeta potential
   b. Used
      i. Papane
      ii. Trypson
      iii. Bromelin
3. Use Anti-Human Globulin Test (AHG)
   a. Detects the IgG bound to the RBCs

Key Words

BSA

PVP

Protamine

Papane

Trypson

Bromelin

AHG