Immunology of transplant rejection

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Types of graft

- **Autograft** is self-tissue transferred from one body site to another in the same individual.

- **Isograft** is tissue transferred between genetically identical individuals.

- **Allograft** is tissue transferred between genetically different members of the same species.

- **Xenograft** is tissue transferred between different species

Types of transplantation

- Orthotopic, graft placed into the same anatomic site
- Heterotopic. Graft placed in different site
- Transfusion, for blood

- The degree and type of response vary with the MHC type blood groups and type of the transplant. Some sites, such as the eye and the brain, are immunologically privileged (ie, they have minimal or no immune system cells and can tolerate even mismatched grafts).
- Skin grafts are not initially vascularized and so do not manifest rejection until the blood supply develops.
- The heart, kidneys, and liver are highly vascular organs and lead to a vigorous cell mediated response in the host.

- Major antigens that cause rejection;
  - Blood group antigens
  - HLA antigens
- The number and variety of histocompatibility antigens tell us that probably no two humans (again, except for identical twins) exist on earth with perfectly compatible tissues and, so,
- successful transplantation of allografts requires some degree of immunosuppression to avoid graft
- The immune response include cell-mediated (late) or antibody mediated (early).
- Bone-marrow transplants effectively bring their own immune system with them, often rejecting the new host, instead of the other way around, in a reaction known as graft-versus-host disease.

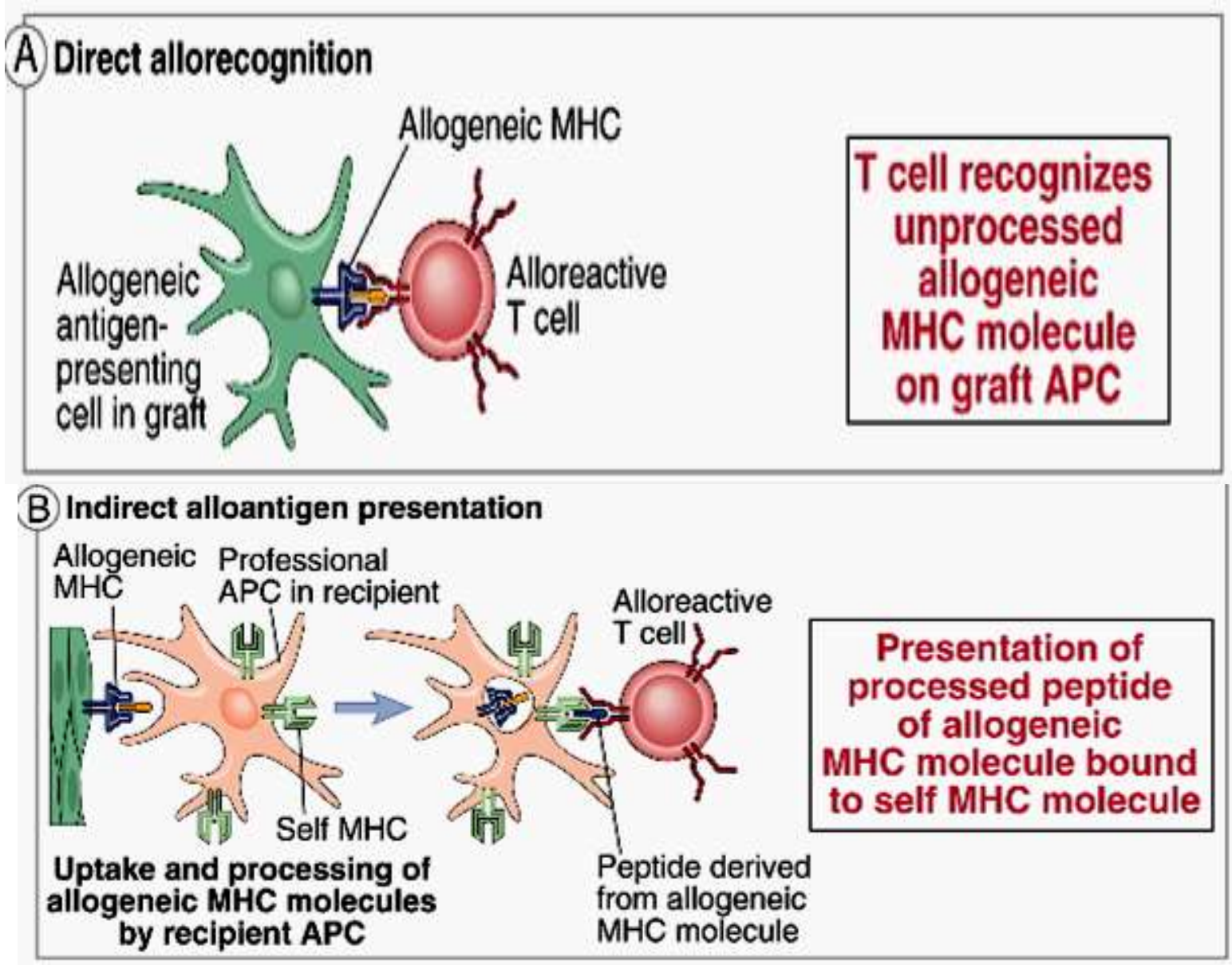
Molecular mechanisms of graft rejection
Direct and indirect allograft recognition

- **Direct**; Initial studies showed that the T cells of a recipient graft recognize intact, unprocessed MHC molecules on the graft APC, the most strong reaction in acute rejection and short lived.
- Cross reaction may play a role, means immune reaction against antigen other than antigen that presented first

  - MHC2 or 1 on APC of donor present antigen to CD4 and CD8 in recipient
  - CD4 primed to TH1 (phagocytosed antigen multiplying within the macrophage's phagosomes)
  - CD8 activation by MHC1 (intracellular antigen)
  - macrophage and NK activation
  - and increase in IL-2 and IFN gamma cytokines.
  - cause tissue damage by Type 4 hyper sensitivity reaction

Follow

- **Indirect**; recipient T cell recognize donor MHC processed by recipient APC and presented on recipient MHC ; in chronic or late rejection. Last long
- Indirect presentation may result in recognition by CD4+ T cells because donor antigen (Alloantigen) is acquired by host APCs primarily through the endosomal vesicular pathway (i.e., as a consequence of phagocytosis) and is therefore presented by class II MHC molecules.
- CD4 T cells provide help to B cells for antibody production which kill the graft by activation the complement cascade
- antibody-mediated damage


A Direct allorecognition
Allogeneic MHC
Allogeneic antigen-presenting cell in graft
Alloreactive T cell
T cell recognizes unprocessed allogeneic MHC molecule on graft APC
B Indirect alloantigen presentation
Allogeneic MHC
Professional APC in recipient
Alloreactive T cell
Self MHC
Uptake and processing of allogeneic MHC molecules by recipient APC
Peptide derived from allogeneic MHC molecule
Presentation of processed peptide of allogeneic MHC molecule bound to self MHC molecule

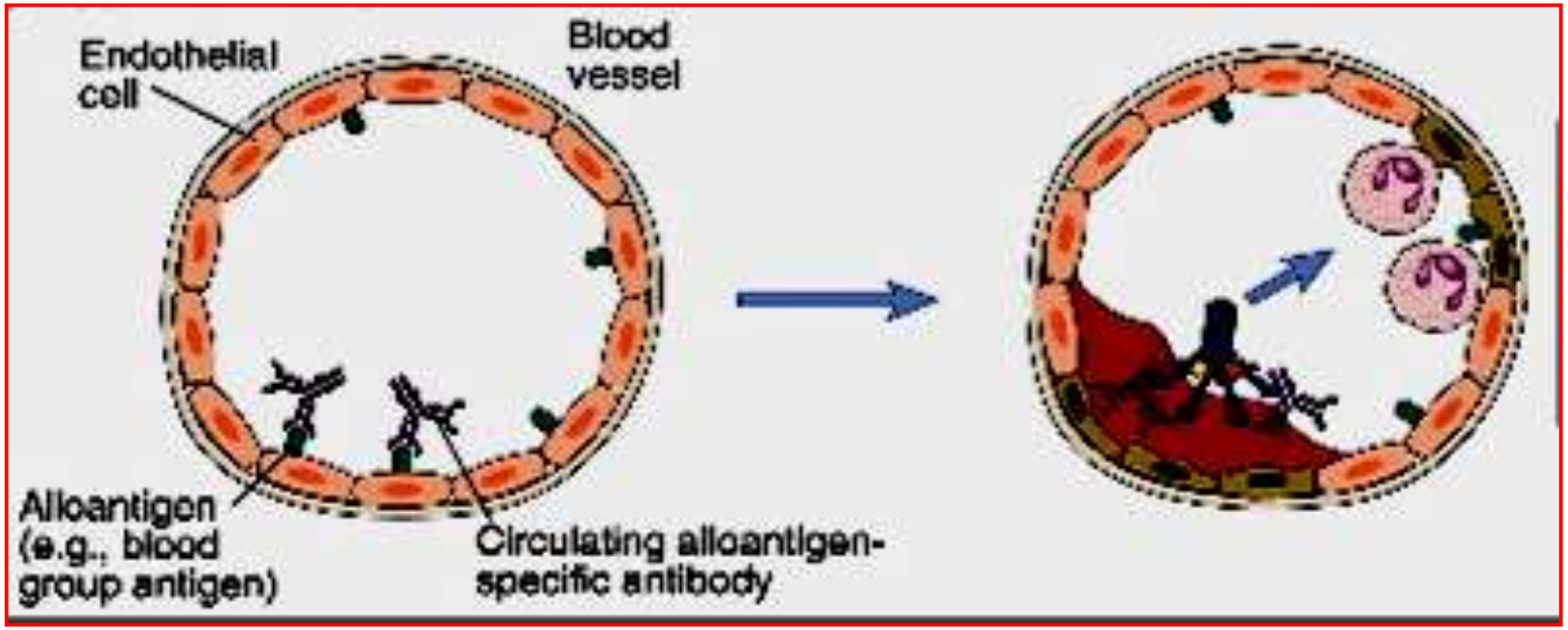
Clinical stages of Allograft Rejection

- Hyperacute Rejection
- Secondary Rejection
- Acute Rejection
- Chronic Rejection

Hyper-acute Rejection

- Or **hemolytic transfusion reaction**; presented by High fever, shock, and disseminated intravascular coagulation. Intravascular hemolysis kidney and liver failure
- The earliest reaction is Febrile non-hemolytic transfusion reactions are the most common reaction reported after a transfusion
- Pre-formed antibodies; IgM Ab attack the RBC ABO antigens of donor, No need to pre-exposure. Or IGG against RH antigen or HLA, need pre-exposure. Plasmapheresis may be used to attempt to remove circulating antibodies, and retransplant
- Type 2 hypersensitivity reaction cause tissue damage from the time of transplant to 48 hours after transplant
- Can be prevented by blood match, with in minutes

Hyperacute Rejection



1. Preformed Ab, 2. complement activation,
3. neutrophil margination, 4. inflammation,
5. Thrombosis formation

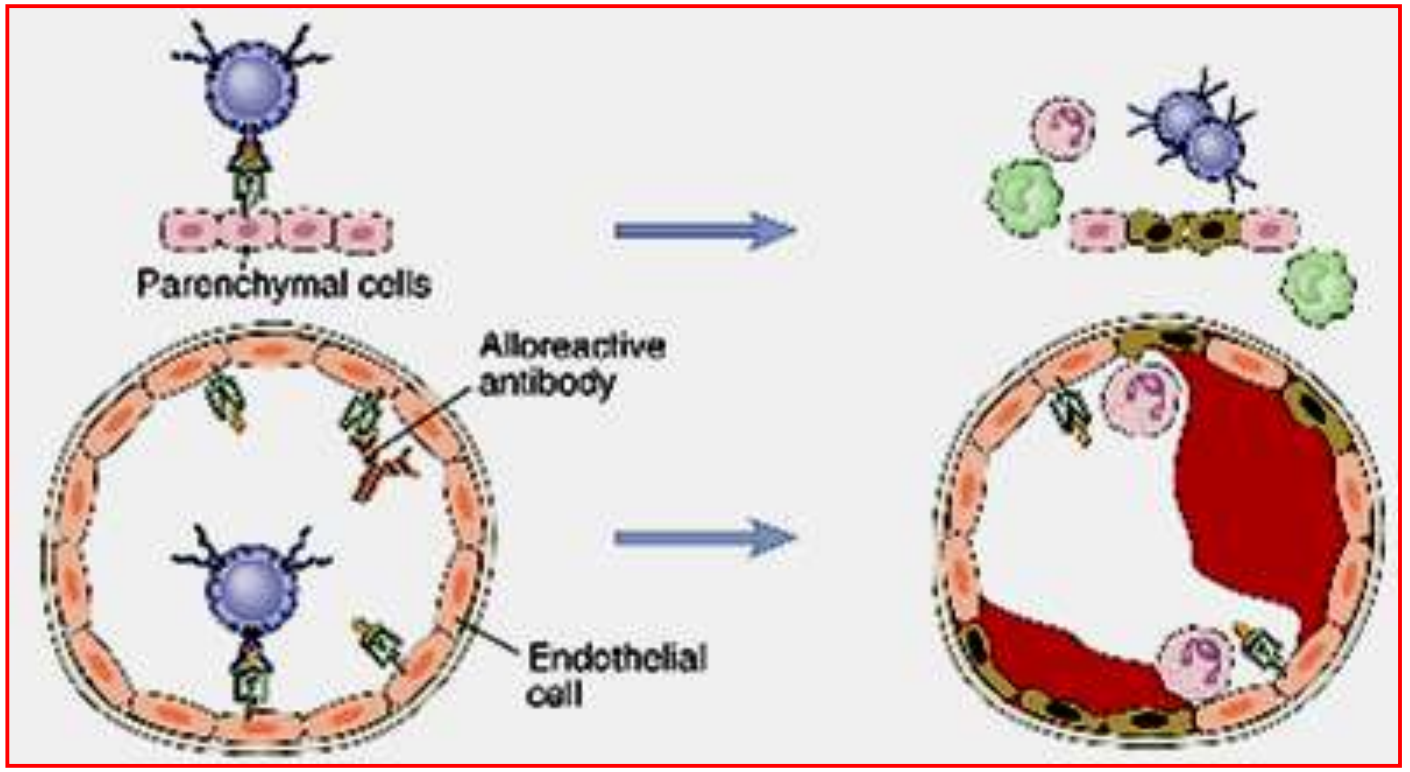
Accelerated or secondary rejection

- HLA mediated
- Transplantation of a second graft, which shares a significant number of antigenic determinants with the first one, results in a rapid (2 - 5 days) rejection. It is due to presence of memory T-lymphocytes during the first graft rejection. Accelerated rejection is mediated by, activation of monocytes and macrophages, and induction of cytotoxic lymphocytes from memory cells
- Type 4 hypersensitivity reaction

Acute Rejection

- HLA mediated
- endothelial and parenchymal injury mediated by
- CTL mediated
- and antibodies (humoral) , Th2 mediated
- usually begin within 3 mon (1-3 weeks) of transplantation
- Treat with steroids

Acute Rejection

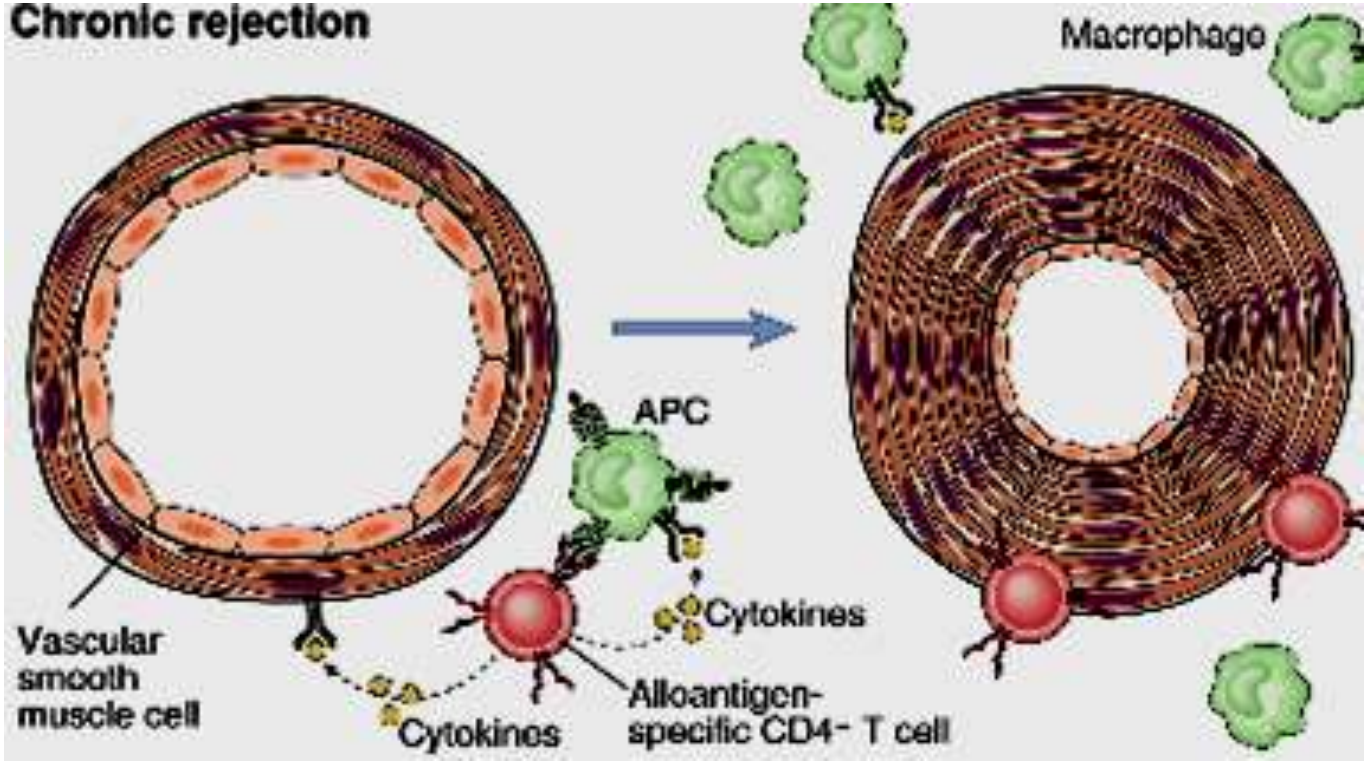


1. T-cell, macrophage and Ab mediated,
2. Endothelial and parenchymal injury,
3. Inflammation at site of rejection

Chronic Rejection

- HLA mediated
- Occurs suddenly after months or years after frequent acute rejection episodes
- Humoral and cellular mechanisms
- A dominant lesion of chronic rejection in vascularized grafts is arterial occlusion as a result of the proliferation of intimal smooth muscle cells, and the grafts eventually fail mainly because of the resulting ischemic
- The use of immunosuppressive drugs and tissue-typing methods to prevent acute rejection, and treat any risk factors has increased the survival of allografts, but chronic rejection is not prevented in most cases

Chronic Rejection



1. Macrophage – T cell mediated and antibody mediated
2. Concentric medial hyperplasia
3. Chronic delayed type hypersesitivity reaction

The following factors increase the risk of chronic rejection:

- Previous episode of acute rejection
- Inadequate immunosuppression
- Donor-related factors (eg, old age, hypertension)
- Reperfusion injury to organ
- Recipient-related factors (eg, diabetes, hypertension, hyperlipidemia)
- Posttransplant infection (eg, cytomegalovirus [CMV])

Bone Marrow Transplantation

- the transplantation of hematopoietic stem cells, usually derived from bone marrow, peripheral blood, or umbilical cord blood.
- Used for Leukemia, Anemia and immunodeficiency, especially severe combined immunodeficiency (SCID).
- Recipient of a bone marrow transplant is immunologically suppressed before grafting, e. g; Leukemia patients are often treated with cyclo-phosphamide and total body irradiation to kill all cancerous cells.
- Because the donor bone marrow contains immunocompetent T cells, the graft may reject the host, causing graft versus host disease (GVHD).
- Other complication is immunediffeciency

Graft vs. Host Disease

- Main complication of bone marrow transplant
- However, graft-versus-host disease can occur in those received stem cells/bone marrow from an HLA matched donor. HLA-identical donors often have genetically different proteins (called minor histocompatibility antigen)
- The mediator is Donor mature naïve T cells against recipient lymphocytes
- Acute GVHD <100 day
  - Characterized by epithelial cell death in skin rash, GIT nausea vomiting, and liver; yellow discoloration.
- Chronic GVHD > 100 days
  - Characterized by atrophy and fibrosis of one or more of these same target organs

Minor histocompatibility Ags

- **Minor histocompatibility antigens**
  - **H-Y**, an antigen encoded on the Y chromosome and thus present in male, but not female, tissue
  - **HA-2**, an antigen derived from the contractile protein myosin.

Treatment of GvH disease

- Injecting donor graft with mono-clonal antibodies to inhibit T cells,
- Increase immunosuppressive drugs to the recipient.
- **Grafts that are not rejected**
  - Privileged sites. These include the brain, anterior chamber of the eye, testis, renal tubule, uterus,
  - Sperm
  - Pregnancy, trophoblast cells not expressing MHC, immunosuppressive hormones produced by fetus (estradiol, progesterone), immunosuppressive layer covered the fetus, blocking antibodies

Prevention of graft rejection

- Proper choice of donor
  - ABO grouping
  - Tissue typing for HLA
  - Cross matching; test recipient serum for the presence of preformed Abs against donor HLA or ABO antigens (indirect coombs test)
- Immune suppression for the recipient

pretransfusion compatibility testing

- Evaluation of the donor includes
  - Testing of the donor unit for infectious diseases
  - ABO/Rh typing
- Evaluation of the recipient includes
  - ABO/ Rh typing
  - Antibody screen. Perform antibody identification if antibody screen is positive to determine the identity of the antibody,
- Crossmatch Tests the compatibility of the recipient's serum with RBCs from potential donor unit

❖Tissue typing or HLA typing

A. Mixed lymphocyte reaction (MLR) mixed leukocytes from donor and recipient in culture; can be used to assess the degree of major histocompatibility complex (MHC) class I and class II compatibility. In mismatch; DNA synthesis and cellular proliferation

B. Molecular HLA typing. (genotyping of the transplanted epitope)

C. In the lymphocytotoxicity assay, recipient sera are tested for reactivity with donor lymphocytes. A positive crossmatch is a contraindication to transplantation

D. Panel-reactive antibody (PRA) lab specialist will test a patient's blood (serum) against lymphocytes (white blood cells) obtained from a panel of about 100 blood donors. test that quantifies the risk of transplant rejection. A high PRA usually means that the individual is sensitized and high percentage of rejection may occure

Immune-suppressive drugs

- Initially, radiation and chemicals were used as nonselective immunosuppressive agents
- Corticosteroids, inhibit immune response, it is a glucocorticoid-based medication that works principally to block T cell and APC derived cytokine. The major elements blocked are proinflammatory cytokines IL-1 TNF and IL-6.
- antiproliferative(azathioprin) inhibit T cell proliferation
- Inhibitor of IL-2
  - Calcineurine inhibitors that inhibits calcineurin. This inhibition ultimately inhibits the production and secretion of IL-2 and prevent T cell activation and growth. e.g.;cyclosporin antibiotic and tacrolimus
  - sirolimus
- antibodies
  - Anti-lymphocyte antibodies; anti-CD3
  - Two antibodies that are IL-2 receptor antagonists (basiliximab and daclizumab)
- Future immunosuppressive agents by induction of tolerance. Under trial. Administration of CTLA-4, high dose of soluble donor MHC. May be useful in chronic rejection

ABO blood antigens

- The ABO antigens are carbohydrates linked to cell surface proteins and lipids that are synthesized by polymorphic glycosyltransferase enzymes
- Most individuals possess a fucosyltransferase that adds a fucose moiety to a nonterminal sugar residue of the core glycan, and the resulted fucosylated glycan is called the H antigen (O antigen).
- A single gene on chromosome 9 encodes a glycosyltransferase enzyme that may further modify the H antigen.

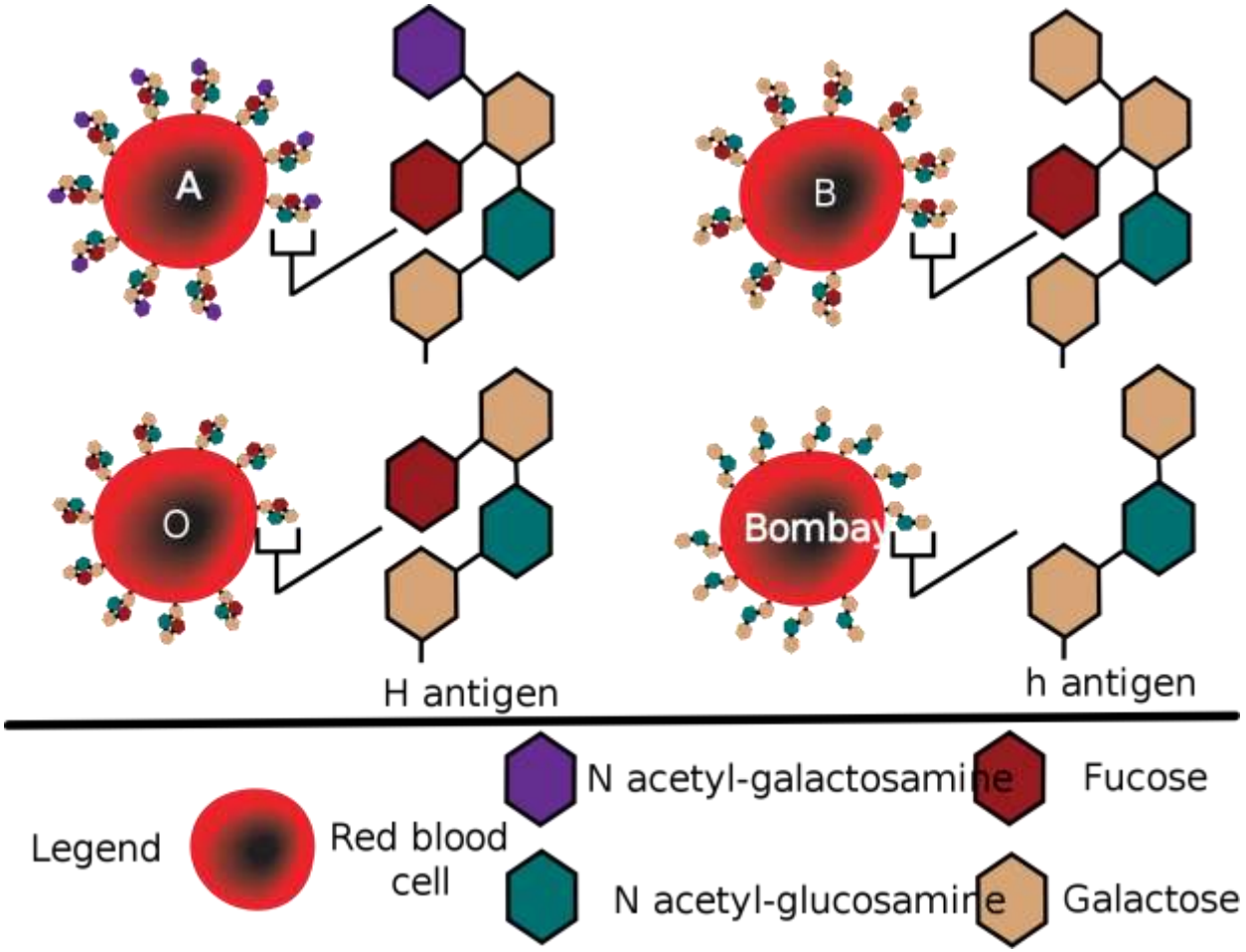
✓ There are three allelic variants of this enzyme

✓ O allele gene product is devoid of enzymatic activity and cannot attach terminal sugars to the H antigen and express

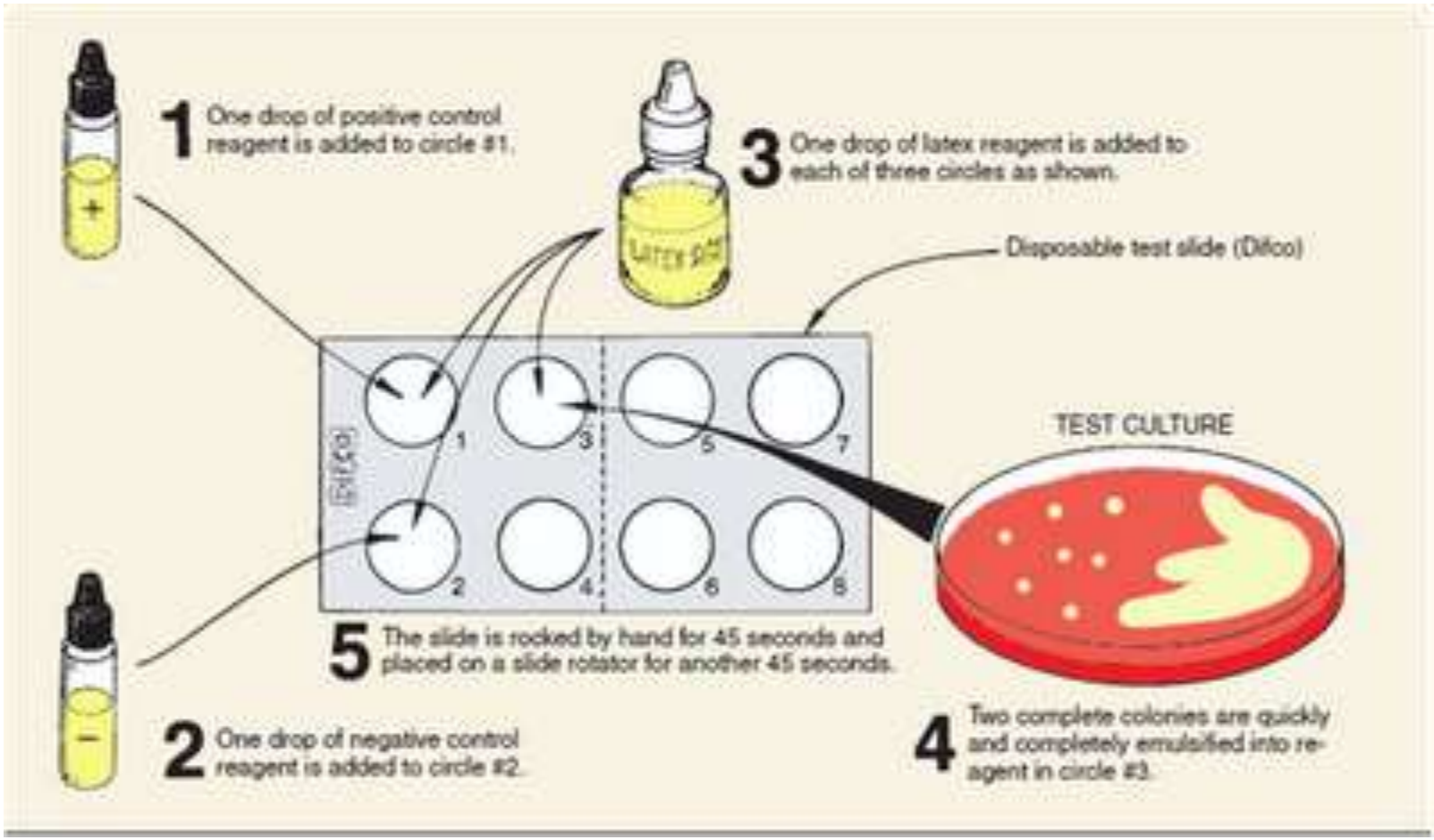
✓ only the H antigen

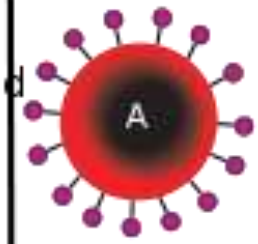
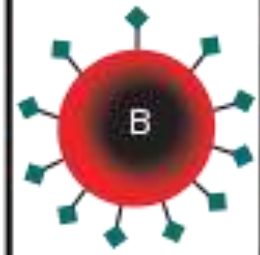
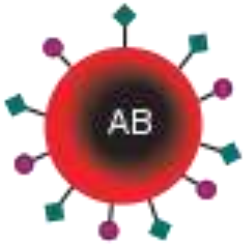
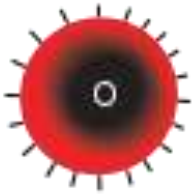
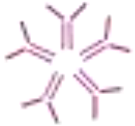
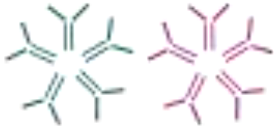
✓ The A allele– encoded enzyme transfers a terminal ***N-acetylgalactosamine*** moiety onto the H antigen.

✓ B allele gene product transfers a terminal ***galactose*** moiety.


A
B
O
Bombay
H antigen
h antigen
Legend
Red blood cell
N acetyl-galactosamine
N acetyl-glucosamine
Fucose
Galactose

- Mutations in the gene encoding the fucosyltransferase that produces the H antigen without fucose are rare; people who are homozygous for such a mutation are said to have the Bombay blood group
- And cannot produce H, A, or B antigens. and cannot receive type O, A, B, or AB blood.

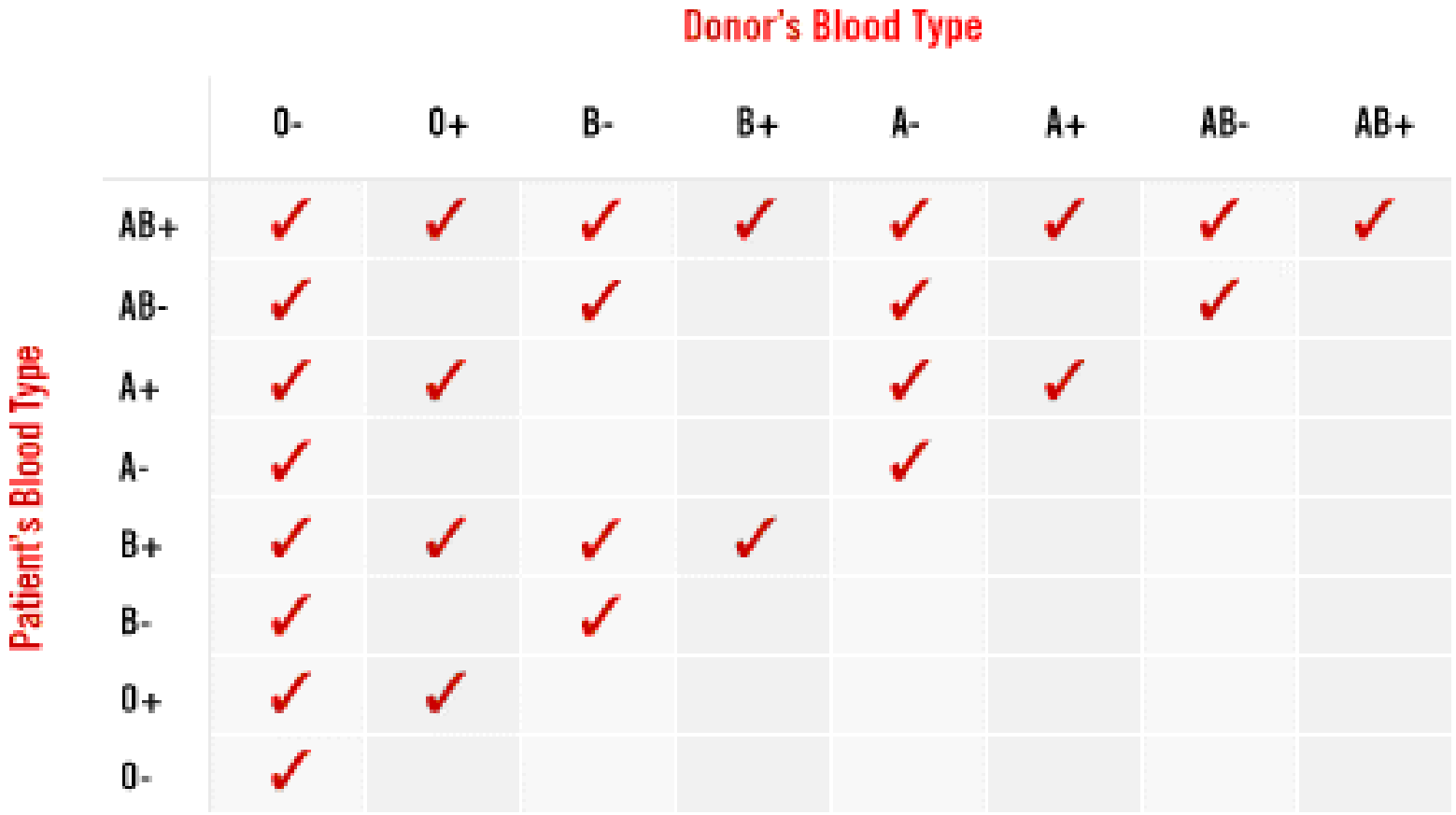

1 One drop of positive control reagent is added to circle #1.
3 One drop of latex reagent is added to each of three circles as shown.
Disposable test slide (Difco)
TEST CULTURE
5 The slide is rocked by hand for 45 seconds and placed on a slide rotator for another 45 seconds.
2 One drop of negative control reagent is added to circle #2.
4 Two complete colonies are quickly and completely emulsified into reagent in circle #3.

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies present	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens present	 A antigen	 B antigen	 A and B antigens	None

Red blood cell compatibility table

Recipient[1] \ Donor[1]	O−	O+	A−	A+	B−	B+	AB−	AB+
O−	✓	✗	✗	✗	✗	✗	✗	✗
O+	✓	✓	✗	✗	✗	✗	✗	✗
A−	✓	✗	✓	✗	✗	✗	✗	✗
A+	✓	✓	✓	✓	✗	✗	✗	✗
B−	✓	✗	✗	✗	✓	✗	✗	✗
B+	✓	✓	✗	✗	✓	✓	✗	✗
AB−	✓	✗	✓	✗	✓	✗	✓	✗
AB+	✓	✓	✓	✓	✓	✓	✓	✓

Most Common Transplantation -Blood Transfusion-



Percentages of the 8 blood groups

- **AB-**negative (. 6 percent)
- **B-negative** (1.5 percent)
- **AB-positive** (3.4 percent)
- A-negative (6.3 percent)
- **O**-negative (6.6 percent)
- **B-positive** (8.5 percent)
- A-positive (35.7 percent)
- **O-positive** (37.4 percent)

- O-negative is the universal blood type, meaning any other blood type may receive it (see our blood type compatibility chart here). This can quickly deplete the stores of O-negative that blood centers have on the shelves. And while 45% of the population is type O, less than 7% is O-negative. So as you can see, the most needed type of blood is also the hardest to collect.
- AB negative is the rarest of the eight main blood types - just 1% of our donors have it. Despite being rare, demand for AB negative blood is low

Blood grouping System	System symbol	Epitope or carrier, notes	Chromosome
ABO	ABO	Carbohydrate) N-Acetylgalactosamine ,galactose .(A, B and H antigens	9
MNS	MNS	Main antigens M, N, S, s.	4
Rh	RH	Protein. C, c, D, E, e antigens (there is no "d" antigen; lowercase "d" indicates the absence of D	1
Kell	KEL	Glycoprotein. K_1can cause hemolytic disease of the newborn (anti-Kell ,(which can be severe.	7
LI	Li	Polysaccharide	6
Duffy	FY	Protein) chemokine receptor .(Main antigens Fy^a and Fy^b .Individuals lacking Duffy antigens altogether are immune to malaria caused by *Plasmodium vivax* and *Plasmodium knowlesi*.	1

RH blood antigen

- Rh antigens are non-glycosylated, hydrophobic cell surface proteins found in red blood cell membranes. 15% of the population has a deletion or other alteration of the RhD allele.
- Rh status is inherited from our parents, separately from our blood type. If you inherit the dominant Rhesus D antigen from one or both of your parents, then you are Rh-positive (85% of us). If you do not inherit the Rhesus D antigen from either parent, then you are Rh-negative (15% of us)

Rh System

- ***Rh Antigens and Encoding Genes***

✓ Subsequently it was confirmed that the ***RH*** locus is on **chromosome 1** and comprises two highly homologous, very closely linked genes, ***RHD*** and ***RHCE.***

✓ ***The Rh blood group system consists of 49 defined blood group antigens,***

✓ ***among which the five antigens D, C, c, E, and e are the most important. There is no d antigen. D antigen is the main that its presence or absence mean RH+ or RH- respectively***

✓ The main antigens are D, C, E, c and e, which are encoded by two adjacent gene loci, the RHD gene which encodes the RhD protein with the D antigen and the RHCE gene which encodes the RhCE protein with the C, E, c and e antigens

✓ The *RHCE* gene has four main alleles; *CE, Ce, ce and cE.*

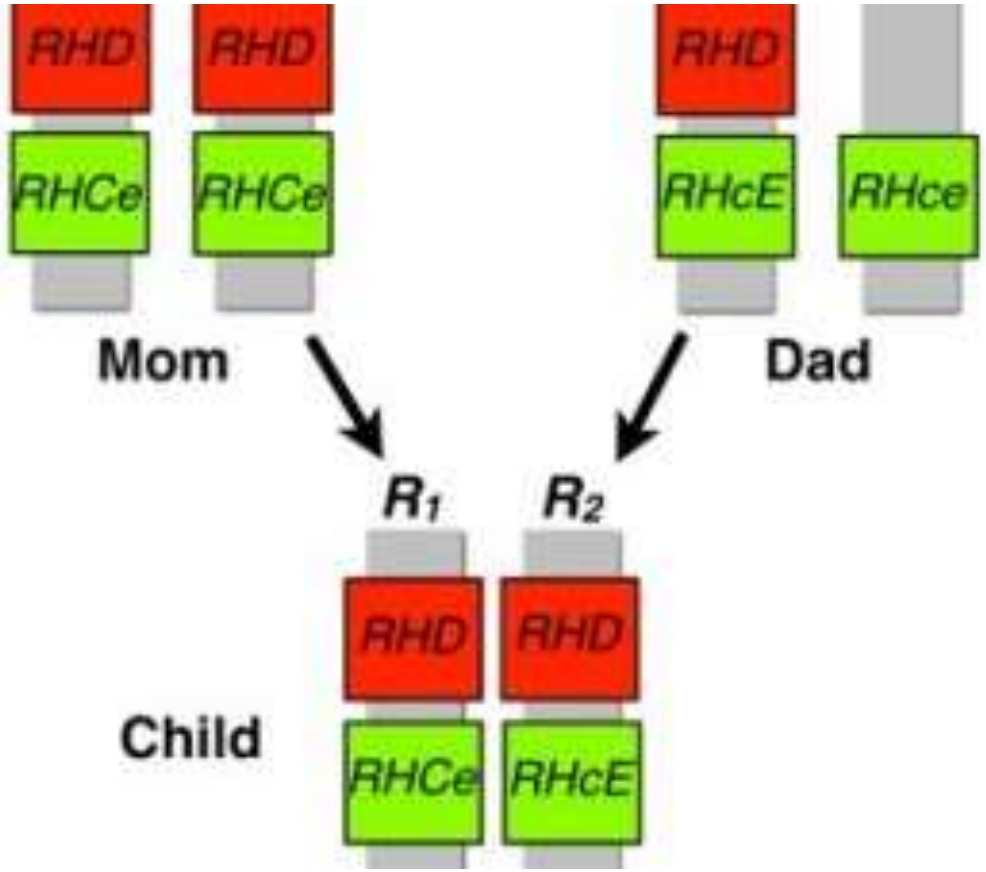
✓ This concept of D and CcEe genes linked closely and transmitted together is consistent with the Fisher nomenclature.

✓ Examples on antigens in RH+ and -

✓ D– C+ E+ c– e+ (RhD–)

✓ D+ C+ E– c– e+ (RhD+)

- Each locus has its own set of alleles which are Dd , Cc , and Ee . The D gene is dominant to the d gene, but Cc and Ee are co-dominant (meaning that all of the inherited alleles lead to expression of the coded antigens).
- Antibodies to Rh antigens can be involved in hemolytic transfusion reactions and antibodies to the Rh(D) antigens confer significant risk of hemolytic disease of the fetus and newborn.



This child is D+C+c+E+e+

Other option is RHD, RHCe, Rhce (D+C+c+E+e+)

This means all loci in one arm of chromosome are inherited together

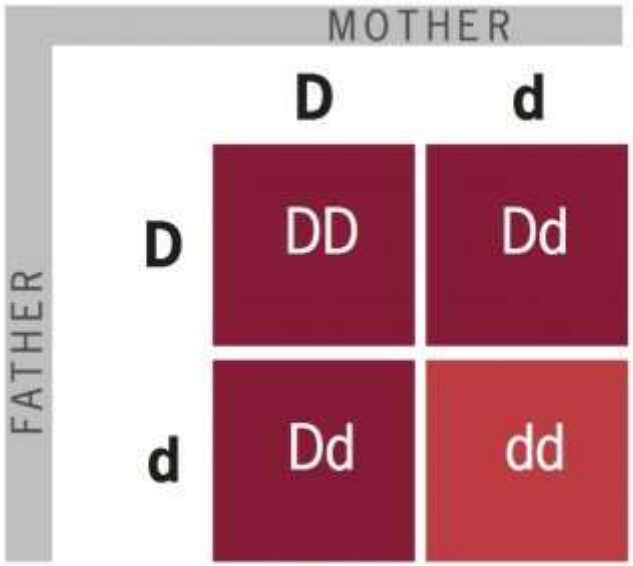

MOTHER
D
d
FATHER
D
d
DD
Dd
Dd
dd

Table 21.7 The Rh haplotypes in order of frequency (Fisher nomenclature) in Caucasians and the corresponding short notations

FISHER	SHORT NOTATIONS	APPROXIMATE FREQUENCY (%)
CDe	R^1	41
cde	*r*	39
cDE	R^2	14
cDe	R^0	3
C^wDe	R^{1w}	1
cdE	*r″*	1
Cde	*r′*	1
CDE	R^Z	Rare
CdE	r^y	Rare

Rh System

- ***Antibodies***

✓ antibodies directed against all Rh antigens, except d, have been described: anti-D, anti-C, anti-c, anti-E and anti-e.

✓ Rh antigens are restricted to red cells and Rh antibodies result from previous alloimmunization by ***previous pregnancy*** or ***transfusion.***

✓ Immune Rh antibodies are predominantly IgG

Rh System

- ***Antibodies***

✓ Anti-D is clinically the most important antibody.

✓ it may cause ***hemolytic transfusion reactions*** and was a common cause of fetal death resulting from ***hemolytic disease of the newborn*** before the introduction of anti-D prophylaxis.

hemolytic disease of the newborn

- When the condition is caused by the Rh D antigen-antibody incompatibility, it is called Rh D Hemolytic disease of the newborn
- The major clinical significance of anti-Rh antibodies is related to hemolytic reactions associated with pregnancy that are similar to transfusion reactions. Rh-negative mothers carrying an Rh-positive fetus can be sensitized by fetal red blood cells that enter the maternal circulation, usually during childbirth. IgG antibodies are generated in Rh-negative mothers. Subsequent pregnancies in which the fetus is Rh positive are at risk because the maternal anti-Rh D IgG antibodies can cross the placenta and mediate the destruction of the fetal red blood cells. This causes anemia, dyspnea, jaundice and erythroblastosis fetalis

Mother's Rh factor	Father's Rh factor	Baby's Rh factor	Precautions
Rh positive	Rh positive	Rh positive	None
Rh negative	Rh negative	Rh negative	None
Rh positive	Rh negative	Could be Rh positive or Rh negative	None
Rh negative	Rh positive	Could be Rh positive or Rh negative	Rh immune globulin injections