كلية المستقبل الجامعة قسم الصيدلة
المرحلة الثانية

PHYSIOLOGY

Blood Types; Transfusion; Tissue and Organ Transplantation

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INTRODUCTION

A blood type (also called a **blood group**) is a classification of blood based on the presence or absence of inherited antigenic substances on the surface of red blood cells (RBCs).

Blood is classified into different **blood groups**, based on the presence or absence of these antigens or agglutinogens.

The ABO blood group is characterized by two glycolipid antigens, called A and B – depending on whether the RBCs have none, only one or both antigens, blood groups are distinguished as type O, type A, type B, or type AB.

Agglutinins of ABO System

Blood plasma contains antibodies or agglutinins that react with non- self antigens. They are absent in a newborn; the ABO antibodies start appearing in the plasma by the age of 3–4 months due to cross reactivity of ABO antigens present in naturally occurring bacteria, viruses, pollen, etc. present in the environment. These antigens are absorbed into blood and stimulate the formation of antibodies against antigens not present in the infants' red cells, i.e. those antigens that are recognized as "non-self" by the body's immune system.

Purpose of blood typing

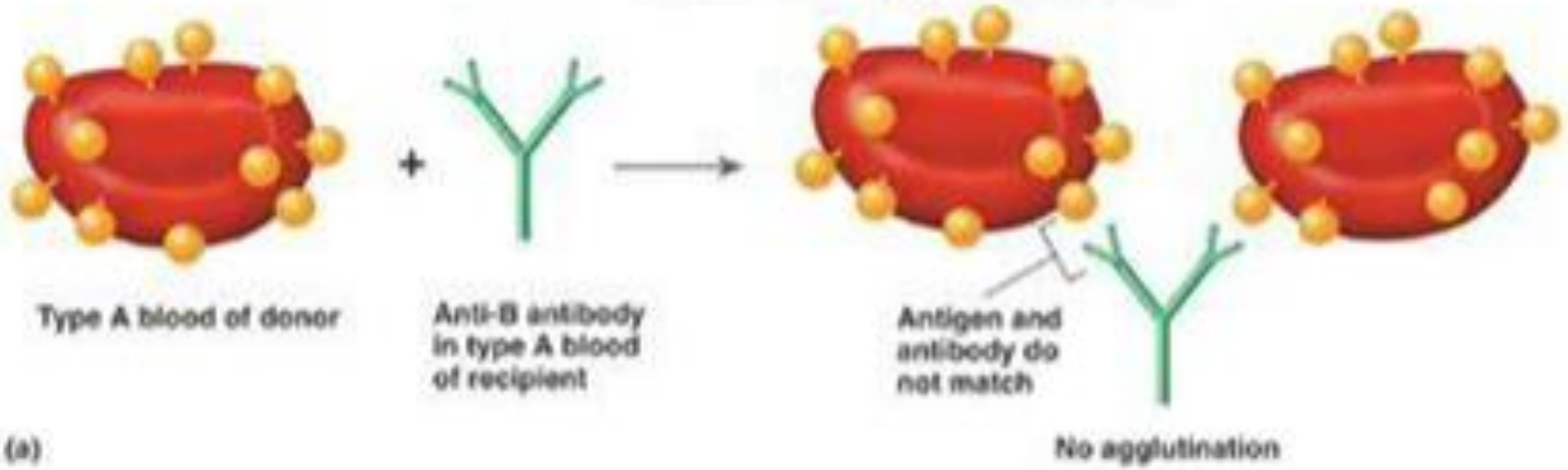
Blood transfusion is a life-saving procedure in all cases of severe loss of blood, and in life-threatening anemias. However, blood can only be given after blood grouping which is an essential requirement before blood is given to any individual.

Agglutination

If someone receives blood of the wrong type, the worst problem is the reaction of the recipient's antibodies on the donor's RBCs.

When the body encounters a foreign antigen, agglutination occurs. Agglutination is the clumping of RBCs due to binding of antibodies (part of the immune system) to antigen, and causes blockage of blood vessels and eventually death. In your blood, you have antibodies for the antigens you don't have.

Agglutination Reaction



Blood Group	Antigens	Antibodies	Can give blood (RBC) to	Can receive blood (RBC) from
AB	A and B	None	AB	AB, A, B, O
A	A	B	A and AB	A and O
B	B	A	B and AB	B and O
O	None	A and B	AB, A, B, O	O

RH factor

In addition to antigens of ABO system, the red cells of humans also contain an additional antigen, called Rh antigen (or Rh factor).

There are several varieties of Rh antigen—C, D, E, c, d, and e—but the D antigen is the most common, and antigenically, the most potent. Therefore, Rh +ve persons are also called D +ve and Rh –ve are called D –ve.

Persons whose red cells contain this additional antigen are called "Rh positive" (Rh +) while those who lack this antigen are called "Rh negative" (Rh –).
However, there are **no naturally occurring antibodies against Rh (D) antigen**.

The Rh (D) antigen is not present in body fluids and tissues, but only on red cells.

Clinical Significance of Rh factor

Although there are no natural anti-Rh antibodies, and they never develop spontaneously, they can be produced only in Rh –ve persons. This can happen in either of 2 ways:

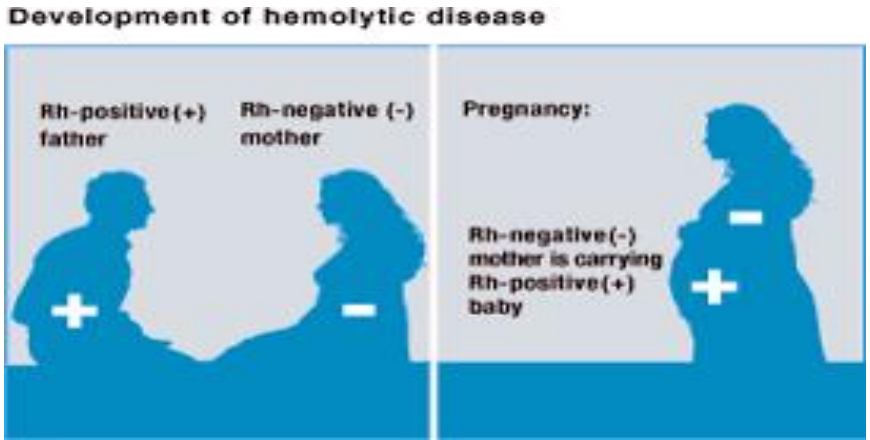
1.In transfusions. When an Rh –ve person receives Rh +ve blood, there is no immediate reaction since there are no antibodies. But during the next few weeks/months, he/she may produce anti-Rh antibodies that will remain in the blood. (Even 0.5 ml of Rh +ve blood is enough to produce immune response). However, if within a few weeks, or even years later, a second Rh +ve blood is injected, the newly donated red cells will be agglutinated and hemolysed, thus resulting in a serious transfusion reaction.

2.*In pregnancy*. The most common problem due to Rh incompatibility may arise when an Rh –ve mother (phenotype dd) carries an Rh +ve fetus

Normally, no direct contact occurs between maternal and fetal bloods. However, if a small amount of Rh +ve blood leaks (at the time of delivery) from the fetus through the placenta into the mother's blood, the mother's immune system will start to make anti- Rh antibodies.

As a result, some mothers develop high concentration of anti-Rh antibodies during the period following delivery. Therefore, the first-born baby will not be affected.

However, during the second and subsequent pregnancies, the mother's anti-Rh antibodies cross the placental membrane into the fetus where they cause agglutination and hemolysis. The clinical condition that develops in the fetus is called ***"hemolytic disease of the newborn (HDN)' or "erythroblastosis fetalis"***2



How can hemolytic disease of the newborn be prevented? What is the treatment of severe HDN?

The condition can be prevented by desensitizing all Rh –ve mothers by giving them injections of massive doses of **anti-Rh antibodies** called Rho(D) immune globulin after every abortion, miscarriage, or delivery. These antibodies bind to and inactivate the fetal Rh antigens (on fetal red cells) present in maternal circulation. In this way, the Rh antigens from the mother's blood are cleared (removed) before they have had time to stimulate production of anti-Rh antibodies.

Why does the ABO-incompatibility rarely produce hemolytic disease of the newborn?

The ABO-incompatibility between the mother and fetus rarely causes HDN. The reason is that the anti-A and anti-B (anti-ABO) antibodies belong to IgM type of gamma globulins (big size) that do not cross the placenta.

N.B With regard to **transfusions of packed red blood cells**, individuals with type O Rh D negative blood are often called universal donors, and those with type AB Rh D positive blood are called universal recipients.With regard to **transfusions of plasma**, this situation is reversed. Type O plasma, containing both anti-A and anti-B antibodies, can only be given to O recipients. The antibodies will attack the antigens on any other blood type. Conversely, AB plasma can be given to patients of any ABO blood group due to not containing any anti-A or anti-B antibodies.

1. Antigenicity Causes Immune Reactions of Blood

1.1. Background

- Before discovering blood typing and matching procedure, blood transfusions were resulted in transfusion reactions and frequent deaths.
- Transfusion reactions involved immediate or delayed agglutination and hemolysis of RBCs.
- Blood typing: Bloods of different people have different antigenic and immune properties.
- Principals:
  - Donor RBC surface contain surface antigen
  - Recipient blood contains antibodies
- Matching procedure: mixing bloods from donor and recipient on a slid
- Result: antigen-antibody reaction → agglutination and hemolysis of RBCs
- Precautions must be taken to determine if transfusion reaction has to occur.

1.2. Multiplicity of Antigens in the Blood Cells

- The surface of human blood cells bearing at least 30 commonly occurring and 100s of other rare antigens.
- Any of which can cause antigen-antibody reactions
  - Most of which are weak, but import for studying the inheritance of genes to establish parentage
- Two types of antigens cause blood transfusion reactions.
  - The O-A-B system of antigens
- The Rh system

Which RBCs are hemolyzed during mismatched blood transfusion

A) Those of donor
B) Those of recipient
C) Both those of donor and recipient
D) Neither those of donor nor those of recipient
E) Those of Rh+ donor

2. O-A-B Blood Types

2.1. A and B antigens "agglutinogens"

- In human: Type A and type B antigens occur in a large proportion on RBC surface.
- These antigens also **agglutinogens**
  - **Inherited** antigens
  - Often cause **agglutination** *[blood transfusion reaction]*
  - People may have:
- **Neither** of them on RBC *(non-antigenic RBCs)*
  - ✓Either of them one on RBC *(either A or B antigens)*
  - ✓**Both** of them on RBC (both A & B simultaneously)

Major O-A-B Blood Types

- Human blood (donors and recipients) normally classified into 4 major **O-A-B blood types**.
- Classification depends on presence **or** absence of A and B agglutinogens
  - *Type O: neither A nor B agglutinogen is present*
  - **Type A**: **only A** *agglutinogen is present*
  - **Type B**: **only B** *agglutinogen is present*
  - *Type AB: both A & B agglutinogen are present*

Group	% of population
O	47%
A	41%
B	9%
AB	3%

Genetic Determination of the Agglutinogens

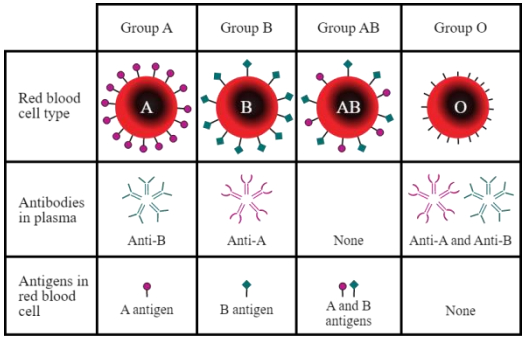
- The ABO blood group genetic locus has 3 *alleles* *(3 different forms of the same gene)*
  - IA also called A – functioning gene → cause **strong agglutinogens** on the cells
  - IB also called B– functioning gene → cause **strong agglutinogens** on the cells
  - IO also called **O** – **functionless** gene → **no** significant type O **agglutinogen** on the cells
  - O and A genes occur frequently, whereas the B gene occurs infrequently

  [The letter "I," stands for "immunoglobulin"].

  [The O allele is recessive to both the A and B alleles, show co-dominance]
- In each individual:
  - **2 chromosomes** encoded for blood type
  - One allele presents on each chromosome
  - 6 possible combinations of alleles:

    (1) OO
    (2) OA
    (3) OB
    (4) AA
    (5) BB
    (6) AB

Genotypes	Blood Types	Agglutinogens	Agglutinins
OO	O	–	Anti-A and Anti-B
OA or AA	A	A	Anti-B
OB or BB	B	B	Anti-A
AB	AB	A and B	–



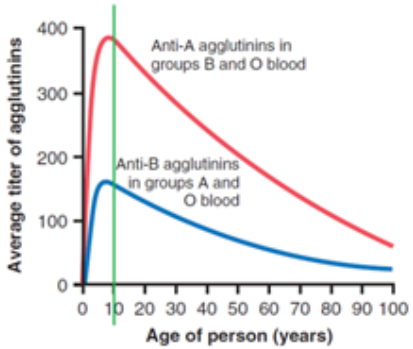
Which is true about blood type gens

A) OA and AA genes encoded for B surface antigens
B) OB and BB genes encoded for A surface antigens
C) OA and AA genes encoded for anti-B antibodies
D) OB and BB genes encoded for anti-A antibodies
E) Alleles A, B and O are used for paternity investigations

2.2. Agglutinins

- Definition: are immunoglobulins of type "IgG" or "IgM" against A or B antigens
- Synthesis: by plasma cells in lymphoid tissues (lymph nodes, bone marrow and spleen)
- Target: Circulate in blood and target RBC surface antigens
- Present: in type A, B and O blood
- Absent: in type AB blood
- When type A agglutinogen *is not present* in a person's RBCs (type O & type B)
  - *Antibodies known as* **anti-A** *agglutinins develop in the plasma*
- When type **B agglutinogen** *is* **not present** in the RBCs (type O & type A)
  - *Antibodies known as* **anti-B** *agglutinins develop in the plasma.*
  - Type AB blood contains both A and B agglutinogens but **no** agglutinins
- Origin: **plasma cells** in reaction to **entrance** of small amount of **A** and **B antigens** (in **food**, **bacteria** and others)
- Titer: **depends on age**
  - *Neonate*: immediately after birth = zero

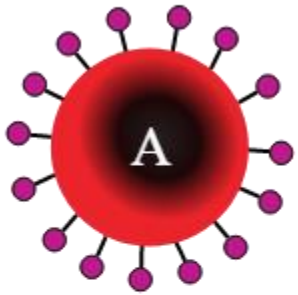
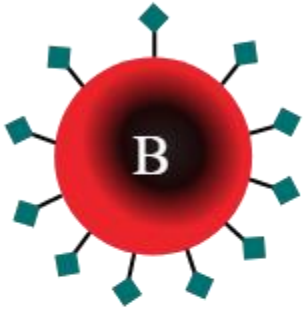
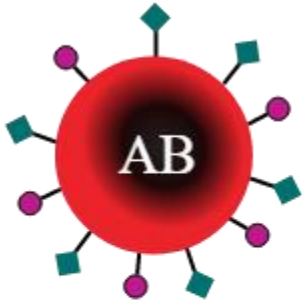
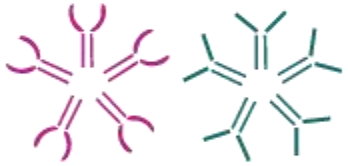
  [Neonate has few, if any, agglutinins; but the formation occurs almost entirely after birth]
  - 2-8 months after birth: start of production
  - 8-10 years: maximum titer reached
  - After 10 years: production declines gradually



- *Note: Infusion of group A antigen into a recipient having a non-A blood type causes a typical immune response with formation of greater quantities of anti-A agglutinins than ever.*

Infusion of type B blood produce agglutinins in which recipient blood
A) Type A
B) Type B
C) Type AB
D) Type O
E) Type A, and type O

When type B agglutinogen is not present, the blood type is
A) Type A
B) Type AB
C) Type AB and type O
D) Type O
E) Type A and O

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

2.3. Agglutination Process in Transfusion Reactions

- Mismatched blood transfusion causes RBCs to agglutinate
- Agglutination: **clumping of RBCs** by attaching themselves to agglutinins
- **Mismatching:**
  - Recipient blood "plasma" contain anti-A mixed with **donor** blood "RBCs" contain **A antigen**
    B + A → Agglutination
    O + AB → Agglutination

  - Recipient blood "plasma" contain anti-B mixed with donor blood "RBCs" contain **B antigen**
    A + B → Agglutination
    O + AB → Agglutination

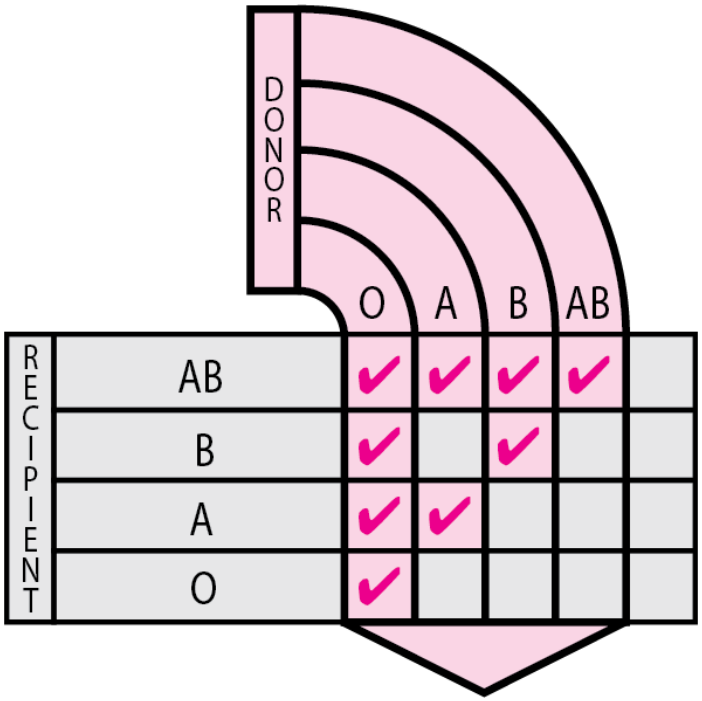
- **Matching**
  - Recipient blood "plasma" contain no anti-A and no anti-B mixed with donor blood "RBCs" contain **A** or **B antigen**
    AB + A → No agglutination
    AB + B → No agglutination *Type AB called Universal recipient & Type O called Universal donor*
    AB + O → No agglutination

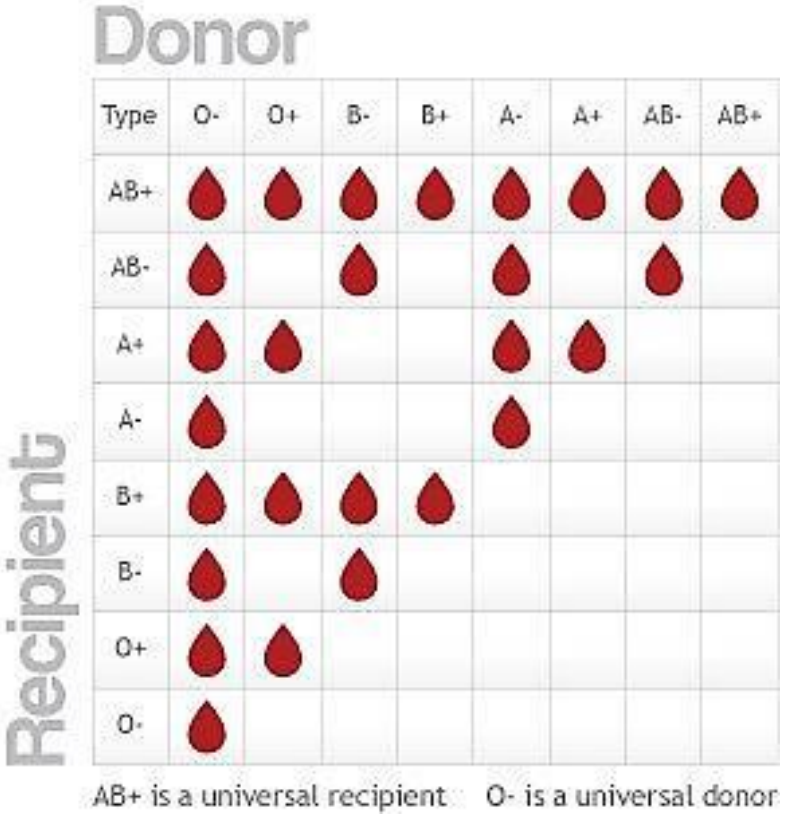
  - Recipient blood "plasma" contain anti-A or anti-B mixed with donor blood "RBCs" contain **No A** or **no B antigen**
    B + O → No agglutination
    A + O → No agglutination
    O + O → No agglutination

- A single agglutinin can attach to 2 or more RBCs at the same time, thereby causing the cells to be bound together " clump" by the agglutinin
  [Recall: IgG is divalent and IgM is decavalent immunoglobulin]
- Clumps **plug small blood vessels** throughout the circulatory system.
- **Prognosis**: *hemolysis of clumps*
  - **Hemolysis**: Destruction of clumped RBC membrane by physical distortion or attack by macrophages and releasing hemoglobin into the plasma.

Which is the universal recipient of blood types
A) Type A-
B) Type B-
C) Type AB-
D) Type O-
E) None of them

Infusion of A+ blood into A- blood produce the following
A) Anti-A antibodies in recipient blood
B) Anti-B antibodies in recipient blood
C) Anti-A antibodies against A+ in recipient blood
D) Anti-A antibodies against A- in recipient blood
E) Anti-B antibodies against A+ in recipient blood


DONOR
O
A
B
AB
RECIPIENT
AB
B
A
O


Donor
Recipient
Type
O-
O+
B-
B+
A-
A+
AB-
AB+
AB+
AB-
A+
A-
B+
B-
O+
O-
AB+ is a universal recipient
O- is a universal donor

Acute Hemolysis Occurs in Some Transfusion Reactions

- Mismatched recipient and donor bloods result in **immediate** or **delayed** intravascular **hemolysis**
- Agglutination causes delayed RBC hemolysis
  - **Immediate hemolysis**
    - Occur in some cases of mismatched transfusions
    - Require **higher concentration** of the **specific hemolysins** antibodies "**IgM**"
  - The antigen-antibody complex **activate lytic complement complex**
    - The activated complex causes rupturing of RBC membrane

Delayed hemolytic transfusion reactions (DHTRs)

- Occurs 3-10 days after transfusion of RBC products that appear to be serologically compatible
  - These reactions occur in patients who have been alloimmunized to minor RBC antigens during previous transfusions and/or pregnancies.
  - **Pretransfusion testing** fails to detect these alloantibodies due to their **low titer**.

2.4. Blood Typing

- Means: determining the blood type of the recipient's and the blood type of the donor before transfusion for proper blood matching
- Procedures:
- RBCs first separated from the plasma and diluted with saline solution
- One portion mixed with **anti-A** and another portion with **anti-B** agglutinin "sera"
- After several minutes, the mixtures are observed under a microscope.
- Presence of RBCs clumping "**agglutination**" means antibody-antigen occurs

Red Blood Cell Types	*Sera*	
	Anti-A	Anti-B
O	–	–
A	+	–
B	–	+
AB	+	+

3. Transplantation of Tissues and Organs

- All body cells (in addition to RBCs) have surface antigens.
- Thus, foreign cells (from donor) transplanted into the body of a recipient can produce immune reactions.
  [Most recipients are just as able to resist invasion by foreign tissue cells as to resist invasion by foreign bacteria or RBCs]

3.1. Autografts, Isografts, Allografts, and Xenografts

- Transplant of tissue or whole organ source categorized according to the donor into

Category	Donor- recipient	Antigens	Immune reaction	Need for Immune suppression therapy	Example
Autograft	Same subject	Same antigens	No	No	
Isograft	Identical twins but Same species	Same antigens	No	no	
Allograft	Different subjects but Same species	Different antigens	Yes	Yes	skin, kidney, heart, liver, glandular tissue, bone marrow, and lung
Xenograft	Different subjects but Different species	Different antigens	Strong (may cause death within 1-5 days)	Yes (intensive)	

- With proper "matching" of tissues between persons, many kidney allografts have been successful for at least 5 to 15 years, and allograft liver and heart transplants for 1 to 15 years.

Fetus represent which type of transplant

A) Autograft
B) Isograft
C) Allograft
D) Xenograft
E) Non of the above

3.2. Attempts to Overcome Immune Reactions in Transplanted Tissue

- To prevent antigen-antibody reactions associated with transplantation the following specific procedures have met with some degrees of clinical or experimental success.

Tissue Typing— The Human Leukocyte Antigen Complex of Antigens

Human leukocyte antigens (HLA):

- The most important antigens for causing graft rejection
- Occur on the WBCs *(as well as on the tissue cells)*
- **6 HLA** of these antigens are present on the tissue cell membranes of each person.
- These 6 HLA show wide diversity (trillion possible combinations)
- It is impossible for two persons (**except** in the case of identical twins "**Isograft**", to have the same 6 HLA antigens.
- Immunity against any of these antigens can cause graft rejection.

  Procedure: - on the membranes of lymphocytes

  (1) Lymphocytes are **separated** from the person's blood
  (2) **Mixed** with appropriate antisera and complement
  (3) **Incubated**
  (4) Tested for membrane damage (by testing the rate of transmembrane uptake by the lymphocytic cells of a special dye)

- **Some** of the HLA antigens are **not severely antigenic**.
- Precise match of some antigens between donor and recipient is not always essential to allow allograft acceptance.
- The best success of matching is between siblings and between parent and child.
- The match in identical twins is exact - transplants between identical twins never rejected.

Which is false?

A) Blood typing is used to detect blood compatibility
B) Blood typing is used to overcome allograft rejection
C) RBC's A and B antigens are used for paternity investigation
D) HLAs antigens are used for paternity investigation
E) HLAs are used to detect blood compatibility

Prevention of Graft Rejection by Suppressing the Immune System

- T cells (but not plasma B cells) kill grafted cells
- T cell suppression is important to **resist** transplant rejection
- Completely suppression of immune system results in no graft rejection.
- Immune suppression therapy is used to prevent graft rejection
- Graft in person whose immune system is seriously depressed can success without therapy.
- Allografts in normal immune person (even with the best possible tissue typing):
  - **Without therapy**: success is not more than a few days or weeks then **rejected**.
  - With therapy: success for years

Immune suppressing agents:

(1) **Glucocorticoids**
- Inhibit genes that code for several cytokines (interleukin-2)
- Suppress the growth of all lymphoid tissue (T cells and B cell antibodies formation)

(2) **Azathioprine**
- Has toxic effect on the lymphoid system block T cells and B cell antibodies formation

(3) Cyclosporine and tacrolimus
- Inhibit formation of T-helper cells (thus block the T-cell rejection reaction)
- Do not depress some other portions of the immune system.

(4) **Immunosuppressive antibody therapy**: Specific antilymphocyte or IL-2 receptor antibodies

Adverse effects:

- Leaves the person **unprotected** from infectious disease (rampant "widely spread" **bacterial** and **viral infections**)

1) **Increase incidence of cancer**: T cells are important in destroying early cancer cells before they can begin to proliferate.

- The current approach to immunosuppressive therapy attempts to balance acceptable rates of rejection with moderation in the adverse effects of immunosuppressive drugs.

Which is false?
A) Corticosteroids inhibit both T and B cell growth
B) Azathioprine inhibit both T and B cell growth
C) Cyclosporine inhibit both T and B cell growth
D) Immunosuppressive antibodies inhibit both T and B cell growth
E) Non of the above is false