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Virology

Doctor 2018 | Medicine | JU

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Parvoviruses and Herpesviruses

- **Viruses** are classified according to certain characteristics, for example**:**

1) The genome: DNA or RNA, and whether it's single or double stranded.

2) Existence of an envelope: Enveloped or naked (non-enveloped)

The DNA or RNA (genome) is enclosed by a protein coat called the capsid and an envelope may be present outer to the capsid.

***red notes all over this sheet are not included, they're just for further understanding.**

Parvovirus	Herpesvirus
DNA	**DNA**
Single stranded	**Double stranded**
Classified as group II viruses in the Baltimore classification of viruses	**Classified as group I viruses in the Baltimore classification of viruses**
Naked (non-enveloped)	**Enveloped (envelope around the protein coat)**

-Parvovirus is the **simplest** DNA containing animal virus.

- The importance (sternness) of naked viruses (non-enveloped):

Naked viruses are **resistant** to environmental conditions; they are extremely resistant to the body's mechanisms to inactivate them. HOW? Naked viruses are **stable** between a pH of 3 and 9 and they **tolerate heat** at (65 c) for 60 minutes. Nevertheless, they can be **inactivated** by oxidizing agents.

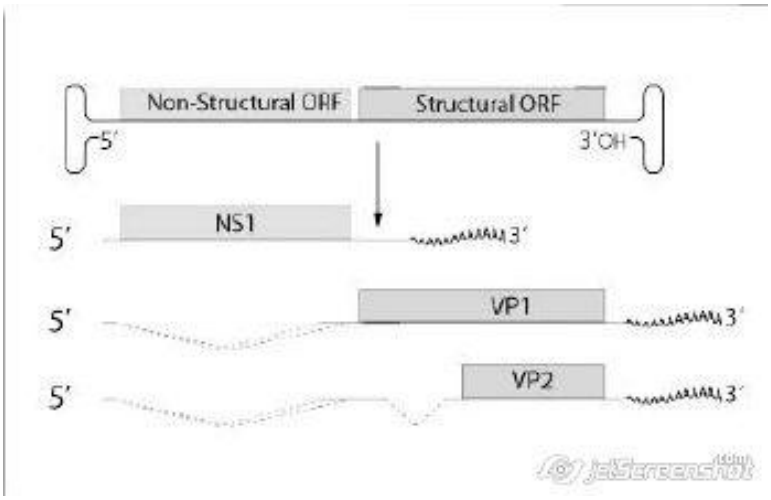
- Parvoviruses are naked viruses as we said, with no envelope, just a capsid. This capsid is made of many copies of just two protein molecules (**VP1**⟶ viral protein 1, **VP2**⟶ viral protein 2) approximately translated from the same area in the viral genome, this is why they are similar in structure as VP2 is identical in sequence to the **terminal carboxy portion** of VP1.((they are encoded by an overlapping, in-frame DNA sequence)).

Look at this picture:

ORF: open reading frame

NS: non structural

(Just look at VP1 and VP2 gene portions how they are overlapped)



- **VP2** represents 90% of the virion proteins; Because of this percentage, antibodies are mainly synthesized against viral protein 2.

Important properties of parvoviruses

Virion: Icosahedral, 18–26 nm in diameter, 32 capsomeres

Composition: DNA (20%), protein (80%)

Genome: Single-stranded DNA, linear, 5.6 kb, MW 1.5–2.0 million

Proteins: One major (VP2) and one minor (VP1)

Envelope: None

Replication: Nucleus, dependent on functions of dividing host cells

Outstanding characteristics:

Very simple viruses

Human pathogen, B19, has tropism for red blood cell progenitors

One genus contains viruses that are replication-defective and require a helper virus

* virion: the entire

* virion: the entire virus particle consisting of the capsid and the inner core of nucleic acid(DNA,RNA).

* icosahedral: a virus with 20 plane faces

* a capsomere is a subunit of the capsid that protects the genetic material of the virus.

"not mentioned in the lecture"

There are **two subfamilies** (-Virinae) of Parvoviridae

Parvovirinae

Densovirinae

Parvovirinae subfamily	***Densovirinae subfamily***
It comprises 5 genera	**It has 5 recognized genera**
Infects vertebrates such as humans	**Infects insects**

Some **genera** (single: genus) of the **parvovirinae** subfamily

Bocaparvovirus genus

An example: human bocaviruses which are three types in this genus.

Erythrovirus genus

-There are three human genotypes in this genus associated with infections of humans.

-The cell's epidemiology differs from one genome to another.

-**Epidemiology**: **is a branch of medical science that studies how often diseases occur in different groups of people and why.**

-The most common member of this genus is parvovirus B19.

Dependoparvovirus genus

-they are called Adeno-associated viruses **because the dependoviruses were first found associated with adenoviruses**.

-It contains members that are defective and dependent on a helper virus (an adenovirus or herpesvirus) for replication.

They are called DEFECTIVE because they have lost essential functions required for replication. Thus, they require the simultaneous infection of a cell by a helper virus.

-Human "adeno-associated viruses" do not cause diseases alone.

NOTE:

-**Parvovirus's replication** is accomplished by 2 methods: **replicating in host cells (autonomous)** or by **co-infection** with helper viruses.

-Parvoviridae can replicate in two ways: 1. **autonomously** (basically: the virus Encapsidates (encloses in its protein shell) some primary DNA strands complementary to its viral mRNA) ⟶ in this way they don't require helper viruses, but they replicate only in actively cycling cells) or 2. by **co-infection** with other viruses (such as dependoviruses with adenovirus or herpesvirus).

-**Autonomous paroviruses rely on cellular factors whose expression is associated with proliferation (S phase of the cycle) and differentiation**.

-**Co-infection includes simultaneous infection of a single cell by two or more virus particles.**

-If you isolate a human sample from a certain location and came out with bacteria and viruses, WHAT **determines** if these viruses and bacteria are pathogenic (associated with disease) or are just components of the normal flora (microorganisms that live normally in our bodies)?

Each has its **specific components** that can be detected by certain methods, which can be used for that.

-The **number** of genes of all the microbes of an individual's microbiome is **much more** than the **number** of genes in the human genome.

Parvovirus's Replication

Parvovirus B19

-Why is it called **parvovirus B19**?

In the past, they knew that the parvovirus B19 infects animals, but they wanted to know if this virus can infect humans too. So, they performed an experiment using a **microtiter plate** which is a flat plate that has multiple wells with numbers ⟶ they isolated this parvovirus from the well number B19. -_-

- Actually, **Parvovirus B19** was the **first** discovered parvovirus that causes human disease, one example of diseases associated with this virus is **((erythema infectiosum))- the "fifth disease".**

The name "fifth **disease**" comes from its place on the standard list of rash-causing **childhood diseases:**

-**First disease** caused by Measles virus.

-**Second disease** caused by Streptococcus pyogenes (bacterial disease associated with problems in the skin known as the scarlet fever).

-**third disease** caused by rubella virus.

-**forth disease** caused by staphylococcus aureus

-**Fifth disease** caused by parvovirus B19.

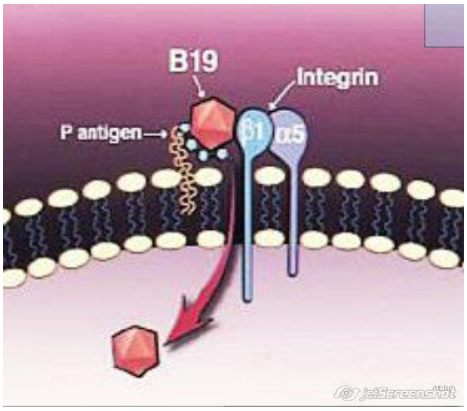
-**sixth disease** caused by two types of herpesvirus. (**Just read**)

- It is difficult to **culture** human B19 parvovirus; because only primary erythroid progenitors are known to be permissive for B19 infection. In other words, Immature cells in the erythroid lineage are targets of B19 parvovirus infection. These cells are found in **the adult bone marrow & fetal liver** ⟶ **the major sites of infection**

NOW THE QUESTION IS

-WHY does B19 infect primary erythroid progenitor (erythroid precursors) cells only?

There are **certain receptors** (integrated to erythroid precursor cells) that can interact with specific proteins on the parvovirus B19 .these receptors are called **P antigens** and exist on erythroid precursor cells.



-**P** is a blood group system (just like ABO system or RhD system) **which can classify human blood based on the existence of any of 3 substances known as (P, P1, Pk) antigens on the surfaces of red blood cells.**

-P antigens exist on **most** individuals' RBCs, so they are **susceptible** to parvovirus B19 (able to be infected by it).

- Only in **some certain** individuals (1 in 200000 people), the antigen P is absent ⟶ they are genetically resistant to the virus (cannot be infected by B19).

-P antigen is a **Globoside** (**a type of glycosphingolipid with more than one sugar as the side chain (or R group) of ceramide**.

- P antigen is expressed on mature erythrocytes, erythroid progenitors, and fetal liver and heart, which helps explain the narrow tissue tropism of B19 virus.
- **Tissue tropism is the specific cells and tissues of a host that support growth of a particular virus or bacterium**.
**Note that P antigen is expressed in mature erythrocytes, but they can't be infected by the virus even when it binds to the P antigen, because mature erythrocytes lack nuclei.

- **The α5β1 integrin is believed to be a co-receptor to the P antigen, and must be existent for the entry of B19 to happen.**

-**Viral DNA replication:** occurs in the nucleus (remember: in erythroid progenitors)
* Viruses just infect dividing cells
* Cellular DNA polymerases are involved in this process.
* The non-structural protein, **NS1**, is required for viral replication.
* Viral replication in infected cells results in cell death, by interrupting red blood cells' production.
* Replication of viral DNA is in the nucleus ⟶ Mature RBCs can't be infected by B19 because RBCs **don't have nuclei**.
* An **Exception**: Poxviridae family ⟶ Replication is in the cytoplasm not the Nucleus.

PARVOVIRUS INFECTION IN HUMANS

- In immunocompromised patients (**having impaired immune system**), **persistent** B19 infections occur (the viral infection will go unabated (non stoped), resulting in chronic anemia (because as we said these viruses infect precursor erythroid cells).
As an example, people with sickle cell anemia or thalassemia when infected by a parvovirus will suffer from transient anemia in the blood (will be explained down in the sheet)
- When parvovirus b19 is congenital (transmitted from the mother to the fetus) it can cause either:
1) **Fetal death**: chronic infection can cause severe anemia to the fetus, leading to its death- especially **before week 20** of pregnancy.
Or 2) **Hydrops fetalis**: usually occurs after the third trimester (28th week of pregnancy).
-details will be discussed-
- **Now lets talk about Bocaviruses:**
*newly discovered to cause infection to humans (in the respiratory tract & GI tract (not strongly proved yet))
* part of the parvovirenae subfamily
*the name "parvovirus" means "the smallest animal virus". Parvo=small

*the name “bocavirus” is related to types of parvovirus that can affect cows “bovine” and dogs “canine” -the first 2 letters of each is taken to name these viruses **Boca**.
* The pathogenesis of human bocavirus infection is not yet known.
* It is prevalent among **children** with **acute wheezing.**
* Because this virus has been detected in 1.5% to 11.3% of **respiratory tract samples** from young children with respiratory infections. It is presumed to infect the respiratory tract and to be mainly **transmitted** by the respiratory route.
* The virus has been detected in about 3% of stool samples from children with acute gastroenteritis. Co-infection rates with other enteric pathogens were high, so any causative role of bocavirus in gastroenteritis is **not yet known.** (**Bocavirus isn’t well linked to the GI tract as it’s well linked to the respiratory tract**.)
* Infection by the virus may also rarely be **transmitted** through **blood transfusion.** So in blood transfusion we make sure that the blood is **parvo-negative**.

Blood test is done to blood samples used for transfusion, this test checks mainly for:

-HIV

-Hepatitis b, c

-Treponema, which is a type of bacteria transmitted sexually

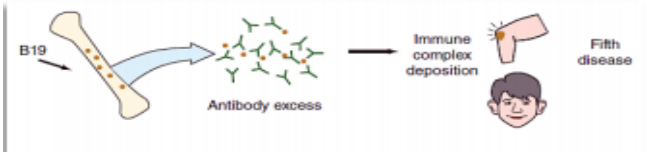
((Checking for treponema is essential in sensitive carriers, because it’s an indicator of the person’s behavior as it’s sexually transmitted)).

-**Manifestations** (symptoms) to any infectious disease are a result of: **the pathogen itself**, the **immune response**, or **both.**
-manifestations of parvovirus diseases are mainly caused by immune response.

Syndrome	Host or Condition	Clinical Features
Erythema infectiosum	Children (fifth disease) Adults	Cutaneous rash Arthralgia-arthritis
Transient aplastic crisis	Underlying hemolysis	Severe acute anemia
Pure red cell aplasia	Immunodeficiencies	Chronic anemia
Hydrops fetalis	Fetus	Fatal anemia

Persistent infections occur in patients with immune deficiencies resulting in anemia, but what's its effect on immune competent patients?

Let's take the erythema infectiosum as an example, this disease usually causes arthropathy and joint pain to **adults,** when infected with B19, due to the deposition of immune complexes in joints (immune complexes are **antibodies** bound to the **antigen** of the virus). This pain can extend for weeks, or even years. The same disease can cause skin rash to **children**, skin rash is also due to deposition of immune complexes.



However, immunocompromised patients, have low immune response → no antibodies produced → nothing can stop the virus from infecting progenitor erythrocytes → reduced number of erythrocytes → anemia.

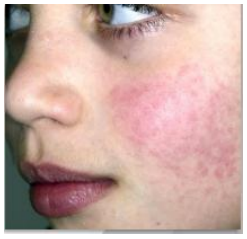
-**Transmission of B19 is by** the **respiratory route**, also can be transmitted **parenterally by blood transfusion** or **vertically** from mother to fetus. There is no evidence of virus excretion in feces or urine.

Now let's discuss each syndrome by details:

A) Erythema infectiosum

This disease **is the most common** manifestation of **B19** infection. It is most common in **children** of early school age and occasionally affects adults, both in **sporadic** (occurring only in few places) and **epidemic** (widespread) forms. Mild constitutional symptoms may accompany the rash, which has a typical **"slapped cheek" appearance**.

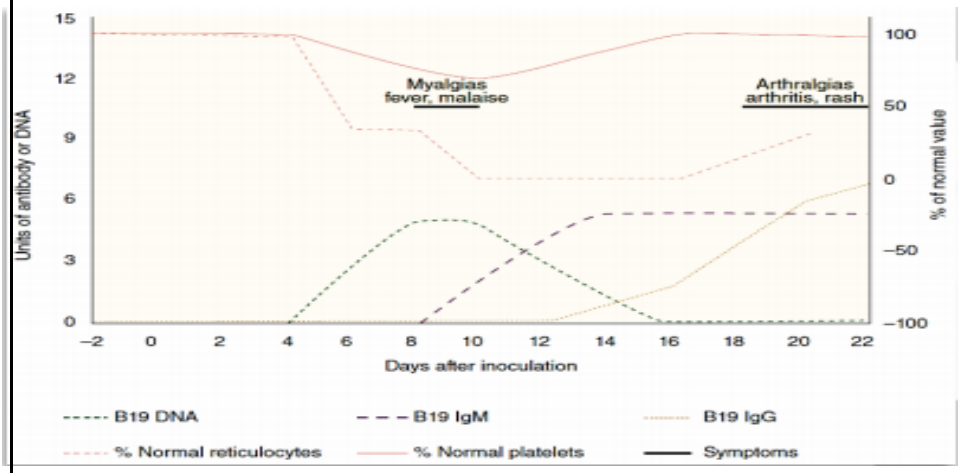
Joint involvement is a prominent feature in adult cases as we discussed, joints in the hands and the knees are most frequently affected. The symptoms **mimic rheumatoid arthritis**, and the arthropathy may persist for weeks, months, or years.



Slapped cheek appearance

The incubation period is **usually 1–2 weeks** but may extend up to 3 weeks (this period differs from one person to another depending on the **infection dose** in each one).

Viremia occurs 1 week after infection and persists for about 5 days **with the upper respiratory tract** as the site of viral **shedding tract** (our respiratory tract is divided into upper and lower). The **first phase** of illness at the end of the first week includes: **fever, malaise, myalgia, chills, and itching** happening **simultaneously with viremia and reticulocytopenia** (**abnormal decrease of reticulocytes in the body, reticulocytes are immature RBCs**) and with detection of circulating **(IgM–parvovirus) immune complexes**. After an incubation period of about 17 days, a **second phase** of illness begins. The appearance of an erythematous facial **rash** (redness and swelling in the face) and **a lacelike rash** on the limbs or trunk may be accompanied by joint symptoms, especially in adults. The illness is **short-lived,** with the rash **fading after 2–4 days**, although the joint symptoms may persist longer**. Specific IgG antibodies appear about 15 days post-infection**



The pic. Shows clinical and laboratory findings during the course of human parvovirus B19 infection in adult volunteers.

***the incubation period (first week)**

***the first phase of illness with flu-like symptoms simultaneous with viremia (days 6–12); igM appear in this phase**

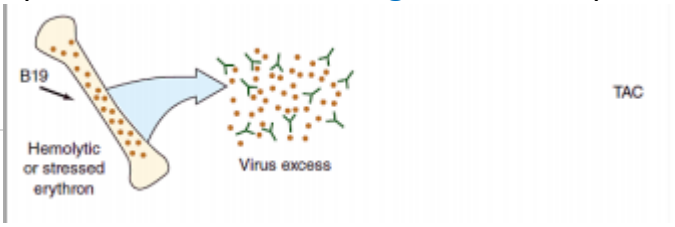
***the second phase of illness with rash appears on about day 18. .IgGs appear in this phase**

B) Transient Aplastic Crisis (TAC)

Parvovirus B19 is the cause of transient aplastic crisis that may complicate in cases of **chronic hemolytic anemia** (such as in patients with sickle cell disease, thalassemia, and acquired hemolytic anemias in adults). Transient aplastic crisis may also occur after **bone marrow transplantation**.

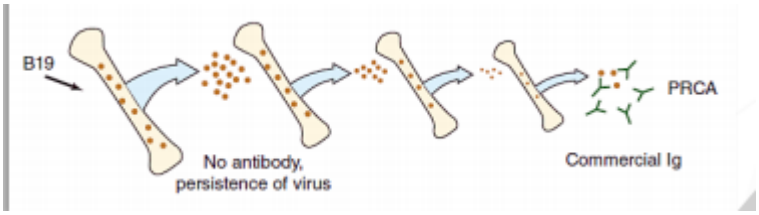
The syndrome is a **severe drop** of red blood cell synthesis in the bone marrow reflected of the absence of erythroid precursors in the marrow, accompanied by a rapid **worsening** of anemia.

The infection lowers production of erythrocytes causing a reduction in the hemoglobin level of peripheral blood. The temporary arrest of production of red blood cells becomes apparent **only** in patients with **chronic hemolytic anemia** because of the **shortened life span** of their erythrocytes. Few anemia patients have a rash. Symptoms of transient aplastic crisis occur **during** the viremic phase of infection.



C) Pure red cell aplasia

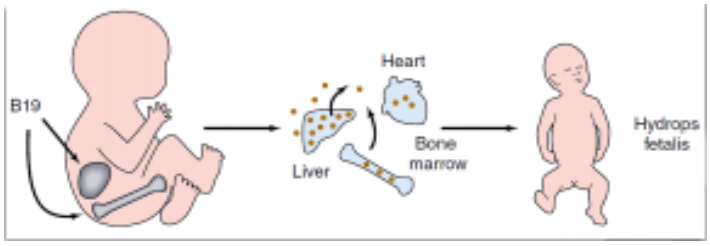
B19 may establish persistent infections and cause chronic suppression of bone marrow functions and chronic anemia **in immunocompromised patients**. The disease is **called pure red cell aplasia**. The anemia is severe, and patients are dependent on blood transfusions. It has been observed in patient populations with congenital immunodeficiency, malignancies, AIDS, and organ transplants.



D) Hydrops fetalis:

Maternal infection with B19 virus may **result in hydrops fetalis OR fetal death** due to severe anemia. Fetal death occurs most commonly **before the 20th week** of pregnancy. Maternal–fetal transmission may occur most commonly in pregnant women **with high plasma viral dose**. **B19 is considered as a **Torch Agent** because it can cross the placenta during pregnancy.

Hydrops fetalis is characterized with edema all over the fetal body. WHY? The infection is through erythroid precursors (which are found in the liver of the fetus mainly) – so there aren't enough erythrocytes being produced, the fetus tries to compensate for this condition by pumping more blood to the body, resulting in heart failure and fluids start to accumulate all over the body **causing** hydrops fetalis.



Diagnosis of parvovirus

1- detecting parvovirus DNA

- Most sensitive tests are used to detect viral DNA, available tests nowadays are:

1) Polymerase chain reaction PCR

2) Hybridization of serum or tissue extracts (outside the body)

3) In situ (in place) hybridization of fixed tissues

*** The most sensitive assay (test) is **PCR**

*** **PCR** is the only currently available assay used to detect **Bocaviruses**.

NOW, where can we find the viral DNA in the body in order to detect it?

→ **B19 DNA** has been detected in **serum, blood cells, tissue samples, and respiratory secretions**.

→ **Bocavirus DNA** has been detected in **serum, saliva, stool samples, and respiratory specimens.**

-During acute infections, viral loads in the blood can reach approximately 10^11 genome copies/mL.

2- SEROLIGIC ASSAYS:

- **Serologic tests are blood tests that look for antibodies in your blood. They can involve a number of laboratory techniques. Different types of serologic tests are used to diagnose various disease conditions.**

-Serologic assays based on **antigens** of parvovirus B19 are used to measure **antibodies.**

- VP2 in virus particles appears to be as the optimal antigen for the antibody detection.

→ Detection of B19 **IgM** antibody is indicative **of recent infection**; it is present for 2–3 months after infection.

→ B19 **IgG** antibodies are indicative of **past infections.** IgG antibodies are produced against **conformational epitopes** on VP1 and VP2, and they persist for years ((although antibody responses against **linear epitopes** decline within months of infection.))

A **linear epitope is an epitope that is recognized by antibodies by its linear sequence of amino acids; In contrast, most antibodies recognize a conformational epitope that has a specific three-dimensional shape and its protein structure. (reread the paragraph above)**

SO, parvovirus has conformational epitopes on VP1 & VP2 that can last for years → these epitopes are recognized by IgG antibodies of the immune system → this means that detecting high igG in blood is an indication of **past infection** of the patient.

Note that Antibodies may not be found in immunodeficient patients with chronic B19 infections. In those patients, chronic infection is diagnosed by detecting viral DNA.

3- Antigen detection assays: can identify high-tittered B19 virus in clinical samples. For example, Immunohistochemistry has been used to detect **B19 antigens in fetal tissues**

and bone marrow. This method depends on detecting antigens found on VP2 in the capsid of the parvovirus.

*** B19 and bocaviruses are difficult to grow in cultures. also, Virus isolation is not used to detect infection.

To summarize, there are 3 ways to diagnose parvovirus infections, either by detecting the viral DNA (by PCR tests or hybridization) OR by serologic assays that detect high amounts of antibodies in blood (IgM for recent infections &IgG from past infections) OR by detecting viral antigens (which are found on VP2).

***Note:** High levels of IgG antibodies in babies may indicate that IgG antibodies have been transmitted from the mother. (Not because of a past infection)

Epidemiologic tests :

-The B19 virus is widespread. Infections can occur throughout the year in all age groups.

-Up to 60% of all adults and 90% of elderly people are **seropositive**. (Giving a positive result in a test of blood serum)

-B19 infection is transmitted via the respiratory tract mainly. Transfer among siblings and children in schools and daycare centers is the **main** path of transmission.

-Many infections are **subclinical**. (with no symptoms)

** Whereas patients with **aplastic crisis** are likely to be infectious **during** the course of their illness, patients with **fifth disease** are probably **no longer** infectious by the time of **onset** (beginning) of rash.

-The epidemiology of human bocavirus is not known. It has been found in young children and appears to be global in distribution.

Treatment, prevention, and control:

- Fifth disease and transient aplastic crisis (TAC) are **treated symptomatically** (treats only the symptoms, not its cause). TAC may require blood transfusion therapy.

- Commercial immunoglobulin preparations contain **neutralizing antibodies** to human parvovirus; they can sometimes improve persistent B19 infections in **immunocompromised** patients and in those with **anemia.**

Neutralizing antibody: **it is an antibody prepared to defend a cell from an antigen or infectious body by neutralizing any effect it has biologically.**

- **Unfortunately:**

There is no treatment for human bocavirus infections.

There is no vaccine against human parvovirus.

There is no antiviral drug therapy.

***Sorry if there were any mistakes, and please Do not hesitate to ask us about your questions ☺**

Remember always that you chose this path, and you have to complete it...

GOOD LUCK ☺