1

Microbiology

Doctor 2018 | Medicine | JU

◉ Sheet ○ Slides

Done By

Moh Al-Raymoony

Contributed in the Scientific Correction

Abdullah Herzallah

Contributed in the Grammatical Correction

Abdullah Herzallah

Doctor

Belal Azab

Virology

So, what is virology, and what is a virus?

Virology is the branch of science that deals with the study of viruses.

A virus is an infectious agent, obligate intracellular parasite (they can't live or reproduce outside their host cell), compromising genetic material (DNA or RNA) surrounded by a protein coat(capsule) and/or a membrane(viral envelope).

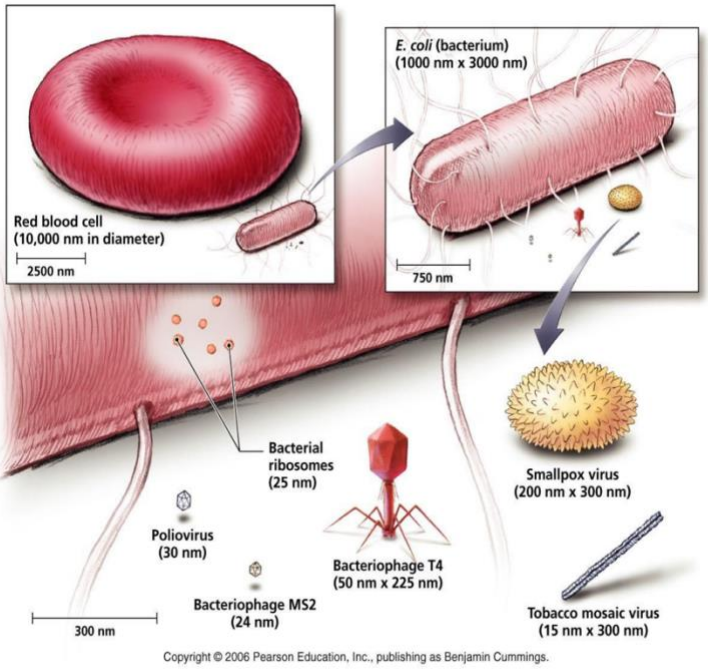
> Bacteria can live in a culture without a host cell whilst viruses can't

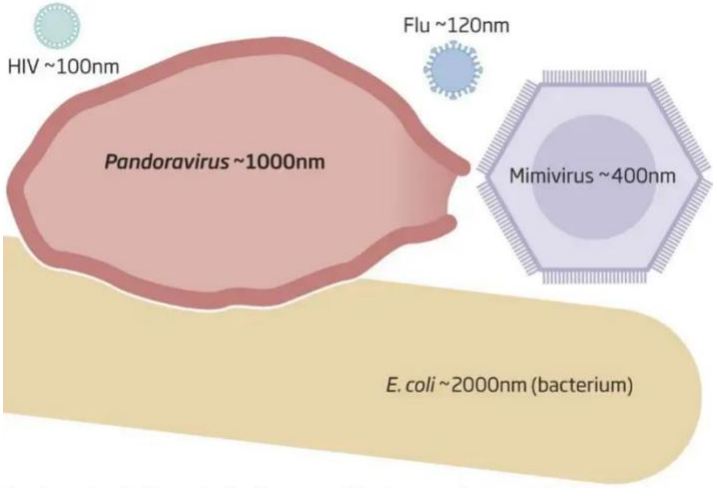
How small is a virus?

Viruses are very small (they are measured in nanometers) and we can only see them using an electron microscope unlike tissues and cells which we can see using a light microscope. However later on some large viruses were discovered like the Mimi virus (400 nm) and the Pandora virus (1000 nm) and we can see them using a light microscope (an exception)

> Note:
>
> Pandora virus is bigger by far than any other known viruses, and rivals' bacteria.

In order to visualize how small viruses are, look at these figures.





Why do we study viruses?

- Viruses are everywhere
  Viruses are found everywhere, in our bodies, in the food we eat, and in the air we breathe.

- Viruses infect all living things.
  They can infect virtually everything, from the smallest bacteria to the human body.

- Viruses cause human diseases
  So, studying them helps to study and treat these diseases.

- Viruses can cross species boundaries.
  Meaning that they can adapt to new species infecting them (e.g. Corona Virus it can infect humans though it comes from camels).
  However, some viruses can infect only certain species like the bacteriophage which only infects bacteria.

- Viruses "R" us
  Meaning they are parts of our body; viruses' genomes are part of the human genetic material. And surprisingly the viral DNA that integrated in our genetic material throughout the course of evolution is more than the amount of protein coding DNA in our genome (The exons are only 1.5% of our DNA while the retroviral DNA contribute to about 8% of our genome)

- Viruses are valuable tools in studying and manipulating biological systems.
  Nowadays scientists are using viruses to cure some genetic diseases (like cystic fibrosis and Retinitis pigmentosa), by putting the normal gene inside the virus and infecting the target tissue with that virus, then the virus will deliver that normal gene to compensate for the mutated gene inside patients.
  And it's already working! e.g. Adeno-associated virus (AVV) is approved by the FDA to deliver gene (RPE 65) for patients with Leber Congenital Amaurosis (LCA eye disease)
  To sum up viruses can be used as delivery vehicles for gene therapy.

Virus classification

Virus classification is the process of naming viruses and placing them into a taxonomic system. Similar to the classification systems used for cellular organisms (the classical hierarchical system:
kingdom→Phylum→Class→Order→Family→Genus→species)

- The international committee on taxonomy of viruses (ICTV), stated that: "A species is a monophyletic group of viruses whose properties can be distinguished from those of other species by multiple criteria".

- Viruses don't obey the classical hierarchical system exactly, it's just slightly different, the **viral classification** starts at the level of Order and continues as follows with the taxon suffix in *italics*:

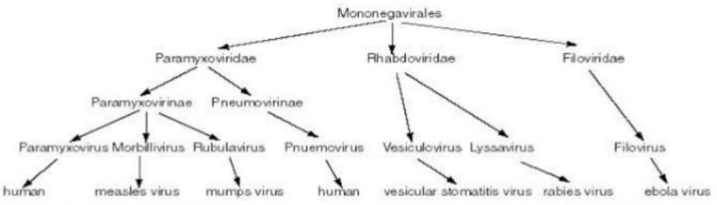
Order *(-virales)*
Family *(-viridae)*
Subfamily *(-virinae)*
Genus *(-virus)*
Species (species names generally take the form [disease] virus).
Species can also show different genotypes

An example (not to memorize):
Family →Filoviridae
Genus →Ebolavirus
Species → Zaire Ebolavirus

Further example, just to understand the general idea of viral classification (not to memorize)

Virus taxonomy

Order	*virales*	e.g *Mononegavirales*	
Family	*viridae*	e.g. *Orthomyxoviridae*	*Herpesviridae*
Subfamily	*virinae*	e.g.	*Alphaherpesvirinae*
Genus		e.g. influenzavirusA	Simplexvirus
Species		e.g. influenza A virus	human herpesvirus1
Informally:			
Type		e.g.	herpes simplex virus 1
Strain		e.g. influenza A/PR/8/34	SC16

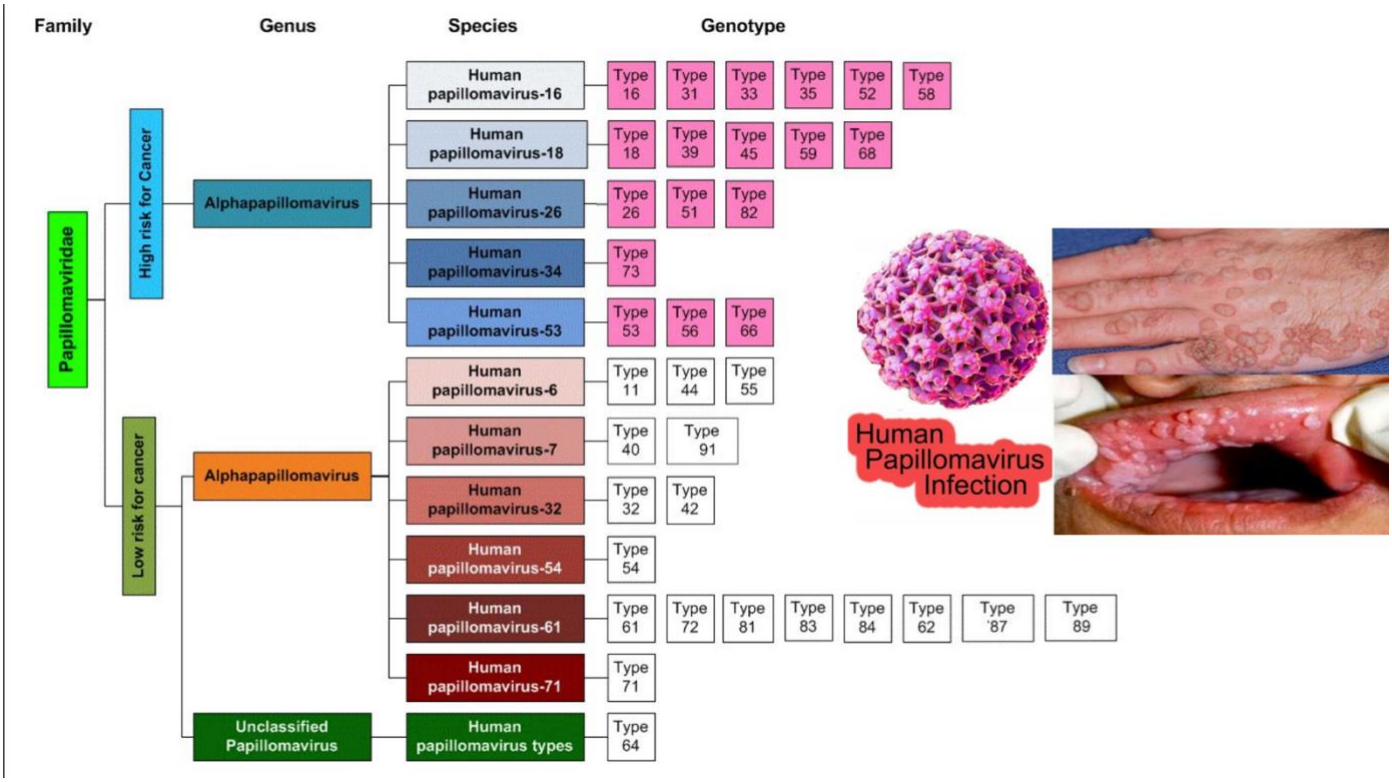


In biology, binomial names are used. e.g *Rattus rattus, Saccharomyces cerevisiae*
In virology, this does not happen:
Tobacco etch potyvirus sounds OK
Influenza A influenzavirus A does not!
Bacteriophage have their own rules

NOTE.
The doctor said there will be a question about the viral classification in the exam, that doesn't mean you need to memorize examples. However, you should know what suffix belongs to what level of classification, for example when you see "alphaherpesvirinae" you should know that it is a subfamily name (because of the suffix *-virinae).*

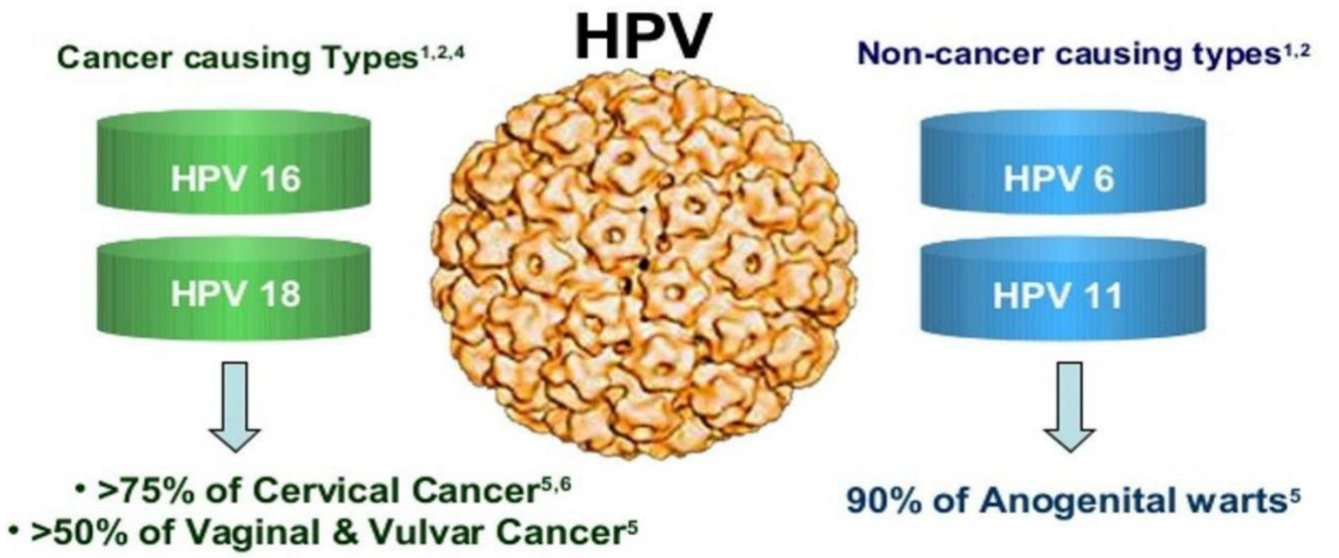
Another example to understand the classification is the Human papillomavirus family

the Human papillomavirus (HPV)



This is not for memorizing, you should only know these points

- HPV is a necessary cause of cervical cancer – 99.7%
- There are 2 genera for the virus one with high risk for cancer and the other with low risk for cancer (the non-cancer genus are responsible for the anogenital warts).
  a- Cancer causing types → HPV16, HPV18 → cervical and vaginal cancer
  b- Non-cancer causing types → HPV 6, HPV11 → Anogenital warts



So now let's move to the important question

How infected are we?

- we are all infected (even the healthy ones) by many types of viruses

-for example, every one of us is infected by some kind of the Herpes virus. We even have at least 2 of them inside our bodies, as there is: (we will discuss what's in bold)

-Herpes-simplex I (HSV-1) **- Herpes-simplex II (HSV-2)**

-Varicella zoster virus (VZV) **- Epstein-Barr virus (EBV)**

-Cytomegalovirus (CMV) - Human herpesvirus 6 (HHV-6)

-Human herpesvirus 7 (HHV-7) - Human herpesvirus 8 (HHV-8)

-We got the Herpes virus quite young in age, most likely from our parents, through their saliva, when they used to kiss us.

-once infected with herpes you are infected for life (there is no cure for Herpes), even if the symptoms are gone you still have the virus.

1 -Herpes Simplex Virus (HSV)

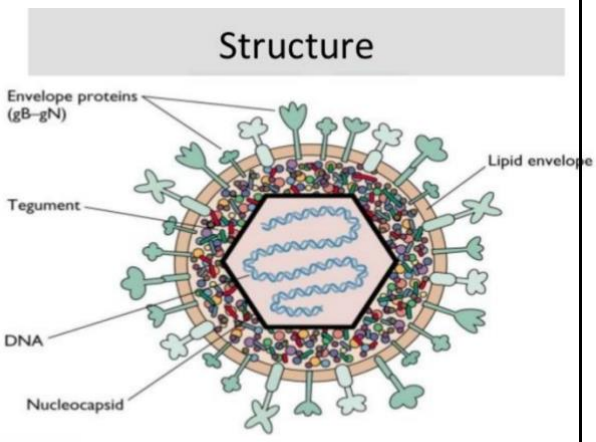
-HSV is a double stranded DNA surrounded by a protein coat (capsid) virus that belongs to Herpesviridae family.

-It contains 3 main structural components:

a- Central core: It holds the viral DNA.

b- Inner core: Surrounded by an envelope (a phospholipid bilayer; made of viral glycoprote spikes) and a capsid.

c- The tegument: Space between the capsid and the envelope.

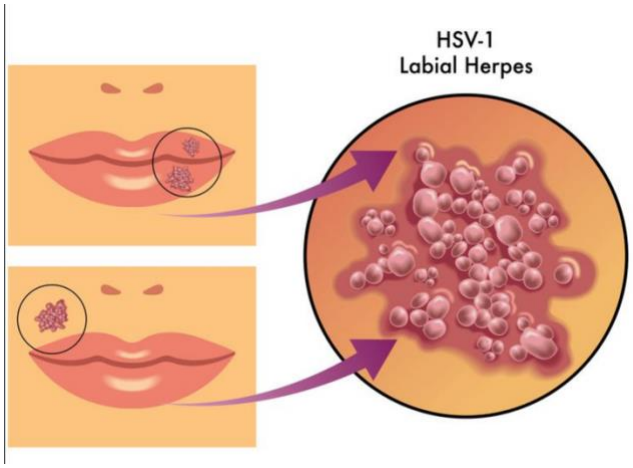


-Various proteins are delivered into the infected cell upon cell fusion.

There are 2 types of Herpes Simplex virus

a- Herpes Simplex virus 1 (HSV1)

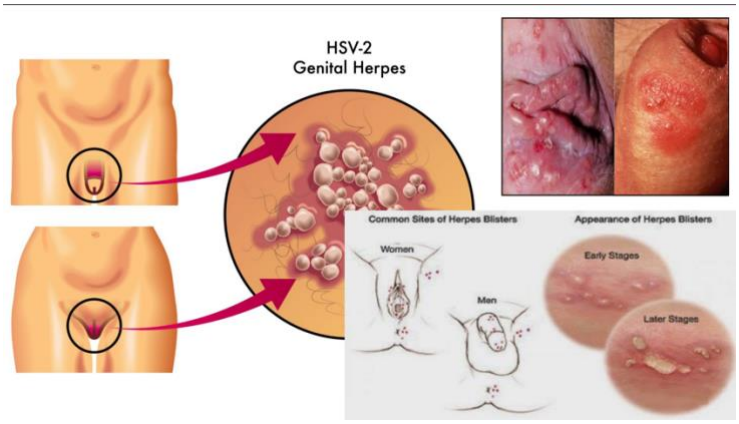
-Known as Labial or oral herpes, and it causes sores and fever blisters around the mouth and on the face.



b- Herpes Simplex virus2 (HSV2)

-Responsible for genital herpes and causes blisters and sores on the sex organs of both males and females.

- HSV1 may contribute rarely to genital Herpes.



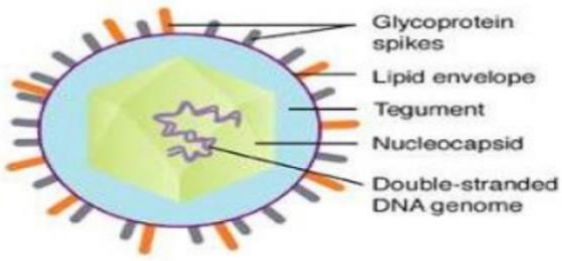
2 -Varicella Zoster Virus (VZV) (HHV3)

-VZV also known as Human herpes virus 3 (HHV3), it belongs to the herpesvirus family.

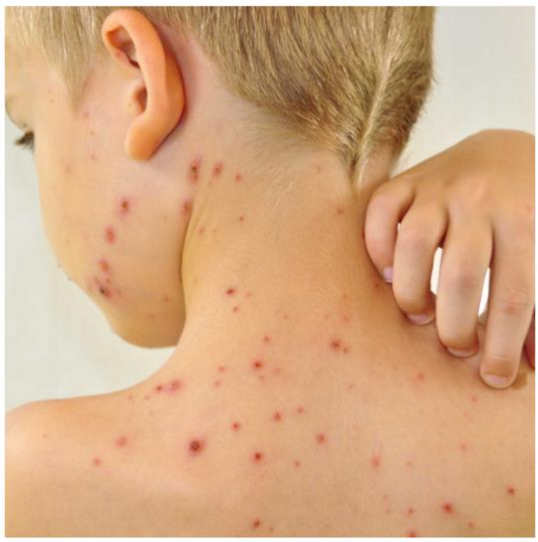
-The envelope is interspersed by spikes made up of viral glycoproteins.

- The VZV genomes is double stranded DNA coiled upon a protein axis.

- It causes chickenpox.



Structure of the varicella-zoster virus particle



3 -Epstein-Barr Virus (EBV) (HHV4)

-Family: Herpesviridae.

-Host: Humans

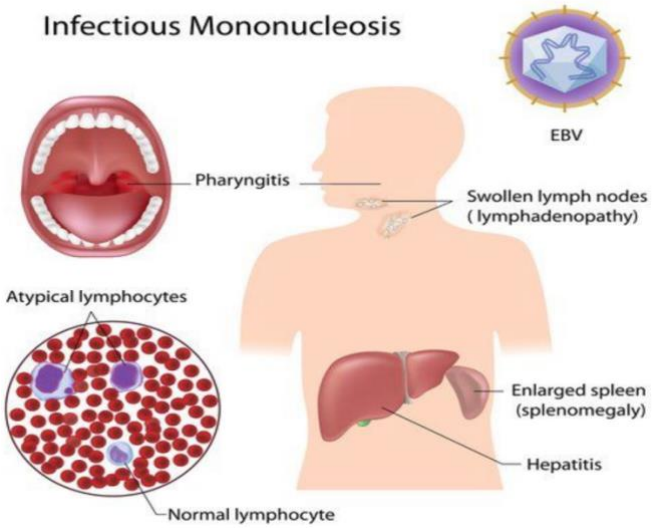
-It is enveloped, Icosahedral (20 faces).

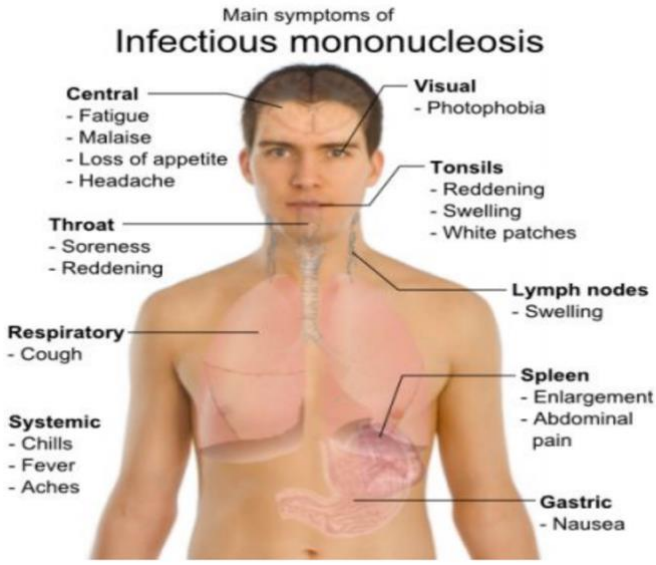
- it's very small

-Genome: Double stranded DNA, linear and made up of 125-240 kbp (no need to memorize the numbers just know that the DNA is really small)

-EBV causes infectious Mononucleosis, also known as Mono, and it's also called the kissing disease (cough love)because it spreads through kissing (especially in kids).

- Mononucleosis affects many systems and has many symptoms throughout the body displayed in the figures below





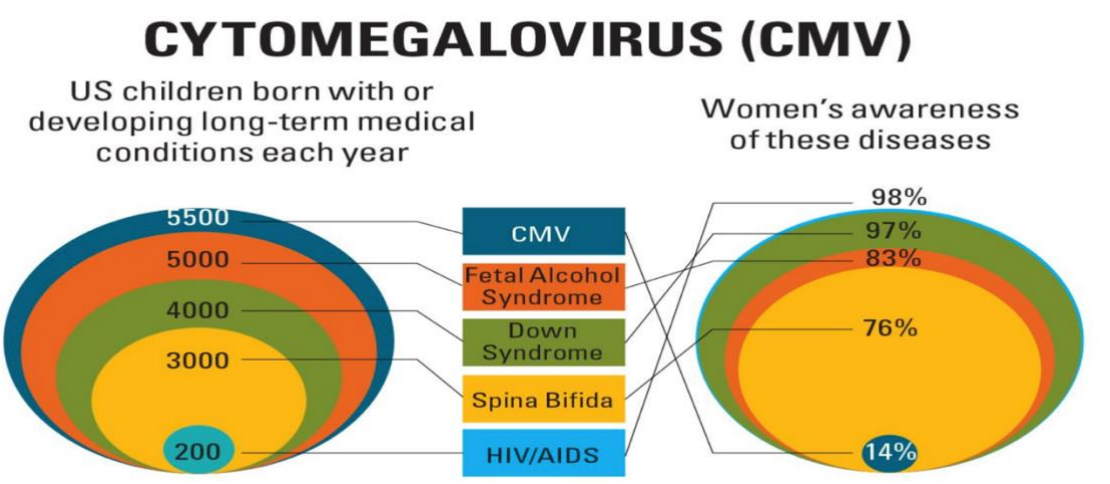
4- Cytomegalovirus (CMV) (HHV5)

- CMV (from Greek cyto- "cell", and -megalo "large") is a viral genus from the family Herpesviridae (or Herpes viruses).

- The species that that infect humans is commonly known as human CMV (HCMV) or human herpesvirus-5 (HHV5) and it is the most studies of all cytomegaloviruses.

CMV is very serious because
1- every hour one child is permanently disabled by CMV and more than 400 children die every year because of CMV
2- It is a leading non-genetic cause of childhood hearing loss.
3- CMV can also cause: Vision loss, mental disability, microcephaly, behavior issues and seizures.
4- 90% of children born with CMV appear healthy at first

- Even with all these serious complications of CMV it has very low awareness between women



Microbiome

So, what is microbiome?

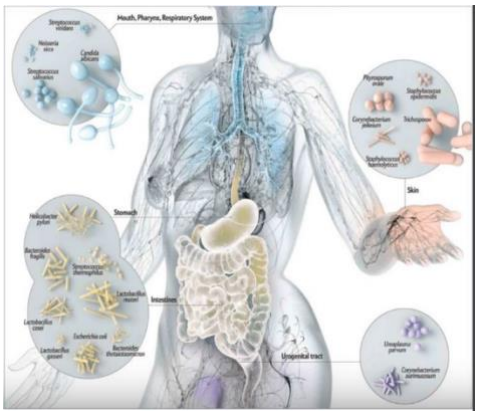
Microbiome: bacteria that colonize every organ system of the body. (the genetic material of all the bacteria that live on and inside our bodies)

How can we discover the microbiome which lives on us?

-Using next generation sequencing (NGS) on a biopsy from any part of the human body will show some sequences that belongs to bacteria, and by sequencing the bacterial ribosomal RNA we can determine what type of bacteria lives on that part of the body , and by doing that we came to know that the bacteria living on your skin for example differ greatly from one person to another, and even from the right to left hand.

-This mechanism is cheap and easy because we don't need to sequence the whole genome to identify the type of bacteria, sequencing the bacterial rRNA would be enough.

-This figure shows the bacteria that inhabit some parts of our body



Virome

- like the microbiome, the virome is the total collection of viruses on and inside the human body

-Even though it is as important as microbiome it doesn't get the same attention. Maybe because it is harder to enumerate(sequence) the virome.

- To study microbiome all you need to do is to extract some nucleic acid from the sample (for example from skin swap or stool sample) after extracting the nucleic acid, you sequence the rRNA, then you will have an idea about what bacteria inhabit the sample.

- The case with the virome is different, because viruses don't have a common sequence (like the bacteria have rRNA) to identify the viruses in the sample, so the Whole genome must be done to identify the viruses in the sample.

The experiment

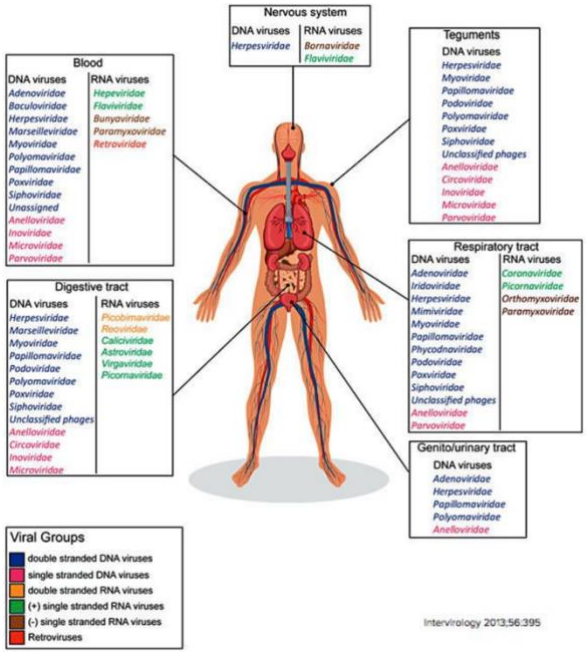
- Scientists took human **blood** from a healthy person and extracted nucleic acid from it and did very high throughput sequencing (NGS).

-The sequencing gave a good idea about the present nucleic acid, and the figure here represent the results from that sequencing.

The results

- 67.7% of the sequences in the blood are viral.
- 9.5% of the sequences are bacteria.
- Only 3.6 % of the sequences are for eukaryotes including human DNA.
- The way we identify the sequences is by comparing it to a database.

- This figure is a more detailed study of the human virome in every part of the body (not only in the blood).
- There's a unique set of viruses that are listed here.
- DNA and RNA viruses at each of these locations.
-These viruses can be found in healthy humans.
- There is a huge virome in the human body and it is always changing.



(the viruses in the picture are not for memorization)

So, the question here, what is the purpose of all these viruses swimming around our body?

The answer is simple, we don't know yet, it's not simple to know whether the virome is beneficial or not.

In the case of bacteria, it's an easy task you can just wipe out all the bacteria with antimicrobials and see what happens, unfortunately this not the case for viruses we don't have that many antivirals that will simply wipe out your virome, it's a hard task as we said but we are moving one step at time, the following study gave scientists an idea about the role of the virome.

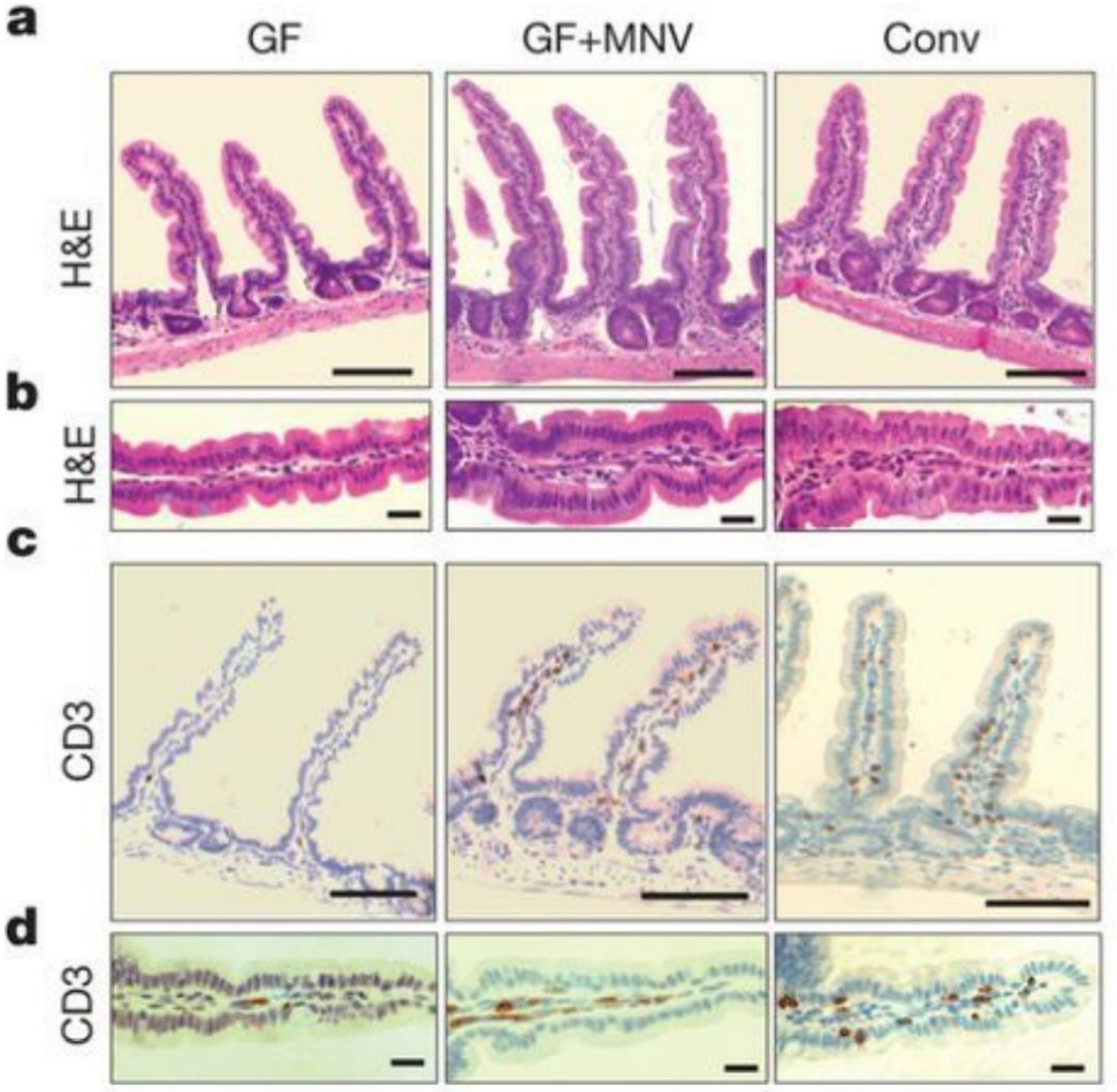
The study on mice

Intestinal microbial communities are known to have profound effects on healthy host physiology. Whereas the role of viruses that are present in the gastrointestinal tract is undefined.

What scientists did is they took a group of mice and heavily infected them with antibiotics to the point they lost their bacteria and became germ free (gf) then they looked at healthy mice and compared the microvilli between the germ-free mice and the healthy mice, and found that the microvilli morphology was disturbed, furthermore using different stains they deduced that the lymphocytes function was lost.

After that they made a third experiment with GF mice infected with a virus called **Murine Norovirus (MNV)**

And the results demonstrated that a common Murine Norovirus (enteric RNA virus) can replace the beneficial function of commensal bacteria in the intestine and restore intestinal microvilli normal morphology and lymphocyte function without causing inflammation and disease. This indicates that eukaryotic viruses have the capacity to support intestinal homeostasis and shape mucosal immunity, similarly to commensal bacteria.



LTR-Retrotransposons & Human endogenous retroviruses (HERVs)

NOTE.
This topic is a bit hard to digest but the doctor went through it fast, so you don't need to know the details just understand the main idea

Some terms before we start (just read them)

-Transposons: are DNA sequences that can change position within a genome (without using the reverse transcription)

-Reverse Transcriptase: It is an enzyme used to generate complementary DNA from an RNA template and vice versa, this process is termed as reverse transcription.

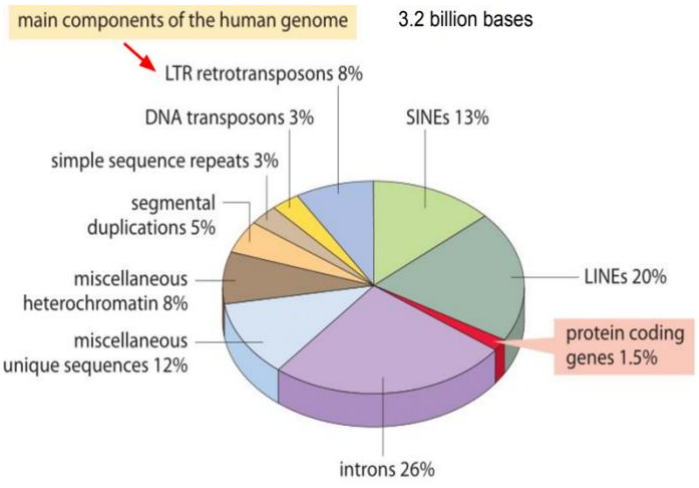
-Retrotransposons: are DNA sequences that replicate within the genome using the reverse transcription method (the DNA is transcribed into mRNA then the mRNA is reversely transcribed to DNA in another position)
it's called retrotransposons because there is an RNA intermediate

- **LTR-Retrotransposons** are retrotransposons characterized by the presence of Long Terminal Repeats (LTRs) directly before and after the coding region.

- **Retrovirus:** an RNA virus that inverts its genome into DNA and then integrates it into the infected cell, they use the reverse transcription method in their working mechanism e.g. HIV.

Now let's start by talking about the **Human Genome**

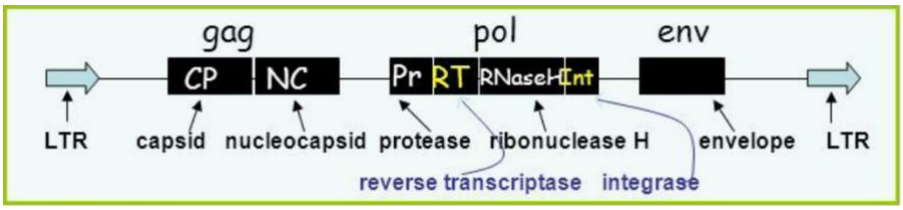
- 3.2 billion bases and it is divided up into the different parts that is shown in the pie chart.
- Protein coding genes are 1.5% of the DNA. (the minority of the DNA actually encodes proteins)
- The rest of the genome is all sort of stuff, like introns, heterochromatin, duplicated sequences, interspersed elements like lines and signs.



8% of the genome is composed of LTR retrotransposons, but only 1% of them has a structure similar to retroviruses, while all the rest are degenerated.

Now what do we mean by "similar structure"?

If you look at the structure of the LTR retrotransposons you will find the same genes that retroviruses have, with the genes coding for the capsid formation and genes coding for the reverse transcriptases, envelope, virus proteins, etc. all inside our genome! that's why they are called Human Endogenous Retroviruses(HERV).



gag: capsid (structural element)
pol: polymerase: reverse transcriptase, integrase, protease, RNase H
env: envelope (structural element)
LTR (long terminal repeat): promoter

Where did these HERV come from?

- Endogenous retroviruses (ERVs) descend from ancient infectious RNA viruses containing a reverse transcriptase gene (found in all retroviruses), which converts RNA into DNA. Then, it is integrated into the chromosomal DNA of the host animal.
- The integrated viral genomes have been vertically inherited for tens of millions of years through generations, having varying degrees of mutations and deletions.
- Human endogenous retroviruses (HERVs) are footprints indicating previous exposure to retroviruses, thus they are named as Fossil Viruses. HERVs constitute approx. 1% of the human genome. - They have similar genomic organization to exogenous retroviruses.

Since we have viruses in our DNA why aren't we infected with them?

The reason is that all the Human Endogenous Retroviruses are mutant; therefore, unable to form infective virions. While in other species like monkeys and chimps they contain infective Retroviruses.

-But even if they're not infecting us it turns out that many of the viral proteins have been exapted.

Exapted protein means you take a virus protein and use it to your own purposes.

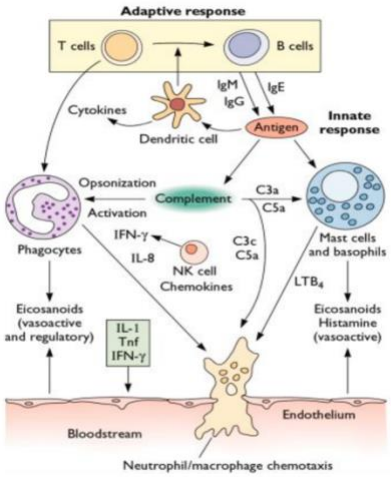
-We have exapted a number of retroviruses genes for our own use over the years

So, dealing with that number of viruses, how come we aren't infected?

Amazingly, the vast majority of viruses that infect us have little or no impact on our health or wellbeing. Why?

1- Most viruses that pass through us "ingest" regularly with food, are non-animal viruses. Metagenomic analysis in human feces revealed that most viral sequences are like plant viruses; 91% of the obtained sequences are for plant viruses.

2- We have an amazing immune system. This figure shows the work of the immune system (just understand). The immune system takes care of any viral infection protecting us.



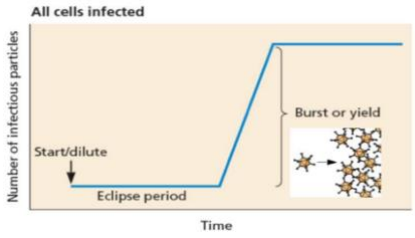
Viruses' replication

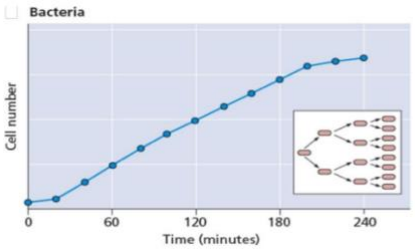
Viruses replicate by assembly of pre-formed parts into many particles. (Parts formed→Assembled into final product)

As the graph shows, viruses go through two phases:

1- **Eclipse phase**, Viruses parts are built like DNA.

2- **Burst or yield phase**, Viruses are assembled.





NOTE.

- Unlike bacteria viruses do not replicate through binary fission, which explains the different curves

- notice the steep increase in the virus replication curve (the Burst phase)

How old are viruses?

Estimates of molecular evolution suggest marine origin of some retroviruses more than 450 million years ago in the Ordovician period.

So, does this mean that viruses appeared before cells?

viruses most likely originated before cells, which can be true since they are simpler in structure, but it's still an ongoing debate (what originated first).

Virus classification

There are many ways to classify viruses, and they can be classified according to:

- Nature and sequence of nucleic acid in virion(virus)

- symmetry of protein shell (capsid)

- presence or absence of lipid membrane (envelope)

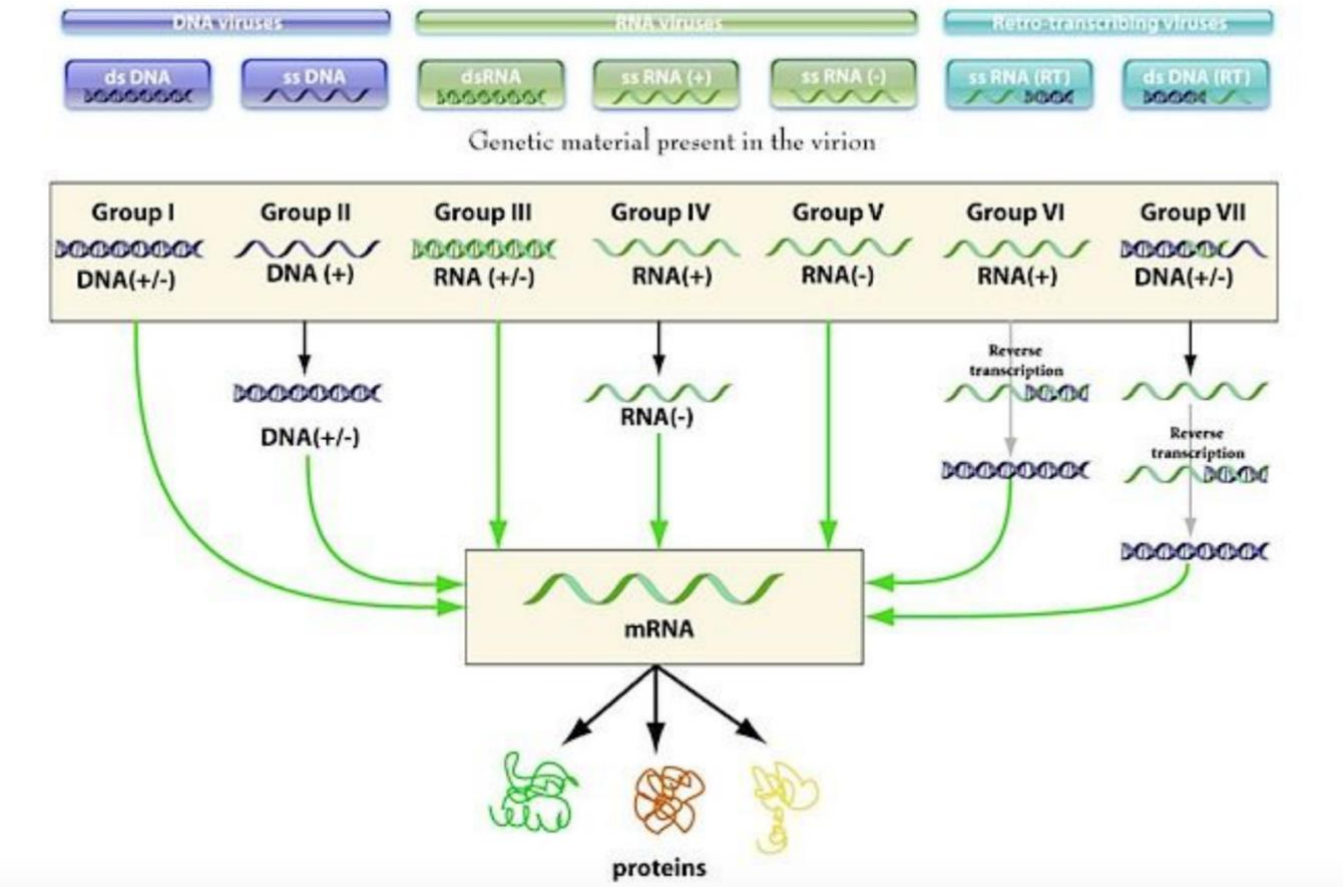
-Dimensions and faces of virion &capsid

We have already talked about viral classification and the taxonomic system (ICTV) before, now another way of classifying viruses is based upon their genetic material and it's called the Baltimore classification
According to the Baltimore classification viruses are arranged into 7 groups

Group I: Double stranded DNA viruses (dsDNA).
Group II: Single stranded DNA viruses (ssDNA).
Group III: Double stranded RNA viruses (dsRNA).
Group IV: Single stranded RNA viruses (+ ssRNA). +/- signs will be discussed later.
Group V: Single stranded RNA viruses (- ssRNA).
Group VI: Single stranded RNA viruses,(ssRNA) but with a reverse transcriptase gene.
Group VII: Double stranded DNA viruses (dsDNA), but with a reverse transcriptase gene.

Note. You should memorize the following table

Class	Nucleic Acid	Examples
I	dsDNA	Herpes virus Poxvirus Adenovirus Papillomavirus
II	ssDNA	Adeno-associated virus
III	dsRNA	Reovirus
IV	(+) ssRNA	Togavirus Poliovirus Foot-and-mouth disease virus Hepatitis A virus Hepatitis C virus
V	(-) ssRNA	Influenza virus
VI	(reverse) RNA	HIV
VII	(reverse) DNA	Hepatitis B virus


DNA viruses
RNA viruses
Retro-transcribing viruses
ds DNA
ss DNA
dsRNA
ss RNA (+)
ss RNA (-)
ss RNA (RT)
ds DNA (RT)
Genetic material present in the virion
Group I
DNA(+/-)
Group II
DNA (+)
Group III
RNA (+/-)
Group IV
RNA(+)
Group V
RNA(-)
Group VI
RNA(+)
Group VII
DNA(+/-)
Reverse transcription
DNA(+/-)
RNA(-)
Reverse transcription
mRNA
proteins

GOOD LUCK.