Microbiology

◉ Sheet ○ Slides

Number : Virology-3

Done by : Sinamis Drei

Corrected by: Abdullah Qaswal

Subject: Viral replication

Doctor: Ashraf khasawneh

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بسم الله الرحمن الرحيم

In this lecture , we are going to talk about virus replication , so there is some terminology we have to go over . More details will be included in the sheet :

- **Plaque forming unit (PFU) :**

  a measure of the number of particles capable for forming plaques per unit volume such as virus particles .
  so , this test is used to see the amount or ***the number of viruses*** that are capable for infection in the first cycle .

But, how is that possible ?

we said that viruses are ***obligate intracellular organisms*** , they need cells to replicate . Thus , you take a sample that you think contains a virus and put it into a cell culture and leave it for , let's say , 12 to 24 hours and then you harvest or take out the media , because if you leave them for a long time , viruses will be produced and replicated and released bake into the media and they're going to , once again, infect the other cells , so you are going to end up with all the cells that are infected by the viruses . however , we want to test the amount of infectious viruses in the sample you have(only) , so you put the sample in the media and after a short period of time you take it out , you just allow for the period of ***adsorption and penetration of the virus into the cell .***

As mentioned earlier , viruses can only grow within cell culture , this cell culture can be either done in a **cell culture flask** which is made of plastic or **cell culture plate** .

- you put the cells and leave them for short period of time (1 to 2 hours) in order for the cells to settle and attach to the plastic , then you add the sample you think that the virus is present in and leave for 1 to 2 hours and then you wash and add a fresh media **or** you wash and add a semisolid media , agarose gel on top of it .
- ***What is the idea behind using a semisolid media (Agarose) ?***
  Because as we said , when using a liquid medium , the virus is going to replicate and be released into the liquid and then travel and infect the other cells .

- Then , you leave it for 6 to 12 hours and you end up with dots (faint dots) , which represent the number of killed cells and also the amount of viruses that were infectious viruses in your sample.

  So briefly , if you leave the viruses for a long time or use a liquid media , the virus is going to replicate and be released from the cell to the media and start a second round of replication , by attacking other cells , but you only want to detect the infectivity of viruses in your sample (viruses that you have put) in the first round , so you shouldn't do that .

Multiplicity of infection (MOI) :

The *ratio of infectious agents to infection targets (the ratio of viruses to cells) , this is applicable in both labs or even human body .*

- ***So ,when we say that the MOI value is 10 , what does that mean ?***
  It means that 10 viruses were added to 1 equivalent cell , you put ***1000*** *cells in the flask and added* ***10000*** *viruses to them , for example .*

Viral replication terminology :

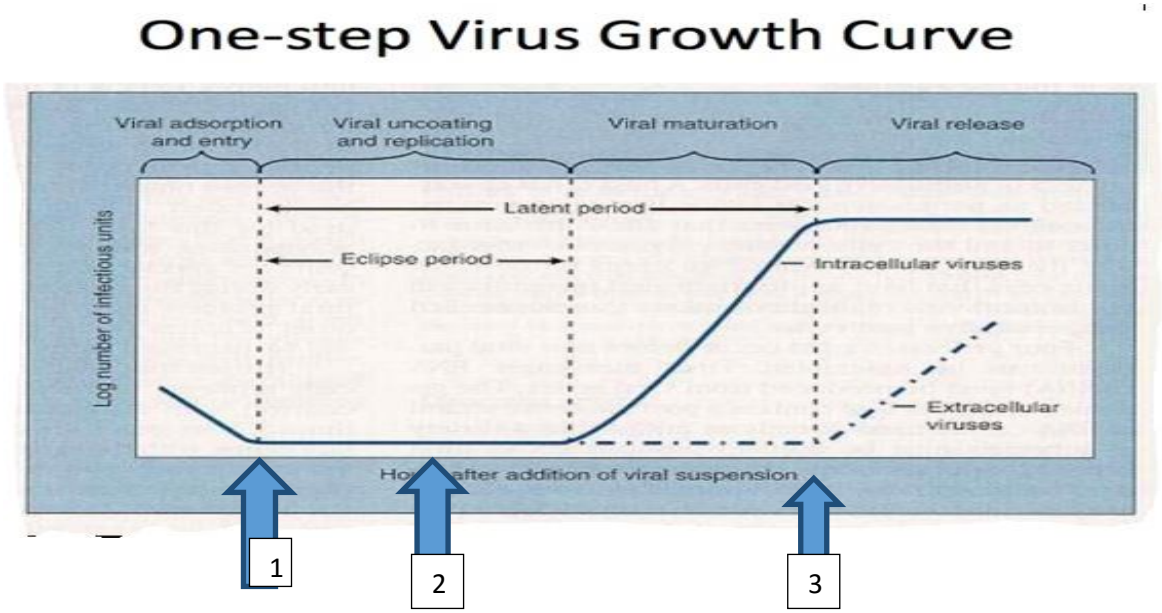
Eclipse phase : the period during which the input virus becomes uncoated (10 to 12 h) .

Synthetic phase : the period during which new virus particles are assembled (4 to 6 h) .

latent phase : the period at which no extracellular viruses can be detected .

Burst size : the amount of infectious viruses produced per infected cell .

To make things more clear and understandable , let's look at the figure that shows the **One – step growth curve (represents the infection of one cell) :**



The arrow(1) : *represents the zero time when the virus inters the cell .*

The arrow (2) : *we have an eclipse period . we said that the simplest definition of virus replication is* ***disassembly and reassembly*** *. so, during the eclipse period , the number of viruses inside the cell is Zero . how come ? the virus has just entered !*
The virus is going to disassemble(dismantle) , so if you are to look to the number of viruses during this time , no viruses will be found .

After the eclipse period , what happens is the replication of the genome and the expression of viral proteins .

***In the latent period** , we have assembly of the virus , but we are still talking about inside the cell (intracellular) . so ,the line pointed(arrow 3) is the margin between the presence of the virus inside the cell and outside the cell .*

***Latent period includes the eclipse period** , the synthetic period where there is replication of genome and expression of viral proteins and it includes the assembly of the virus inside the cell .*

So , does the figure represent a naked or enveloped virus ?
Naked virus** is released from the cell by cell death or lysis , so all the viruses are going to exit the cell all at the same time . on the other hand , **enveloped viruses** will start to exit the cell acquiring the envelope of the virus from the cell membrane , the cell can compensate for this loss of the cell membrane by regenerating it until it reaches a point at which no more compensation is beneficial and it dies . **and so , it's an enveloped virus .

- *So back to the figure ,the number of viruses will be increasing until reaching a plateau , this plateau is called the **burst size** , if a cell is infected with one virus and produced 200 viruses , then the burst size is 200 and so on . it differs from one virus to another , hepatitis C is a virus that has one of the largest burst sizes .*

- ***Another general rule from the figure :** the **first thing** to be translated are t**he non - structural enzymes** (in the early stage of protein synthesis) , and **lately** you have the **structural enzymes** translated .nucleic acids and late protein synthesis may occur at the same time . certain viruses like herpes virus have **3 distinct phases** : immediate early , early , and late (here we talk about protein synthesis) .*

  So briefly :
  Early protein synthesis : non-structural enzymes and proteins .
  Late protein synthesis : structural enzymes and proteins + replication of the genome .

***Virus replication can be divided into 8 stages**(the number is not that accurate) :*

- *NOTE :This doesn't mean that they're all distinct , but we will talk about them as if they were detectable as distinct stages in viruses .*

- *The first step is **attachment or adsorption** followed by the **penetration or entry** of the virus into the cell followed by **uncoating** which contains the disassembly of the capsid and the release of the genetic material into the cytoplasm and then we have the **synthetic phase** where the genome is going to be replicated and proteins are going to be synthesized early and late followed by the assembly of the virus then the virus is going to leave the cell in a step called **release** and the last step is **maturation** , which can occur to the virus within the cell or after the virus is released , maturation step is just a matter of time , when it occurs inside the cell , it requires cleavage by certain enzymes and if it occurs after release , it*

will require further modification for the viral proteins by certain enzymes , and the virus will be totally infectious and mature and we call it the virion .

- ***So once again , the steps are :***

1. Adsorption or attachment .

2.penetration and entry of the virus .

3. uncoating

4. replication of the genome and synthesis of proteins .

5. assembly

6. release and maturation .

- *When we talk about the adsorption or the attachment step , we need to talk about the enveloped and the naked viruses .*

Virus attachment consists of specific binding of a viral attachment protein to a cellular receptor , target receptor molecule can be a protein .

- ***What do we need to know about the adsorption or the attachment step ?***
  First of all , we need to know how the adsorption of the virus to the target cell occurs ? Do cells attract viruses by producing certain substances so that viruses come and attack them? Or does the virus have certain proteins that can detect the cell ?
  The answer is no .it ***occurs as a random collision*** *, depending on the number of viruses and cells .*
  The higher the number of viruses , the higher the chance of adsorption or attachment of the virus to the cell .

- ## *What do we need for adsorption ?*

In case of enveloped viruses , we need an envelope - embedded glycoproteins or spikes . also , the cell has to have a cell receptor . so , the glycoprotein on the virus will attach to the receptor on the target cell .

- ***Is one viral glycoprotein or spike only present on each virus ?*** *No. viruses may have one glycoprotein* ***or*** *they may have a* ***complex glycoprotein*** *made of two or three units* ***or*** *they may have two glycoproteins but one is embedded within the other like HIV virus it's made of two : GP120 and GP41 , once the GP120 binds to the CD4 on the CD4 cells , GP120 is going to fall off exposing the GP41 and then will continue the penetration of the virus to the cell . also , in HIV , the cell receptor and the glycoprotein alone* ***are not enough*** *for the entry of the virus , it needs a* ***co-receptor*** *which is a* ***chemokine co-receptor*** *which is either CCR5 or CRCX4 .*

- *Is one type of receptors enough for all viruses to enter into the cells ? The answer is no , certain viruses interact with 3 to 4 receptors on the target cell , Hepatitis C , for example,*

interacts with 3 to 4 receptors on the target cell in order to allow for the entry of the virus into the cell .

- ***How many Glycoprotein-receptor complexes are required to initiate the entry of the virus into the cell ?or is one glycoprotein-receptor complex enough to initiate the entry of the virus into the cell ?***

***The** answer is no , it requires from **3 to 5 glycoprotein-receptor complex** to initiate the entry of the virus .*

- ***What about the penetration step ?***

What occurs during the penetration step is an approximation between the envelope of the virus and the target cell membrane and fusion of the viral envelope with the cellular membrane .

- ***Host Range :***
  The collection of hosts that the organism can utilize as a partner .what do we mean by that ?
  host range means does the virus infect horses or birds or … , an example of this is influenza .
  *so , the host range talks about what **species** does the virus target .*

- ***Cellular tropism :*** the cells and tissues of a host which support growth of a particular virus.
  *Hepatitis virus , for example , infects the liver . thus , the tissue or cellular tropism of Hepatitis virus is **hepatocytes** .*
  Another example
  Is the Influenza virus , which infects the upper respiratory tract .
- *The cellular tropism for HIV virus is **T-lymphocytes** .*
- *Herpes virus has 8 genera which differ in their cellular tropism :Herpes simplex infects the skin , human Herpes virus 4 causes infection in the **B-lymphocytes** .*

- ***A conclusion :***

  - *a glycoprotein binds to receptor on the target cell facilitating the entry of the virus*

  - Glycoprotein-receptor complex is not enough in certain enzymes such as HIV virus , it needs a coreceptor .

  - certain viruses may have more than one glycoprotein that play a role in the attachment and penetration process .

  - Certain viruses use more than one receptor on the target cell (Hepatitis C) .

**** so , this is all about enveloped viruses , what about naked viruses ?***

*Do naked viruses have glycoproteins or spikes '? No .they have something called **surface proteins** , and certain other naked viruses don't have surface proteins , what they have are **slits or grooves** within the capsid of the virus .*

Do they still need to attach to receptors on the target cells ?yes .naked viruses have to attach to receptors on the target cell to initiate the entry of the virus to the target cell .

Are naked viruses superior to enveloped viruses in terms of evasion from the immune system ?

Maybe yes *, the immune system may not be able to get access to the slits and grooves on the capsid and thus doesn't recognize them . However , if the virus has surface proteins , it is still exposed to the immune system and can be recognized by the immune system .*

- *So , naked viruses with surface proteins on the capsid are exposed to a higher risk of being recognized by the immune system than those with slits and grooves , but still superior to enveloped viruses in terms of evasion from the immune system .*

- ***What needs to be memorized from (table 6-6) in the slides :***

  - *HIV virus requires a receptor , which is* ***CD4 molecule*** *and a* ***chemokine coreceptor which is CCR5and CRCX4 .***

    The receptor of Influenza A virus is ***Sialic acid .***

    Epstein – Barr virus has more than one cell receptor .

- *As mentioned earlier ,sialic acid is the receptor of Influenza A virus . However , this receptor can also be attached to by other viruses such as rota virus .so ,the receptor can be used by more than one virus .*

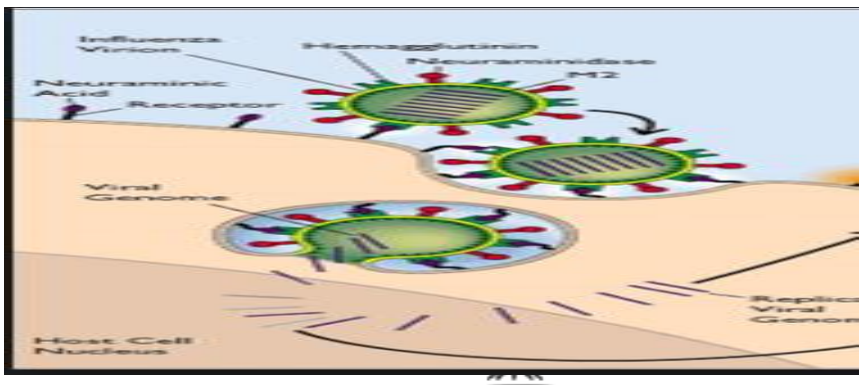
- ***Virus attachment proteins :***

  - *In HIV , it's GP120 and GP41 (as mentioned).*

  - *In Influenza A virus , it's* ***HA (hemagglutinine)*** *and* ***NA(neuraminidase)*** *,* ***but*** *the one which plays a major role in the attachment and initiation of virus entry to the virus is HA , while neuraminidase (NA) plays a minimal role in the fusion step and initiation of the entry process .*

  - *Rhinovirus has a complex glycoprotein made of three units .*

as shown in the figure to the left , there is an interaction between the sialic acid receptor on the target cell and HA glycoprotein (which has a major role in the attachment and initiation of the entry process) .



- ***Penetration***

Is the entry of the virus into the target cell .

- *Penetration of the target cell normally occurs **a very short time** after the attachment of the virus to its receptor in the cell membrane .*

- *Unlike attachment , cell penetration is **an energy – dependent process** , i.e, the cell must be metabolically active for this to occur .*

- *In enveloped viruses , it requires approximation of the viral envelope to the cell membrane and the fusion of the two membranes facilitating the penetration and the entry process .*

- ***Three main mechanisms are involved :***

- *When we talk about the fusion of the viral envelope to the cell membrane , only two mechanisms are involved :*

*1. **translocation** : by binding of the virus to the receptor of the target cell , and then the **internalization by flipping** (this mechanism is rarely seen among viruses and poorly understood)*

***2. Endocytosis :** it almost requires glycoprotein- receptor interaction , followed by **Receptor – mediated endocytosis** .*
Endocytosis occurs in both naked and enveloped viruses .

***3. Fusion :** the envelop of the enveloped virus is left within cell membrane of the target cell and the nucleocapsid is released to the cytoplasm of the cell*
So, fusion only occurs in enveloped viruses unlike endocytosis that occurs in both naked and enveloped viruses .
So the virus attaches and enter an endocytic vesicle which will later enter the cytoplasm .
*Once the endocytic vesicle is inside the cytoplasm , **how will the virus inside this vesicle escape to the cytoplasm ?***

- ***The triggering step** is a drop in the PH of endocytic vesicle .*
- *Then ..for enveloped viruses , this will lead to fusion between the envelop of the virus and the membrane of the endocytic vesicle , thus opening the vesicle and releasing the nucleocapsid to the cytoplasm .*
- *For naked viruses , drop In the PH will lead to the exposure of hidden domain within the capsid protein and these hidden domains will attach to the endocytic vesicle membrane causing the exposure of the nucleocapsid to the cytoplasm **.(protein changes in conformation due to PH changes) .***
- *Another mechanism for naked viruses , is the lysis of the endocytic vesicle and release of nucleocapsid to the cytoplasm .*

***In fusion** , the envelop is left as part of the cell membrane and the nucleocapsid enters .*

- ***Third step is uncoating** (which occurs after penetration) :*
  - *Uncoating is the disassembly or dismantle of the capsid and the genome is being released into the cytoplasm .*

- *For uncoating , we will take about DNA viruses . DNA viruses* ***replicate in the nucleus (except for pox virus which replicates in the cytoplasm).***

- *Some viruses which are too small (like parvovirus , polyomaviridia) , so they uncoat in the nucleus , so the nucleocapsid enter the nucleus through nuclear pores and uncoat there , while large viruses uncoat in the cytoplasm .*

- *All viruses can be divided into 7 viruses according to replication :*

1 .dsDNA

2. ssDNA

3. dsRNA

4. ssRNA (+)sense

5.ssRNA (-) sense

6. ssRNA (+) sense with DNA intermediate in life cycle ex: HIV .

7. partial DNA with RNA intermediate in life cycle ex Hepatitis B .

So , group 6 and 7 apply for only one virus .

Also, sdDNA apply for only one virus that is parvovirus .

Made with love

Sorry for any mistake „ Good luck all ☺

Done by : Sinamis Drei