University of Jordan
Faculty of Medicine

Medical Committee
The University of Jordan

Introduction to

Microbiology

Title :

Viral life cycle

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: 9

- ○ Slides
- ○ Handout
- ● Sheet

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In this lecture the doctor explained the following:

1-The definition of plaque forming unit and how to calculate it.

2-Some important terms in viral replication.

3-Mentioned all replication steps briefly but explained the first two steps,and the rest will be explained in the upcoming lectures.

Viral Life Cycle

Plaque forming unit (pfu) :a measure of particles capable of forming plaques per unit volume.

***pfu** is a measure of infectivity of a virus.

*How to create an appropriate medium to calculate pfu is as follows

1-place the cells in a flask or a dish and leave them for a couple of hours so that they stick to the bottom of the flask or the dish & a monolayer of cells will be formed.

2-add the virus to the cells' medium for another couple of hours.

3-translocate the media containing the virus/cells mixture.

4-wash the cells/virus mixture

*the monolayer shows how confluent cells are

(confluency is a term used as a measure of the number of cells in a culture/dish,so a 100% confluency means the dish is completely covered by a monolayer cell in our case)

*If we don’t spread cells and by spreading we mean diluting;then cells might die.

5-after translocating the monolayer to another dish with the viruses,you should cover it with a semi solid matter.(why)

in order to make sure neighboring cells won't get infected.Since we want to see the effect in cells placed in our dish.

*Not necessarily all cells would get affected ;they will be seen as pointed dots.

Check Slide number 2

shows infected insect cells of the type (sf9),it was infected by a virus ,notice that surrounding these infected cells are non infected ones painted in pink and red.

Slide 3

shows 2 dilutions of a virus one is 10 to the power -7 and the other is 10 to the power -6.(when you add **more virus** you get more cells infected ,with **more dilution** you get less cells infected)

*the plaques are less where the medium is diluted

The plaque forming unit formula

Plaques number * dilution * volume

For the second picture where the dilution was 10 to the power -7 apply the formula:

211 * 10 to the power 7 * 1=2.11 * 10 to the power 9 pfu/ml

** we diluted 10,000,000 times so applying in the equation we multiply by 10 to the power +7 and NOT -7

Viral replication terminology:

1-Multiplicity of infection : ratio of infectious agent(viruses) to infection target(target cells).

Example:if you seeded 100,000 cells and add 10,000 viruses calculate multiplicity.

Multiplicity=infectious agent/infection targets
=10,000/100,000=0.1

*this term is used in labs to measure infectivity of virus on cells.
*when they get 100 % they might need to use hundred of multiplicity infection to make sure that each cells is infected by at least one virus.

*in our calculations we calculate all viruses added even if they didn't infect the cells under examination.

2- Eclipse phase : the period during which the virus becomes uncoated.

*differs from one virus to another with a range up to 12 hours.

*first step in viral replication is disassembly ,once the virus enters it gets uncoated and loses its infectivity.(to be explained later in detail)

3-synthetic phase : time during which new viruses are assembled.

(virus does genome replication and synthesizes structural and nonstructural proteins)

4-Latent period : a phase during which no viruses can be detected extracellularly.

(it is the period extending from the moment of virus entry to the presence of infectious viruses within the cell)

**there is an overlap between these 3 phases .

5-burst size : amount of infectious viruses produced by a single cell.

**check the curve in the slides

-the burst size is noticed to be reached in the plateau region

-burst size varies from one cell to another(10 up to 10,000 viruses)

-the increment of the curve goes on until we reach the plateau

-burst size in the curve we have is 200

Virus replication

*replication is divided to either 6,7 or 8 parts but as a base it is 6 steps.

*the virus must undergo these steps to replicate successfully.

*these steps are distinctive in each virus.

*some steps can be summed up in one step.

Steps of viral replication:

1-adsorption

2-penetration

3-duplication/synthesis

4-assembly

5-release

the doctor read the life cycle of **enveloped viruses from slide number 7 where they are perfectly illustrated with pictures.

*note that step 2 and step 5 showed we are talking about enveloped viruses.

*after being released not all viruses are mature ones(virions)they might need time to be mature,infectious for neighboring cells.

Viral replication steps (Detailed)

1- Adsorption

*also known as attachment

*it represents the attachment between virus specific protein or anti receptor to a cellular receptor.

*receptors on cell surface might be glycoproteins or (carbohydrates on glycoproteins or glycolipids)

*some complex viruses use more than one receptor and have alternative ways to be uptaken by the cell.

*enveloped viruses have proteins;glycoproteins or spikes attach to specific receptors.

*naked viruses might have surface proteins that can recognize receptors on receptor cells and bind to them to initiate entry.

***naked viruses are better and stronger in adsorption step than enveloped viruses(why)

> And that is because enveloped viruses contain proteins and spikes which can be recognized by our immune system which will produce antibodies to fight these enveloped proteins.unlike naked ones that the immune system can't produce antibodies against it.
>
> ***how many viruses do we need to initiate viruses' entry to the cell?
>
> We need 3 to 5 viruses;forming 3 to 5 virus receptor complexes.
>
> ***why do we need more than one virus to initiate viral entry?
>
> Because we need a force that brings viral envelope closely to the cellular membrane to attach them to each other.

*there are 10,000-10,000 receptors on cellular surface.

-Host range: collection of hosts that an organism can utilize as partners.

*in our study the **host is the human**

*some viruses have a wide range of hosts like:

-influenza:seen in birds like swans and in human beings

-rabies:seen in dogs and human beings

-HIV:first discovered in monkeys and affected human beings as well.

Cellular tropism : meaning that each virus has at least one target cell like:

- influenza virus of respiratory tract.

-hepatitis:affects hepatocytes

-HIV:affects T helper cells

Check table 6-5 and table 6-6

**receptors are not required to be memorized,the doctor wants us to know the following:

1- Some viruses are able to attack multiple cells
2- There are many kinds of receptors
3- some receptors can be shared by more than one virus
4- some viruses might need one or more receptors like in HIV virus that has two receptors CD4 and chemokine co-receptor.(why)

attachment of glycoprotein of HIV which is(gp120) to cd4 is not enough because this attachment induces a conformational change in which the glycoprotein(gp120) falls of allowing the attachment of a co-receptor which is from chemokine family.

*chemokine has two types of receptors :

1-CCR5 2-CRCX4

So,a virus might target one cell line or multiple cell lines,the simplest form of attachment is one glycoprotein with:

A- receptor
B- multiple receptors
C- receptor with co-receptor

** some notes

1- we might have two receptors for the same virus.
2- Receptors might be glycoprotein or carbohydrate on top of glycoprotein.
3- Herpex Simplex Virus and Adenovirus share the same receptor(heparin sulfate)
4- The viral attachment proteins might be single or make a complex(more than 1) like 2 or 3 proteins.
5- In influenza the viral attachment protein is hemagglutinine and in HIV it is gp120 (not required to memorize)

Influenza virus attack (in brief)

Once hemagglutinine(the viral attachment protein)attaches to sialic acid(the receptor on the target cell) this initiates virus entry,keep in mind that we need 3-5 viruses to initiate viral entry.

Herpes Simplex Virus (in brief)

Once glycoproteins GB and GC (viral attachment proteins)attaches to heparin sulfate(the receptor on target cells) this initiates virus entry and we need 3-5 viruses to enter as well.

** Influenza hemagglutinine(abbreviated as HA) is one of two glycoprotein spikes on surface of influenza virus,each glycoprotein is a trimer,the HA spikes are responsible for binding to influenza receptor which is sialic acid.

*HIV target cells are the T cells and the receptors are CD4 and chemokine co-receptor.

The second step of viral replicatin

2-penetration

*Occurs a short time after virus's attachment to cell membrane

* this step needs energy,so the cell needs to be metabolically

we don't need energy in adsorption

*Three mechanisms are involved in penetration(two are mostly used but the third is rare) and these mechanisms are:

➔ Translocation (the rare mechanism)

- In this mechanism the entire virus particle crosses cell membrane of the cell after attachment.
- A change occurs where the protein mediates the virus entry.
- Occurs between virus capsid and specific membrane receptor.

➔ Endocytosis(the most common mechanism)

- Doesn't require any specific virus protein other than glycoprotein, with receptor on the target cell.
- Relies on the formation and internalization of coated pits and cell membrane.
- Receptor mediated endocytosis is an efficient process for taking in the extracellular macromolecules.
    - For enveloped and non-enveloped viruses
    - In this mechanism there is an attachment between the glycoprotein and the receptor (remember that we need 3-5 viruses for penetration to occur)
    - What happens next is engulfment of the virus by endocytic vesicle and internalization to cytoplasm
    - The process is the same for naked viruses

- In naked viruses endocytosis is known as viropexis

➔ Fusion(the last mechanism)

- Needs energy so that viral envelope and cellular membrane can come close together,then a small gap opens between them allowing their fusion,then the neucleocapsid enters the cell.
- We need 3-5 viruses for penetration to occur.
- Enters directly or by being held by a cytoplasmic vesicle
- Next step is uncoating

**For enveloped viruses,after getting endocytosed by a vesicle how are they set free to the cytoplasm?

They are set free by a drop of pH within the endocytic vesicle.

-Examples of viruses penetrating the cell membrane by some of the mentioned mechanisms:

1- Fusion:Paramyxovirus,Herpes virus

2- Endocytosis:Influenza virus,some Togaviruses and Rhabdoviruses
3- Viropexis: Poliovirus,Adenovirus,Reovirus

Good Luck !