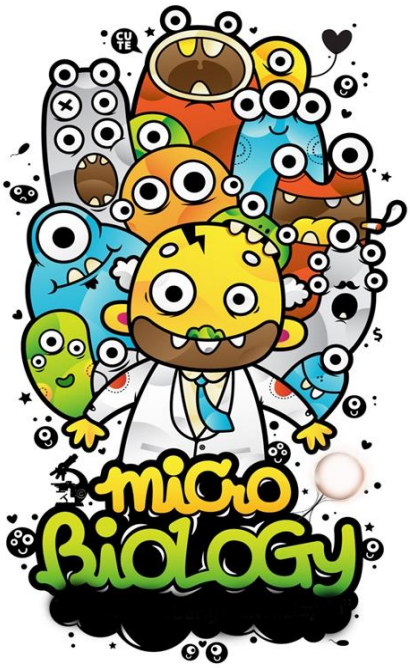

VIROLOGY

Subject: Viral genetics and Anti-viral drugs

Lecture No. : 6

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Last time we talked about viral genetics and we will continue talking about it in this sheet. {*Slides covered: 11-24}*

First of all you should know that genetic changes could happen by :

1. Mutations, mentioned in the previous sheet

2. Recombination, which can be:

A. Classical recombination:

Occurs in DNA viruses and it is the exchange of information (carried on parts of the genome) between two different genomes.

B. Copy choice recombination:

It happens in RNA viruses which either have [RNA dependent RNA polymerase] or [RNA dependent DNA polymerase] (reverse transcriptase) and those enzymes have the ability to jump from one template to another before finishing processing the first one, it binds to strand 1 and starts making a complementary strand for it, then it jumps into strand 2 with that complementary strand ,which it has already made, is still attached.
Once it jumps into strand 2, it can come to the middle, beginning, or close to the end of strand 2:

- If it come to position similar to the one it has left from (in strand 1) then continued reading, the size of the end product will be conserved
- If it came at earlier point, it will be longer and the virus wont be defective because there's no missing genes
- If it come nearer to the end, it will be shorter which means that it is missing some genes so the virus will be defective .

What is the percentage of newly produced viruses that is infectious (not defective) ؟

The answer is that the majority will be defective and the percentage varies between different viruses.
(In our discussion we presume that the ratio is 60% defective, 40% infectious)

As you know, RNA dependent RNA polymerase and RNA dependent DNA polymerase lack proof reading system so there is a mutation every 2500-10000 nucleic bases. And because there is more than one copy of viral genome in one cell, when the transcription happens for the first time, the place of mutation that occurred in the first strand is different from that which occurred in the second strand, so when there is recombination between both, so there's a possibility that recombination happens between already mutated strands.

***Note**: there is another type of recombination that occurs in a cell which is infected by two (or more) viruses so the recombination happens between these different genomes.

***Note**: here we're talking about continuous genome.

This resulted recombinant strand may face a change in [RNA dependent RNA polymerase] and introduce a new point mutation again while making a complementary strand. This new one could make a recombinantion with strand 1. So that's how mutations and copy choice recombination may occur and cause changes in the viral genome. (This is about slide 14 file 5)

[minutes: 00-11]

C. REASSORTMENT

It is a form of recombination and it's very efficient, it happens ONLY to viruses that have segmented genome (can occur naturally)

When we're talking about reassortment, we should remember segmented-genome viruses: Influenza virus which has 8 segments as its genome and rotavirus which has 11 segments.
What happens here is that there is a chance for a single cell in an animal - a pig for example - to be infected by pig, bird and human influenza viruses. When these viruses inter the cell, they will replicate and produce proteins (structural and nonstructural), and then when assembly occurs there will be collecting of segments and each newly formed virus must contain 8 segments. These 8 segments have different probabilities, they could all come from the human influenza in that cell-which have pig, bird and human influenza- and they could be a mix. That's what we call reassortment, the result is a new virus that has a mix of genes and proteins from different species of viruses.

How is that going to affect the virulence of the virus ?

It's going to increase!

***External note**: Our concerns are about viral genetic changes that produce modified copies of the virus which are more pathogenic or more virulent.
On the other hand, viral genetic mutation may result in producing immature or defective copies of the virus - for example, if the genetic change removed some genes that code for viral capsid or envelop - but we are less concerned about these resultants.

Influenza viruses are constantly changing, they can change in two different ways **:**

1. **Antigenic shift (Reassortment)**

Most probably occurs in animals not humans

2. **Antigenec drift**

Results from point mutations affecting the sequence of codons – can affect the genes that code for every protein in the virus but it is more pronounced when it is in the genes which code for the glycoprotein (spikes).

The mutation is more signeficant when it affects the glycoproteins because it is the most antigenic part of the virus that will activate the body's immune response. To make it simple, suppose that the glycoprotein has semicircular shape, after the mutation it becomes triangular or semicircular with a small triangle on the surface. As a result, the efficacy of the virus increases and the human body isn't sesitized or doesn't have antibodies against that virus (that virus becomes more virulent).
The degree of change in the shape depends on the mutation location.
If the mutation affected the shape in a minimal way, most probably the antibodies produced against this virus upon previous exposure are still functional and effective, so they are going to confer a degree of protectoin against this infection and the patient comes with mild symptoms.

This occurs in annually, but it could be more severe every 3-5 years because of relatively huge change in the shape of glycoproteins.

***Note**: when we talk about the extent of viral virulence or infectivity, this is tightly related to the response of our immune systems against viruses (for example, high-virulence virus indicates that immune systems are unable to deal with them).

***Note**: Common cold viruses infections are more common in winter than influenza virus infection.
***Note**: Avian Influenza is commonly known as bird flu, and Swine Influenza is an alternative name of pig influenza.

In antigenic shift, If the whole or some of the glycoprotein are coming from avian or swine segments, humans have never exposed to them so there is no antibodies, whereas in antigenic drift of human influenza we have previous exposure to them, for that reason antigenic shift (reassortment) is more sever and virulent than drift.
While influenza viruses are changing by antigenic drift all the time, antigenic shift happens only occasionally **.**

Antigenic shift may occur every 5-10 years and it could make a huge outbreak. The last outbreak happened in 2009 when an H1N1 virus with a new combination of genes emerged to infect people and quickly spread, causing a pandemic.

[Minutes: 11-24]

In vaccines we depend on our previous knoledge of the most infectious viruses in the previous year or couple of years, we don't depend on predicting the future – predicting what modification or what virus will emerge.

***Note**: influenza has three types: A,B, and C . A is the most infectious one while C is the least infectious and least pathogenic type.

Influenza vaccine includes two type A influenza viruses and one type B and it is made by putting segments of genomes of these three viruses (especially the ones that code for glycoproteins) in one virus so there will be expression of the glycoproteins of the three on the surface of this virus (this will make your immune system produce antibodies for the three viruses included).

There are two types of Influenza vaccine :

- **Injectable (IM)**

- Mostly used worldwide.
- Inactivated or killed virus vaccine.
- We bring viruses and make reassortment or grow each one individually.
- Then they are treated with formalin (causing their disassembly), but glycoproteins are still present within this vaccine.
-When we give it IM, the immune system will make antibodies and memory cells for influenza viruses included.

- **Nasal**

- Live attenuated viruses, meaning that it is capable of infecting the cell and replicating but the infectivity and the virulence are very weak.
- The idea is that vaccine is going to mimic the actual infection, deliver the glycoproteins (antigens) and trigger the immune response in order to form antibodies and memory cells against influenza .
- It is contraindicated to be given to immunosuppressed patients because the virus always tries to return to its wild type by introducing mutations.

Defective Viruses

• They are noninfectious copies of the virus, lacking gene(s) necessary for a complete infectious cycle.

• helper viruses provide missing functions

A mutation or recombination (at protein level) in the glycoprotein is the reason behind the decreased infectivity of defective viruses. Also, if there is a mutation in the gene which code for RNA dependent RNA polymerase there won't be any replication and the virus is defective. So any gene in a virus could be mutated and this will mostly affect the infectivity or replication of the virus.

How could the defective virus survive?!

If it entered the cell with a helper virus and that's called **COMPLEMENTATION**, the defective virus could use the glycoproteins, capsid, or RNA dependent RNA polymerase of the helper virus inside the cell if they infect it at the same time.

There is a phenomenon called **von Magnus phenomenon** after the scientist who observed it for the first time. He was working on influenza virus and he was growing it in the lab, they used to grow it in checken eggs, (even the viruses that are used in vaccines are grown there so one of the things to do before giving that vaccine is asking if the pateint has an allergy to eggs). He found that growing the virus at high MOI was associated with a decrease in production of infectious virus and vice versa.
***MOI**: multiplicity of infection. So if the MOI was high, the cell may be infected with more than one virus and if it was low, the cell is mostly infected by one virus only.

To illustrate that observation suppose that there are three cells:
The first one was infected by one infectious virus , it will produce new copies of the virus, 40% infectious & 60% defective.
The second one was infected by one defective virus and it was its cemetery (because there is no helper virus to aid it).
And the third one was infected by two viruses: infectious and defective ones, they found here that there is competition between the two so the amount of defective viruses will be more than 60%.
(the third cell is an explanation of van Magnus phenomenon)

***Note**: defective viruses are still defective even after complementation.

[Minutes: 24-40]

Anti-viral drugs *{slides covered: 1-5}*

• Viruses have no cell wall and made up of nucleic acid components

• Viruses containing envelopes which have spikes that are antigenic in nature

• **Viruses are obligate intracellular parasite**

• They do not have a metabolic machinery of their own so they use host enzymes (This point most probably describes DNA viruses because RNA viruses occasionally carry their own enzymes with them)

Certain viruses multiply in the cytoplasm but others do in the nucleus.

The general rule is that DNA viruses replicate in the nucleus (except poxvirus) whereas RNA viruses replicate in the cytoplasm (except HIV & Influenza)

• Most multiplication take place before diagnosis is made. This because viruses infect and need an incubation period during which our symptoms appear. There are certain diseases in which you can infect the surrounding even before you know that you're infected! In those situations like influenza, Corona, and common cold, the earlier you treat, the most effective the treatment is because they have short incubation period and short illness. So in the first day you could have runny nose, in the next day you could have fever and in the third day you might realize that you are infected! Two days after, the illness should be gone away.

In such these cases, what is the best antiviral drug to give?

The answer is that we don't give antiviral drug because it is a short lived illness,acute lytic infection and self limiting. We can drink lemon juice, take strepsils and antipyretic drugs which treat the symptoms.

• Many antiviral drugs are Purine (A & G) or Pyrimidine (C & T) analogs (i.e. have a chemical structure similar to these nitrogenous bases). These analogs will insert themselves instead of the nitrogenous bases thereby inhibiting genome replication.

• Many antiviral drugs are prodrugs, They must be phosphorylated by viral or cellular enzymes in order to become active. If the drug was activated by viral enzymes, the drug will be more specific so low side effects could happen and the drug will remain inactive in the uninfected cells.

• Anti-viral agents inhibits active replication only! and the viral growth resumes after drug removal.
• Current anti-viral agents do not eliminate nonreplicating or latent virus (e.g. Herpes virus)

• Effective host immune response remains essential for the recovery from the viral infection.
• Clinical efficacy depends on achieving inhibitory concentration at the site of infection within the infected cells. (subtherapeutic conc. will lead to resistance)

[Minutes: 40-50]

I will not give up untill I reach 💪