Sheet 24

Completion of viral genetics and viral diagnosis and detection

Note: this sheet followed the sequence of slides presented by the doctor.

Viral genetics

Revision:

last time we talked about mutations and their different types, and recombination; we need two genomes in order for recombination to occur between them.

-Classic recombination: seen mostly in DNA viruses where two segments are exchanged between the two genomes.

-copy choice recombination: occurs mainly in RNA viruses because the reverse transcriptase and RNA dependent RNA polymerase have the ability to jump from one template to another, so they start reading or replicating on template A, and after finishing a segment it jumps (with that segment) onto another template and finishes from there. As a result you might end up with longer genome, shorter genome (which is the case most of the time), or same length genome

Last type of recombination: reassortment.

- Reassortment (antigenic shift) Occurs in segmented viruses : rotavirus and Influenza virus.
- Taking Influenza :

  We have another term antigenic drift (also in influenza) which causes the antigenic variations in the spikes or glycoproteins of influenza (due to mutations) so they need new antibodies, and that's why we have to make a new vaccine for influenza each year, you can never get antibodies or memory cells against influenza, these changes in glycoproteins are called **antigenic drift** which differs from **reassortment "antigenic shift"**. Genetic modification change in a virus might reach 5%-10%. We see an epidemic every every 2or 3 years. (Epidemic: more rate of infection in one country). Antigenic variations that come every 3-4 years are more severe than the epidemic occurring every year (for example in every winter (year) we have influenza epidemic, the rates are higher but when the antigenic variation/epidemic is seen every 3-4 years it will be more associated with complications).

  *What really occurs in **reassortment** for influenza specifically?
  We have a wide host range: mammals (swine/porcine flu), birds (avian flu), and humans. So, if the infection occurs for example in an animal like pig (a porcine cell), the cells can be infected by a human, avian or porcine influenza virus, if a cell harbors the three of them, their segments (genomes) will be mixed up within the cell, so during assembly of viruses, the new virus's genome might be a mixture of 2 or 3 types of segments, from the different infecting viruses might get a mixture of segments between 2 or 3 types of these infecting viruses (it might have

segments from human, bird and mammal influenza virus).The variation in the genetic component of the newly produced virus might reach up to 50-60%, this will produce a virus that is more pathogenic and upon infection it will be more associated with complications. We can see a pandemic every 10-20 years. (Pandemic means in more than one country while epidemic means more rate of infection in one country).

***It is used in some newer vaccines against influenza virus and rotavirus.**

We talked about influenza vaccines last time; we have two types: shots (IV) and intranasal (in which we use reassortment), given intranasally. In shots vaccine, we might use the whole virus or the antigenic components alone (the spikes).

* In **shots** if we use the whole virus it should be a killed virus.

*But when we talk about the **intanasal** vaccine produced using reassortment phenomena, it is a live attenuated virus.

-How do we use reassortment in this vaccination?

you look the most pathogenic viruses in the previous year (this is the same for shots and intranasal) take the segments of the glycoproteins and introduce them to this life attenuated virus, then you replicate it in eggs or a cell line, harvest this virus and after that put it in the intranasal spray, when you introduce it in the upper respiratory tract, it won't cause any disease it will just replicate, so the body will produce antibodies and memory B cell for it.

It's a temperature sensitive vaccine, given intranasally, approved in 2003 by FDA, not widespread and they started using it just couple of years ago, still shots are more commonly used.

***2 points concerning influenza vaccination:**

1-taking the influenza vaccine, intranasal or shots doesn't mean that you will be totally protected against influenza for that year, because they take the most 3 serotypes, 2A and 1B serotype (most pathogenic taken as a segments) Even with vaccination, you might still get infected.

2-all these Vaccinations are made outside our country region (in Europe, US. etc.). So they don't take the viruses most pathogenic viruses in our region which may reduce the efficacy of the vaccine. It will more efficacious if these vaccines depended on most pathogenic viruses for our regions.

Rotavirus vaccination (rotavirus can perform reassorment) are:

Uses same concept, live attenuated, you take segments from the most antigenic part of the virus and add them into a live attenuated vaccine, which is given as drops, goes to GI, replicates there, body produces antibodies and memory B cells and that how you get protected.

Note: Rotavirus vaccine is not part of the national vaccination program in Jordan, it is only available in private clinics (But polio-vaccine is given).

However: there was an outbreak of rotavirus infection (3-4 weeks ago) in مبرة الملك حسين للأيتام and more than 50 cases were reported and 26 cases were hospitalized. It's an acute infection with no consequences if you just replace the fluids so that they won't get dehydrated. So, hydration and monitoring of patients were provided for these cases and they were all released. But due to this outbreak a decision to include rotavirus vaccine in our national vaccination program was made, but hasn't taken any effects now, still it is not a part but it will be.

***The non-segmented negative stranded RNA viruses** do not have classical recombination nor Copy choice recombination nor reassortment. So, they have the least ability to exchange genetic material.

Now we come to another term:
Defective interfering particle (DIP)

-Also called "von Magnus Phenomenon" A scientist who found that phenomena while he was working on influenza virus (then this phenomena was applied to the other viruses) discovered that the passage of influenza virus at high multiplicity of infection the number of infectious viruses/particles will drop steadily. When we talk about infectious viruses/ we are talking about the viruses released into the media.

***passaging can be for viruses or cells:**

1-Passaging the **cell**: we put a cell line in a flask, it forms a monolayer on the bottom of the flask (cant stack on one another), after that we add the virus into the media, once the cells are fully confluent (cover 100% of the surface of the bottom of the flask. If they cover 90%, we say 90% confluent. [Here we are talking about a specific continuous cell line later we will talk different cell lines], if you started with 10-20% confluent, after 2 to 3 days they will reach 100% confluence. But if you leave them 100% confluent they will die because they won't have any space to grow, and since they cannot stack on top of each other, they will die. That's why we shouldn't leave them there after they reach 100%). Take out the media, wash the cells, add an agent like trypsin that can break the links between cells with each other and between the cells and the bottom of the flask, then you resuspend them in a media, if you resuspend in a 5 ml and take 1 ml and put in in a new flask, what is the confluence of the cells in the new flask? The answer is: 20% confluence (divide 100% by 5).

This action; taking part of the cells, putting them into a new media to grow once again is called **passaging.**

2-Passaging for **viruses** : taking the media which has the virus and adding it into a flask with new non-infected cells.

* So, what does" passaging the influenza virus at high multiplicity of infection (multiplicity: the ratio between no. of viruses to no. of cells. When we say it is high we mean that there is more than one virus for each cell) leads to steady drop in no. of infectious particles" mean?

First of all, you should know that most of the newly produced progeny viruses are defective viruses as a result of 2 things:

1- Due to mutations that occur.
2- Copy choice recombination that occurs.
   Note: even normally in our body, when a virus infects a cell, most of the produced viruses are defective viruses, not fully infectious.

   *When you passage them at high multiplicity, at least one of these viruses that are infecting a single cell is infectious. So, the cell is being infected by an infectious virus and a defective one (or more),so why more defective viruses are produced?
   The infectious virus complements يُكَمّل the defective one, so the defective virus has problems in many genes (missing) but still it can replicate its RNA, but for the other missing parts, it uses those of the infectious virus, in this way they are called **"defective"** due to mutations and copy choice recombination it is attenuated and can't infect a cell by itself, and **"interfering"** because it can still compete with the infectious ones for the enzymes, nucleotides and everything in the cell, and produces more defective particles.

   -**To get rid of the defective interfering particle**:
   The passage of viruses should be at low Multiplicity of infection.

* If you pass it at multiplicity of 0.1 you will make sure that:

1-Not all the cells are going to be infected

2- The cell that will be infected will be infected by either an infectious or a defective virus (not both due to low multiplicity). The defective will not replicate because it has nothing to support it (to complement it) while the infectious viruses will replicate and give new infectious viruses.

*The dr. read slides 20 and 21, and then said in slide 21 "you can see an infectious virus genome and a defective virus genome which is shortened by copy choice recombination."

The last topic in genetic is **complementation of viruses:**

Slide 23: this is an example of a cell infected by two viruses. In reassortment we said that the mixing occurs at the genome level, while in complementation in occurs at the protein level. So, if we call these two viruses A and B:

1- if reassortment happened, the newly formed virus Genome could be : A or B, or a combined genome of both A and B.
2-if complementation happened, the progeny virus of A will have A genome and the progeny for

B will have B genome; no mixing of A and B at genome level. But the mixing will be at the level of structural or nonstructural proteins, but the effect will be seen on structural ones like glycoproteins, will be a mixture of A and B.

With mutations of the antigenic variations, complementation and mixing of glycoproteins, a new and unrecognizable strain by the body, will be produced.

Done with viral genetics.

Virological test and diagnosis of viral infection:

Wide expanding role for diagnostic biology labs, because:

1-nowadays we have too many immune-compromised patients who are more susceptible to viral infections, so you need to get the diagnosis early to treat them early.

2- And they have increasing no. of antiviral agents so you have to diagnose the infectious agent so you can give the right antiviral agent.

Methods in virology:

1. Election microscopy
2. Viral culture.
3. Detection of viral antigen.
4. Histopathology.
5. Serological tests.
6. Detection of viral nucleic acid.

Specimen choice and collection:

There are certain factors that have a role to get the proper diagnosis:

1. Proper specimen collection site.
2. Proper timing of specimen collection; we mean during the virus replication cycle.
3. Effective timing and processing of sufficient specimens, you should process it as early as possible.

Specimen routine tests:

- Infections involving CNS, such as meningitis and encephalitis, we can take samples from blood, CSF and feces. Because most of the viruses that affect CNS start or are transmitted by fecal-oral route or secondary viremia.
- Respiratory viruses through blood, throat swap, nasal wash, bronchoalveolar lavage.
- Hepatitis: blood.
- Gastroenteritis: feces.
- Skin lesion: from the lesion itself.

*the dr. said that the table (from which these were taken) is not for memorizing except for the things he had mentioned (these things above).

Specimen Storage:
1-Blood sample should be stored at room temperature, others should be stored at 4 degrees.
2-If you have to delay the test for more than 24 hours, you should keep the samples at -70C, not -20C (Because at -20C the virus may lose it infectivity and you might get negative false results).
3-naked viruses are more stable than enveloped ones. Why?
Because in enveloped viruses, if you need to delay the test for more than 24 hours, the freeze-thaw cycle, will affect the envelope, making it less infectious, and you could get false negative results or lower reading in the patient than the actual one.

Diagnosis of viral agents:
-diagnosis of viral diseases is harder than other diseases, because viruses need to grow on cell culture (cannot grow on agar), you will use a cell line which is not easy to maintain.
-once you suspect you should take the history, physical examination, the clinical picture, investigations, and diagnostic tools, in order to reach the appropriate diagnosis.

*This is a plan to test for different types of viruses:
-take a proper sample, infect the cell culture, look for characteristic cytopathic effects in the same culture, screen for parts of viruses (this is done through the serological methods), and detect for antibodies using serological or molecular techniques.
Diagnostic methods:
1.Direct
2.Indirect
3.sereology

Direct : includes:
1.***Electron microscopy :***You can put any sample under light microscope and look for the viruses. The morphology of the virus particles can be seen using EM and you can also add a fluorescent labeled antibody which will attach to the antigenic part of the virus and you should look for that under the microscope, if there is binding between the antigen and the antibody; you will see the antibody fluorescing on the surface of the virus itself.
2. ***Light microscopy***: you can't use it to look for viruses because of the size of the virus (20-450 nm) but it can be used to look for cytopathic effects that occur in cell.
3. ***Viral genome detection***: there are many methods, we will talk about PCR: polymerase chain reaction.

Indirect methods of examination:
1.Cell Culture

2.Eggs
3.Animals

1st: Animals: you might take a sample, you don't know the virus in there so you inoculate part of that sample into the mouse/ rabbit, and look for development of symptoms or death.
2nd: Cell Culture: take part of that sample, inoculate it to the cell culture, and look for cytopathic effects. We can also add immunofluorescent antibody (***How they work***: Let's say that we are looking for suspected influenza virus, and antibodies of influenza virus that are fluorescent – Labeled were inserted. After two hours, the next step is washing the media and the cells, if it is not bound, it will be washed away, if it is bound, you can see it under the microscope (getting rid of any unbound antibody. By observing the specimen under EM, the presence of florescent antibodies would confirm that it is an influenza virus. Otherwise, it's not!)
3rd: Eggs: used to grow influenza virus.
-In the egg there are a lot of sites you can inoculate the virus in.
One is the chorioallantoic membrane. Viruses in egg can grow to form inclusion bodies.

Now we will talk about **Haemagglutination** and **Haemadsorption**

We are talking about testing for the presence of the virus in the lab setting (not in the body). And both of those tests involve the addition of RBCs. This explains "haem".

We infected the cell with the virus, enveloped viruses leave the cell leaving their glycoproteins on the surface of the cell membrane, when you add RBCs they bind to the glycoproteins on the surface of the cell, and this is called **haemadsorption.**

There is another term: **haemadsorption inhibition**, this involves antibodies. If we take a sample containing antibodies, we are not sure they are for which virus, and we have 3 different virus infected cells (by influenza, respiratory syncytial virus and Parainfluenza virus), so we add the antibodies to these cells; first, if we added them to influenza virus infected cells, then added RBCs and didn't see haemadsorption upon adding RBCs, this means that these antibodies are for the influenza virus. If you add these antibodies to respiratory syncytial virus or to Parainfluenza virus infected cells, for example, the antibodies will not bind so they will haemadsorb after addition of RBCs.(result: those antibodies are for influenza virus)

Haemagglutination: we are talking about the released viruses not the infected cells, the released viruses have the ability to bind RBCs with glycoproteins but in the setting of the viruses themselves, this forms a large clot, collection of RBCs, with the viruses trapped in.
***To sum up*: haemadsorption : Binding of RBCS to spikes of viruses on the infected cell surface While haemagglutination: is binding of RBCS to spikes on released virus itself.**

haemagglutination Inhibition ***:*** same concept if you add antibodies it will bind to the antigens preventing haeagglutination (the only difference is that antibodies are added to the viruses themselves not the infected cells).
***Inhibition of haemadsorption or haemagglutination means you are using the right antibody.**

Sereology : We have multiple sereological methods , some are classical others are newer : of these mechanisms we have :
1. Complement fixation

2. haemagglutination inhibition test

3. Immunofluorescence techniques

the slide mentions more and they all will be discussed in upcoming lectures.

This part of the sheet discusses already mentioned methods for detection but detailed now:

Back to cell culture:

- Cell culture viruses are obligate intracellular organisms that require living cells for virus isolation and detection.
- Advantages of cell culture: Relatively sensitive and specific. We said "relatively" because when we will talk about disadvantages sensitivity is an issue for cell culturing

- Can (the cell culture) detect many different viruses, and provide virus isolation for further characterization, serotyping, genotyping, susceptibility information about the virus.

*When we talk about cell culture you need to know that:

1- we cannot grow all viruses that are known now in a cell culture, there are certain types that we still haven't succeeded in growing them in a cell culture, which limits our knowledge about these viruses, because as we said before cell culture allows us to study them at different stages of their replication and know more information about them (serotyping, genotyping, susceptibility).

2- Certain viruses can grow in multiple cell lines and certain viruses can only grow in a single cell line.

***what are the types of cells that we can use?**

1-**primary cells**: Best cell culture system available. However, they are expensive, and often it is difficult to obtain a reliable supply. We can only passage for 1 or 2.

*Most of the time an animal organ is used to make this culture (e.g. Monkey kidney) -> we crush the cells and put them in a nutrient media, after that we put the virus which can grow in these cells but we can't maintain these cells for long time, we only can change the media, and the new media or nutrients, maybe 2 or 3 passages and the cells will die.

2. **Continuous cells**: the easiest to handle but the rate of viruses supported is often limited and they are the cells that are most widely used in research labs, but certain viruses have only one cell line to grow in and some can't grow in any cell line that we know. They have indefinite passaging theoretically speaking (we say theoretically speaking because with each passaging the cells change their characteristics minimally). So, they are good for 30 to 40 passages after that they lose their characteristics and become unreliable.

3. **Semi-continuous cells**: we can passage for 20-50, theoretically speaking, so once they reach 10 or 20 passages there are not good as you started with, they become unreliable for diagnosis, they are only good for 10-20 passages.

*another difference between semi-continuous and continuous is that semi continuous are human embryonic kidney cells or skin fibroblasts, so they are normal cells while the continuous

are cancerous cell line with faster replication, ex. Vero cells.
***note: with each passage, the cells change or lose their characteristics and you might be unable to use them**.

***As we said before in cell culture we look for cytopathic effects in the cell culture such as:**

1-Ballooning of the cell (Herpes and adenovirus)

2-Multinucleated giant cells (syncytia formation) such as HIV, RSV, herpes viridae.
3-Haemadsorption of cell-culture: seen in orthomyxo and paramyxo viruses such as: Measles, influenza, Parainfluenza and mumps …etc. The concept is insertion of viral glycoproteins into the host cell membrane (during the release of enveloped viruses) which promotes the attachment of RBCs of certain species like guinea pigs or rabbits to attach or adsorb to cell membrane.

Problems or disadvantages of cell-Culture:
1.Long period (4 weeks) to get results
2.susceptible to bacterial contamination (though we add antibiotics to media)
3.susceptible to toxic substances; certain viral products or proteins might be toxic to the cells themselves and kill them.
4. Many viruses won't grow in cell culture (we are still unable to do that).
5. Very poor sensitivity

Egg culture: used for isolation of influenza virus. The inoculation sites include:
1.Aminotic sac
2.Yolk sac
3.Allantoic membrane
4.chorioallantoic membrane

Electron microscopy: quick method used for many viruses, useful for unknown pathogens, less prone to cross contamination, expansive equipment, needs an expert to read the results.

As mentioned before: specimen collection could be form:
1.Feces : in case of gastroenteritis; viruses that cause it; rotavirus, adenovirus, astrovirus, calici virus, by fecal-oral route.

2. Vesicle fluid (herpes simplex, varicella zoster or chicken pox).
3. Skin scrapings (papillomavirus or molliscum contagiosum)
*there is a picture under EM adenovirus, rotavirus; icosahedral capsid and you can see parainfluenza virus where the capsid is helical.

Light Microscopy: once again you inoculate the cells with the virus and look for cytopathic effects and negri bodies or cytomegalic inclusion bodies and the other cytopathic effects that we talked about.