University of Jordan
Faculty of Medicine

Medical Committee
The University of Jordan

Introduction to

Microbiology

Title :

Viral Genetics

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- ○ Slides
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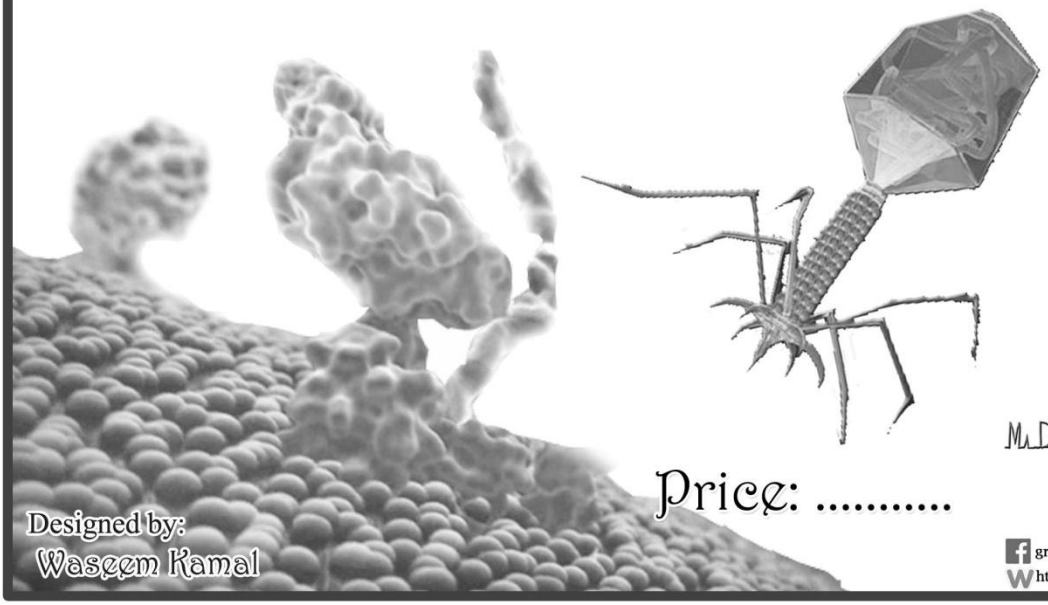

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Virology Lecture – November 6th, 2013

Review:

The basic structure of an immunoglobulin:

- **Y-shaped structure**
- Two **heavy chains** and two shorter **light chains,** they are bound to each other by disulfide bonds.
- **Two Fab regions**: antigen-binding variable region.
- **Fc region** binds to macrophages and complement system proteins in order to destroy the antigen.

We have five different types of immunoglobulins: IgM, IgG, IgD, IgA and IgE.

IgM: This immunoglobulin is the first one to be produced in an acute viral infection. It is a pentamer which consists of five subunits of IgG bound together by disulfide bonds and joined by a single J-chain.

IgG: IgG is a monomer and it is formed later in the case of chronic infection.

IgA: Can be in the form of a monomer in the serum or a dimer in secretions. It is found in bodily secretions such as saliva, respiratory secretions, tears and intestinal contents. It is the main antibody involved in immunity against respiratory viruses and in gut immunity associated with enteric virus infection.

Cell mediated immunity plays an important role in the response of the body to viruses.

Children with congenital deficiency of cellular immunity are abnormally susceptible to viral infection and often (although not always) develop unusually severe diseases. Those with humoral immune deficiency, on the other hand, respond normally to viral infections.

- Humoral and cellular immunity play a role in the clearance or containment of viral infection. Humoral immunity neutralizes the virus **before** it infects the cell and the cell mediated immunity takes part **once the virus is intracellular**.

- Deficiencies in humoral immunity (production of antibodies) are not as important as deficiencies that could occur in cell mediated immunity. Cell mediated immunity is more important when it comes to viral infection because once the virus ends up inside the cell nothing will remove it from the cell, and therefore the body, except the cell mediated immunity (especially the CD-8. We will talk about this later.)

Cell mediated immunity is the mechanism for the elimination of virus infected cells, and therefore, the virus from the body.

T, or thymus dependent lymphocytes, are the principal cells involved in this.

We have two main types:

1) **CD4-positive helper T-cells** 2) **CD8-positive cytotoxic T-cells**

We will talk about the CD4 and CD8 T-cells but before that we will discuss antigen processing and antigen presentation.

- An **epitope** is a binding site which is located on an antigen and is recognized by the immune system. The antigen could have multiple epitopes, each one being different than the other.
- Macrophages and dendritic cells are what we call antigen presenting cells. They present antigens through the use of major histocompatibility complexes (MHC). There are two types (classes) of MHCs: **MHC-1** and **MHC-2.**

MHC-1 is present in all body cells except for erythrocytes (RBCs).

MHC-2 is mainly present on the macrophages and dendritic cells and these are what we call antigen presenting cells.

How does this antigen presentation occur?

- Once the virus infects the cell, proteosomes within the cytoplasm cut up the viral antigens and viral proteins into smaller antigens. These are taken up by the endoplasmic reticulum via transporter associated antigen presentation (TAP) and will then be associated with the Major Histocompatibility Complex. They then travel together through the Golgi apparatus and eventually end up on the surface of the infected cell.

When we are talking about cells in general we will be referring to MHC-1 (this type is present on all body cells while MHC-2 is mainly present on antigen presenting cells).

Which cell is then responsible for identifying the antigen on the MHC-1?

It is the **CD-8.**

If we are talking about **MHC-2 on the surface of dendritic cells → it will be CD-4** which recognizes it.

(Remember: **MHC-1 → CD-8, MHC-2 → CD-4**)

Cell-mediated immunity (*continued from slides*):

1) CD4-positive helper T-cells carry CD4 receptors as markers on their surface. The most important cells in the cellular response, they liberate cytokines that activate and modulate cellular immune responses. They require MHC class 2 antigens to be presented in association with the target antigen for their activation. They also interact with B-lymphocytes for antibody production.

2) CD8- positive cytotoxic T-cells carry the marker CD8 receptor on their surface and are MHC class 1 antigen-restricted. They lyse target cells such as virus-infected cells and tumor cells; the main mechanism for elimination of virus-infected cells from the body; also release cytokines.

   *Once a CD8 recognizes a MHC-1, it functions by lysing and destroying the cell by the release of **perforins and granzymes** (like natural killer cells) which introduce pores into the cellular membrane and lead to cell death.

   Suppressor function: note that CD4 and CD8 cells can suppress as well as activate the cellular response.

- See slide 18 + 19-

→Last time we said T helper zero can differentiate into T-helper 1 and T-helper 2. T-helper 1 activates cell-mediated immunity (CD8) and T-helper 2 activates humoral immunity (helps in the activation of the B cells to give us the plasma cells which give us the antibodies) with the help of the cytokines such as interleukins and interferons and TNF.

Properties and roles of memory cells:

What do memory cells do?

- They survive even after the infection is cleared.
-Their number is more than naive cells (un-activated cells)
-Respond to antigen challenge more rapidly than naïve cells do
-Memory T cells: migrate to tissues, some live in mucosal tissues and skin.
-Memory B cells: produce high affinity antibodies

* As we said upon second exposure we have memory B cells so the response is going to be more rapid than the first time. The antibodies have higher affinity (stronger binding) to the antigen because it was sensitized the first time and the second time it is introduced it will produce a rapid response.

-Provide rapid protection against recurrent or persistent infections.
-Goal of vaccination is to induce effective memory

*Most vaccinations we are talking about affect memory B cells. This leads to the production of antibodies upon second exposure to the antigen which will affect the virus by neutralizing it; they do so by binding to its glycoproteins and so preventing the entry of the virus into the cell.

-See slide 25-

-This figure shows both the innate immunity and adaptive immunity playing two roles. 1st is protection against infection and the 2nd is eradication**. In the protection process of innate immunity** we have the interferons as well as the first line of defense (skin, respiratory system and the GI).
- **In the protection process of adaptive immunity** we have the B cells and the antibodies which as we said function by binding to the antigen neutralizing the virus and preventing it from entering into the cell.

-**In the eradication process of innate immunity**; use of natural killer cells.
-**In eradication process of adaptive immunity** we talked about CD8 destroying or killing virus infected cells by the same mechanism of producing perforins, granzymes which introduce pores and kill the infected cell.

How do viruses evade the immune system?

Antigenic variation; we see this in influenza, HIV and rhinoviruses. Most of the time this results from introduction of mutations and this will lead to antigenic variation. When we say antigenic variation we mean there are changes or mutations which are introduced mainly into the glycoproteins or the spikes.

Inhibition of MHC-1 antigen processing pathway

- different viruses use different mechanisms
- natural killer cells are the host adaptation for killing MHC-1negative infected cells

Production of immune modulators

- Some viruses produce soluble cytokine receptors which attach to cytokines and inactivate them and so they evade the immune system. They function as decoys (traps).
- Immunosuppressive cytokines, e.g. IL-10

Infection of immune cells;

- Especially in the case of HIV, infects the immune cells, the T-cells and it suppresses the immune system.

Efficacy of vaccines:

Vaccines work best against microbes that:

-Do not vary their antigens

As we said before, vaccinations induce production of memory B cells and have been useful in generating protective antibodies, not generating effective cell-mediated immunity. Vaccines work best against microbes that do not vary their antigens. For example, every year there is a new vaccine for the influenza virus as a result of antigenic variation. The most pathogenic strain of the previous year is used as a vaccine to decrease its severity. The fact that there are always new strains being developed means we will not be fully protected by the vaccine. (The vaccine consists of either a whole killed, attenuated live virus or the antigenic parts which are the glycoproteins or spikes).

Do not have animal reservoirs.

Since there is an animal reservoir there is a viral infection and replication going on, so even if you give humans the vaccine, the virus will still be within an intracellular environment. Being in an intracellular environment means it is prone to mutations or errors. This may give us new strains which the human body has no immunity against. Vaccines are more protective when humans are the only hosts of a virus.

Do not establish latent infection within host cells.

No drugs are active against the latent infection, the virus is still in the body but you don't know about its existence until reactivation.

Do not interfere with the host immune response

(like HIV, It interferes with the immune cells).

Viral Genetics

We know that viruses reproduce rapidly so when a virus infects a cell it has the ability to produce up to tens of thousands of progeny viruses.

Replication cycles are usually not very long.

DNA viruses undergo proof reading, while RNA viruses do not.
What do we mean by this?

- DNA viruses use cellular machinery for their replication so they have access to proof reading so any mistakes will be corrected. Certain RNA viruses such as Pox virus, Herpes virus and Adeno viruses encode for their own enzymes and are prone to changes resulting in mutations. When we talk about mutations in viruses, RNA viruses are the targeted viruses, the enzymes they have such as RNA dependent polymerase and reverse transcriptase in HIV lack proof reading ability.

Genetic mutation is of two types:

1. Mutation

We have spontaneous mutation which can occur through two mechanisms. 1st is Polymerase or Reverse Transcriptase errors. For every 2,500 to 10,000 base pairs especially for RNA polymerase, there is a mistake introduced. For Reverse Transcriptase, for every reverse transcribing of the RNA there are five to six mistakes (at least) introduced.

The second mechanism is due to tautomeric forms of bases. We have the bases Thymine, Adenine, Cytosine and Guanine. Most of the time, we see the bases in a certain form according to the hydrogen bonding of the base. Hydrogen bonding is important for the attachment of these bases or nucleotides together, so any changes in the hydrogen bonding will affect the binding or the sequence of nucleotides and may affect the protein. Therefore, spontaneous mutation rate is usually higher in RNA viruses (which lack proof reading) than in DNA viruses.

Physical induction (UV light, x-ray) and chemical induction are also origins of mutations.

What are the types of mutations that could occur?

Point mutation: A mutation which occurs at a single nucleotide or a single base.

Codon: A sequence of three adjacent nucleotides constituting the genetic code that determines the insertion of a specific amino acid in a polypeptide chain during protein synthesis.

If there is a point mutation at the last (third) nucleotide of a codon, what will be the outcome?

As a general rule (there are exceptions) most amino acids have 4 codons encoding them which differ only in the 3rd nucleotide (this is general, some have only 2, others only one). Therefore even if the first 2 nucleotides are the same and the third varies, the same amino acid is produced. This is called a **silent mutation**.

If change occurs in the first or second nucleotide a change would definitely be noticed in the amino acid. The mutation could go unnoticed in the protein as a whole, or it may turn out to be a non-functional protein and form aggregates. This depends on the location of the mutation in the protein and it is called a **missense mutation**. (Affects the protein, whether it is non functional, minimally functional, still functional or suppressed)

Nonsense mutation: A mutation which changes a codon into a stop codon, so after this point, translation will not occur.

Start codon is AUG. Stop codons: UAG, UGA and UAA.

Frameshift mutation: a genetic mutation caused by a deletion or insertion in a DNA sequence that shifts the way the sequence is read. This leads to production of different amino acids. Deletion alters the reading frame.

Genotype and phenotype:

The **genotype** is the information encoded in your genes (the sequence of nucleotides). The **phenotype** is the expression of those genes which gives us observable physical or biochemical characteristics and this is how we vary in our eye color, nose shape etc.

Phenotypic changes

- There will not be a mutation in the genes of a virus but there will be a change in the virus' characteristics.

Conditional lethal:
A mutation that is lethal under one condition but not lethal under another condition. The wild type (original virus) can grow at a range of temp for example 25-37 degrees Celsius. The mutation that is introduced into the virus renders the virus sensitive to high temperatures. So if the temperature goes above 28, the virus might die. This is conditional lethal.

Plaque size:
When we do the plaque assay to measure pathogenicity of the virus, we may see a drop in the pathogenicity of the virus.

Drug resistance:

- Important in the development of antiviral agents.

- While taking antibiotics, the patient must complete the entire course, and not be satisfied with only completing part of it even if the patient feels better.

  If you take the drug for one day it will **partially** kill the bacteria. On the 2nd day the drug will be cleared and it will be at a sub-optimal concentration, this will give the opportunity to the bacteria to introduce compensatory mutations. So the next time it infects the body, even if the antibiotic is taken at the therapeutic dose it might not be effective against it because of this compensatory mutation.

Enzyme-deficient Mutants:

Some genes can be 'optional' in certain circumstances.
This means when mutations are introduced into viruses they may affect certain enzymes. Certain enzymes may not be produced at all. This may not affect the replication of the virus, because in certain viruses, there are optional enzymes which the virus can live without.

Attenuated Mutants:

- Mild or no symptoms
- Attenuated viruses are also used in the vaccine development.

- Are mutations good or bad? This is relative, depends on whether you're asking if they're good for humans or for the virus itself. Antigenic variation is an advantage to the virus, while attenuation is an advantage to humans.
- Most antiviral drugs are only efficient if given straight away, because most of the acute viral infections, especially common ones (influenza, cold, rhino, corona), will only last a couple of days (5-7 days).

2. Recombination

- It is the exchange of information between two genomes.

a. Classic recombination

- Most commonly seen in DNA viruses because they have Recombinases (enzymes). They carry out recombination.

b. Copy choice recombination

RNA viruses are more prone to this recombination pattern. (We mentioned this before when we said that cellular proto-oncogenes undergo copy choice recombination to become viral oncogenes)

-See slides-

RNA dependent RNA polymerase and Reverse Transcriptase in HIV have the ability to jump from one template to another. They start reading strand 1 and continue until they read the stop sign.
There are three scenarios that could occur then:
The Polymerase or Reverse Transcriptase will jump from strand 1 to strand 2. It might jump to the site exactly opposite to it, starting to read the second strand at where it stopped on the first strand, and so we will have the same length of the genome. It may also start reading the second strand at a site proximal to the 3 prime or a site distal to the 3 prime. If distal to the 3 prime it will give us a longer genome. If proximal it will give us a shorter genome.

An example: **HIV**

How does recombination occur in HIV?
Reverse transcription occurs in sub-viral particles, before release into the cytoplasm, so there will be no mixing with other templates or other genomes of other viruses. But recombination still happens since HIV is unique in the fact that it is diploid. We have two strands of positive sense ssRNA so recombination can occur between these two strands. We mentioned that the reverse transcriptase and polymerase are prone to mutations, and so the two strands may differ at certain points, and this is how we end up with recombination.

Positive single stranded RNA enters into the HIV progeny by mRNA (we mentioned before that a part of the mRNA goes for translation and the other goes into the viral progeny). What we might get sometimes is the packing of mRNA which is cellular instead of the viral mRNA, so we get one viral and one cellular, instead of getting two viral mRNAs into the viral progeny. This is another recombination between cellular and viral genes. Viruses with this recombination are more carcinogenic.