**Immunity And Laboratory Diagnosis Of Viruses Lecture - 5-**

**Immunity against viral infection**

Immunity that is developed against virus infections is known as viral immunity. It is developed by a variety of specific and non-specific mechanisms.

1. **Innate (non-specific) immunity** refers to those elements of the immune system that can clear virus or virus infected cells immediately upon or shortly after viral exposure and which are not dependent upon immunologic memory. Non-specific immunity may include:-

a. Phagocytic cells (neutrophils and monocyte/macrophages).

b. Cytokines (e.g., interferons) and chemokines.

c. Natural killer cells.

d. Complement proteins MAC can disrupt the viral envelope

The most effective mechanisms of the innate response against viral infections are mediated by **interferon** and by the **activation of NK cells.**

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| **Interferon** is a **cytokine** with **three different types: α, β**and**γ** .The first two are mainly produced by monocytes-macrophages and to a lesser extent by fibroblasts. However,  interferon- **γ** is produced by CD 4 and CD 8 lymphocytes and NK cells. Interferon has a strong anti-viral action.  |

Virally infected cells produce and release Interferon which prevent replication of viruses, by directly interfering with their ability to replicate within an infected cell.

**IFN can control viral infections by**

- Binding receptors on infected or uninfected cells

- prevents further spread of the virus

**IFN binding to IFN-R**

– inhibits synthesis of viral proteins

– Increase expression of MHC class I molecules

• Enhances the destruction of infected cells by CTLs.

• prevents uninfected cells from being killed by NK cells**.**

IFN binding to receptor on NK cells increases their ability to destroy

cells that have decreased MHC class I expression and/or are

coated with antiviral antibodies.

**The interferon works according to the following:**

1. Viral infection …..Interferon release.

2. Interferon binds to the surface of uninfected cells.

3. Production of antiviral protein blocks translation of mRNA of the virus.This substance is produced by an infected cell. It then reacts with other cells to:-

* + - 1. Activate an RNA endonuclease causing mRNA degradation or
			2. Cause phosphorylation of eIF2 (Eukaryotic Initiation Factor 2) essentially turning off cellular protein synthesis.

**Natural Killer (NK) cells**

•The virally induced MHC class I downregulation

- triggers NK cells to kill the infected cells by release toxic substances **(perforin** **&** **granzymes)** inside the target cell , they initiate a process known as programmed cell death or **apoptosis**  causing the target cell to die

• Recognize infected cells coated with antiviral antibodies

using Fc receptors ( FcR).

• and kill them through antibody dependent cell mediated

cytotoxicity (ADCC).

• Produce increased amounts of IFN.

– Binds IFN-R to prevent production of virus.

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**Adaptive immunity** acts against bothviral particles and infected cells. The most important mechanisms against viral particles are antibodies **(Humoral immunity )**  while the cytotoxic mechanisms are the most important against infected cells. This is mediated by cells (CD8+), antibodies and cells (ADCC) or antibodies and the complement (classical pathway) .

Viruses can also be removed from the body by **antibodies** before they get the chance to infect a cell. Antibodies are proteins that specifically recognise invading pathogens and bind (stick) to them This binding serves many purposes in the eradication of the virus:

• Firstly, the antibodies neutralise the virus (**IgG, IgM and IgA**), prevents the virus from entering the cells.

• Secondly, many antibodies **(IgM)** can work together, causing virus particles to stick together in a process called **agglutination**. Agglutinated viruses make an easier target for immune cells than single viral particles.

• A third mechanism used by antibodies to eradicate viruses, is the activation of phagocytes. A virus-bound antibody binds to receptors, called Fc receptors, on the surface of phagocytic cells and triggers a mechanism known as **phagocytosis**, by which the cell engulfs and destroys the virus.

• Finally, antibodies can also activate the complement system, which opsonizes and promotes phagocytosis of viruses.

 

 **Agglutination of virus**

**Particles by antibodies.**

****•**The antibody can recognize viral antigens on the membrane of the infected cell.**

•**Cell is lysed through activation of complement or activation by**

**ADCC by activating NK cells expressin FCR.**

**Viruses and Acquired Immunity Antibody dependent control of Viruses.**

**Acquired Immunity ( Cell dependent control of viruses)**

Effector cells are **Cytotoxic T lymphocytes (CTLs).**

Cytotoxic T cells have specialised proteins on their surface that help them to recognise virally-infected cells. These proteins are called **T cell receptors** (**TCRs**). Each cytotoxic T cell has a TCR that can specifically recognise a particular antigenic peptide bound to an MHC molecule. If the T cell receptor detects a peptide from a virus, it warns its T cell of an infection. The T cell releases **cytotoxic factors (**Perforin/granzyme pathway **)** to kill the infected cell by **apoptosis** (programmed cell death).

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**Cytotoxic T cell Mechanism**

**Laboratory diagnosis of viruses**

A variety of methods exist to diagnose viral infections with the recent

trend being toward molecular diagnostics**.**

**Diagnostic Methods in Virology**

1. Direct Examination

2. Indirect Examination (Virus Isolation)

3. Serology

* **Direct Examination**

**1- Antigen Detection** in body fluids (e.g., respiratory tract for respiratory viruses) or blood (e.g., cytomegalovirus) or lesion scrapings (e.g, for herpes simplex virus or varicella-zoster virus) with specific immune sera linked to fluorescence or enzyme detection immunoassay (**immunofluorescence, ELISA**).

2. Examination of tissue samples by **light microscopy** for viral induced cytopathology .

3. Examination of body fluids or tissues by **electron microscopy**.

This is not an efficient method and is dependent upon sufficient

numbers of virions being present to permit detection.

4- Viral Genome Detection

PCR amplification , hybridization with specific nucleic acid probes to detect viral nucleic acid in body fluids or tissues.

* **Indirect Examination** **(Virus Isolation)**

**Generally three methods are employed for the virus cultivation** **or isolation:-**

a) Cell Culture [cytopathic effect (CPE) , haemadsorption, immunofluorescence ] .

b) Chicken embryo Egg **[** pocks on chorioallantoic membrane**(**CAM**),** haemagglutination **,** inclusion bodies]**.**

c)Animals (disease or death).

* **Serology**

Demonstration of significant increase in viral Ab in Patient serum during the course of illness. It includes the following tests:

1. Complement fixation test (CFT)
2. Immunoflourescent assay (IFA)
3. Radioimmunoassay (RIA)
4. Enzyme linked immunoassay (ELISA)