3- Laboratory Diagnosis and Treatment of Viral Infection

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Objectives

1. Understand principles of laboratory diagnosis of viral infections
2. Differentiate methods of viral detection and isolation
3. Describe viral reaction to physical and chemical agents
4. Describe and apply common methods of Inactivating viruses
5. Understand principles and classes of anti-viral agents
6. Understand principles, types, and application of viral vaccines

Difficulties

- Can not be seen under light microscope
- Can not be cultivated easily
- Do not grow on culture media
- Treatment was not available

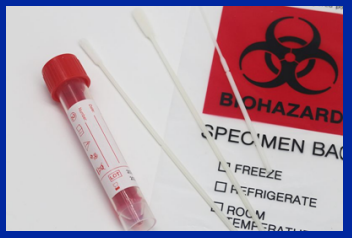
Changed situation

- Rapid techniques
- Screening for Blood transfusion
- Treatment available

Specimens

- According to the disease
  - Respiratory – Throat swab
  - CNS – CSF
  - Eyes- Conjunctival scrapings
  - Viremia – Blood
  - GIT and Liver - Stool
  - Skin - Scrapings

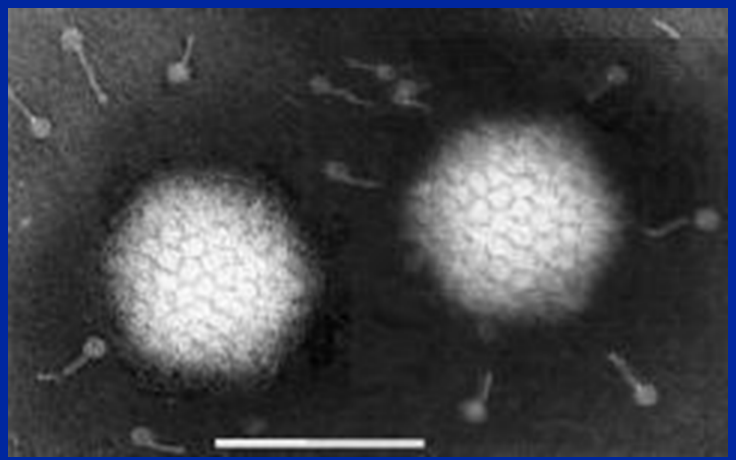
Specimen Storage and Transport

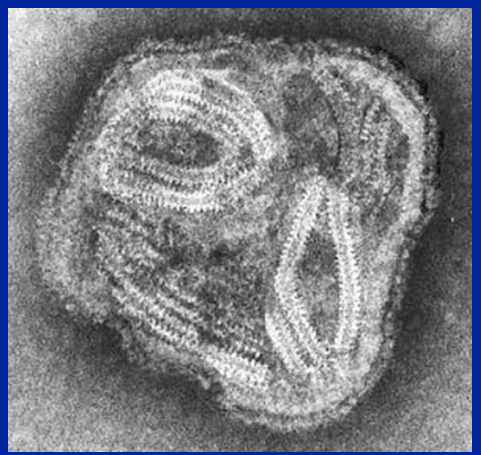


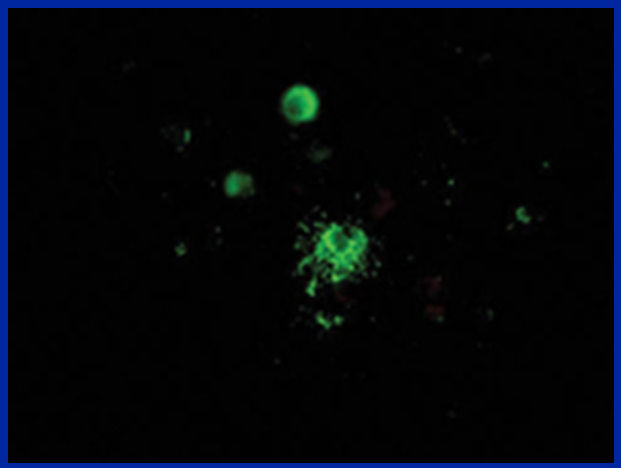
- Keep specimens other than blood at 4ºC
- If delay >24hrs, freeze at -70ºC or below.
- Avoid any storage at -20ºC: greater loss in infectivity
- Nonenveloped viruses more stable than enveloped
- Viral Transport Medium
  - Salt solution – ensures proper ionic concentrations
  - Buffer - maintains pH
  - Protein - for virus stability
  - Antibiotics or antifungals – to prevent contamination

1. Microscopy

- Electron Microscope
- Light microscope – Inclusion bodies
- Fluorescent Microscope -Fluorescent antibody technique

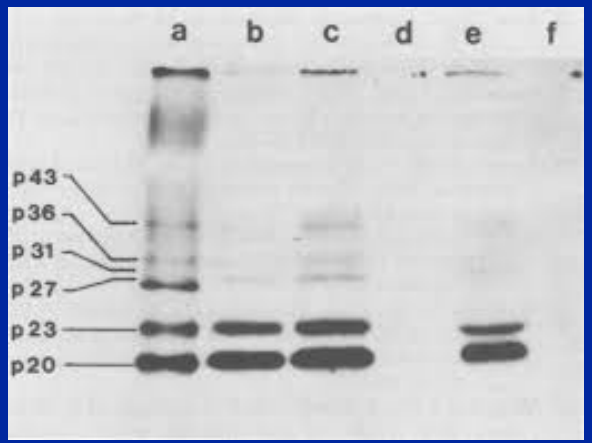


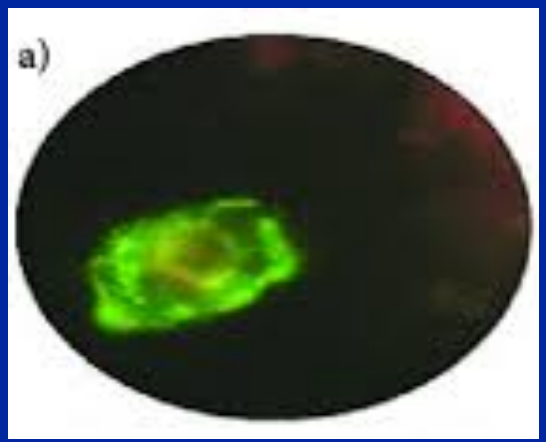




2. Demonstration of Viral Antigens

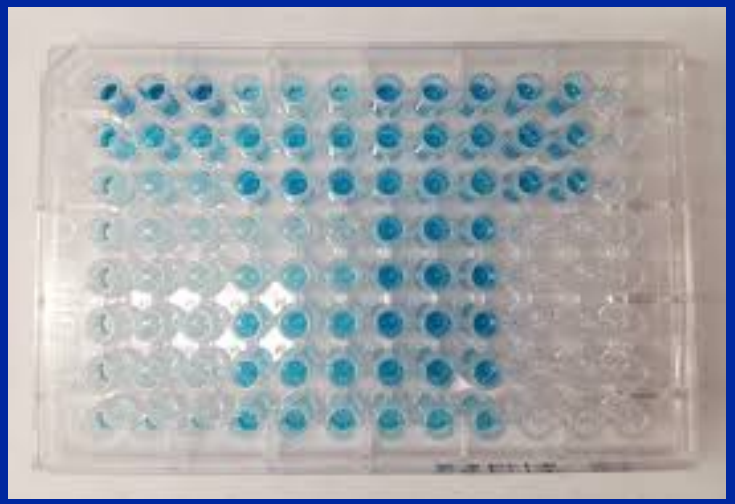
- Precipitation on gel eg HBsAg
- Immunofluorescence
- Enzyme Linkes Immuno Sorbant Assay (ELISA)

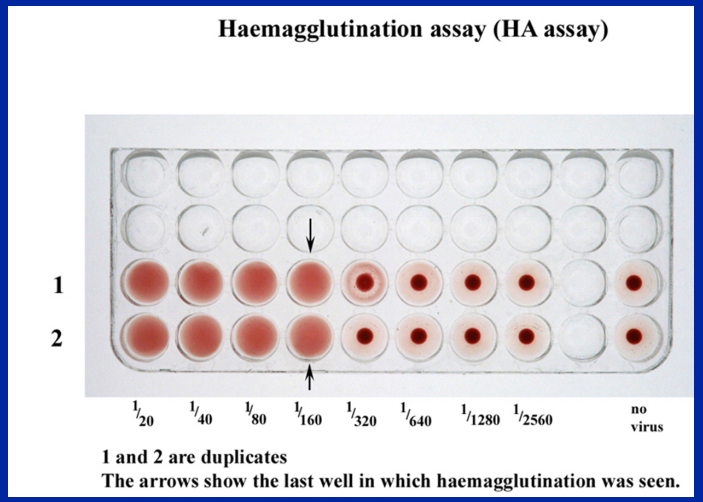




3. Serological Reactions (anti-viral antibodies)

- Rising titre of antibody in paired sample of sera for IgG antibody
  - First sample – At the earliest
  - Second sample – After 2 weeks
- Single sample IgM type of antibody detection
- Techniques –ELISA, Haemagglutination Inhibition (HAI)Test





4. Molecular Techniques

- Nucleic acid amplification techniques such as polymerase chain reaction (PCR) can be used to detect viral genomes in clinic material.
- To detect RNA, an initial reverse transcription step is performed (converts RNA into cDNA). After this, PCR can be performed.
- Molecular assays are very sensitive (able to detect only a few viruses in a clinical sample.)
- They can also be used to measure the amount of virus (viral load) in a patient's sample.

5. Viral Isolation and Culture

Primary purposes of viral cultivation
- To isolate and identify viruses in clinical specimens
- To prepare viruses for vaccines
- To do detailed research on viral structure, multiplication cycles, genetics, and effects on host cells

Using Live Animal Inoculation
- Specially bred strains of white mice, rats, hamsters, guinea pigs, and rabbits
- Animal is exposed to the virus by injection

Using Bird Embryos
- Enclosed in an egg- nearly perfect conditions for viral propagation
- Chicken, duck, and turkey are most common
- Egg is injected through the shell using sterile techniques

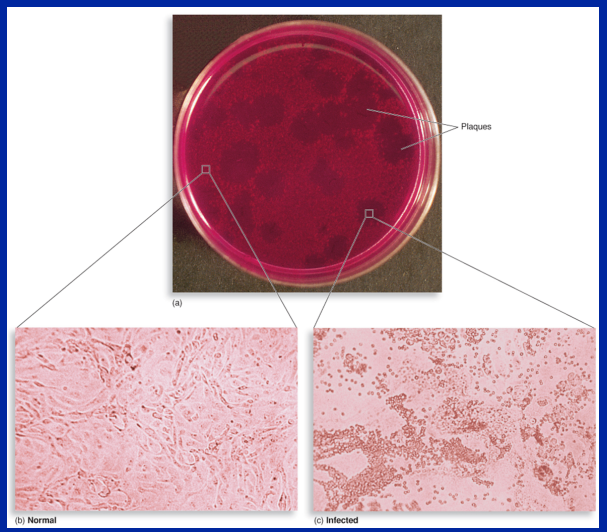
Cell culture for viral identification

Cell Culture

Routinely used for growing viruses
Classified into 3 types:
Primary cell culture – normal cells freshly taken from body & cultured, limited growth
- Rhesus monkey kidney
- Chick embryo fibroblast
- Human amnion cell culture

Diploid cell strains – cells of single type (fibroblast cells) that can be subcultivated for limited number of times, mostly 50
- WI-38: human embryonic lung cell
- HL-8: Rhesus embryo cell

Continuous cell lines – malignant cells, indefinite subcultivtion
- HeLa: Human Ca of cervix cell line
- HEP-2: Human epithelioma of larynx



Detection of Virus Growth in Cell Cultures

- Cytopathic effects (CPE) – morphological changes in cultured cells, seen under microscope, characteristic CPE for different groups of viruses
- Metabolic Inhibition – no acid production in presence of virus
- Hemadsorption – influenza & parainfluenza viruses, by adding guinea pig erythrocytes to the culture
- Interference – growth of a non cytopathogenic virus can be tested by inoculating a known cytopathogenic virus: growth of first virus will inhibit the infection by second
- Transformation – oncogenic viruses induce malignant transformation
- Immunofluorescence – test for viral Ag in cells from viral infected cultures.

Reaction to physical and chemical agents

1. Heat and cold:

- Icosahedral viruses tend to be stable, while Enveloped viruses are much more heat labile
- Viral infectivity is generally destroyed by heating at 50–60°C for 30 minutes
- Viruses can be preserved by storage at subfreezing temperatures

2. Salts:

- Many viruses can be stabilized by salts in order to resist heat inactivation

3. pH:

Viruses are usually stable between pH values of 5.0 and 9.0. Some viruses (eg, enteroviruses) are resistant to acidic conditions. All viruses are destroyed by alkaline conditions.

4. Radiation:

Ultraviolet, x-ray, and high-energy particles inactivate viruses

5. Detergents:

Solubilize lipid constituents of viral membranes and disrupt capsids into separated polypeptides

6. Formaldehyde:

Formaldehyde destroys viral infectivity by reacting with nucleic acid

7. Quaternary ammonium, organic iodine, low-concentration chlorine, and Alcohols are relatively not effective against viruses

Common Methods of Inactivating Viruses

- Sterilization may be accomplished by steam under pressure, dry heat, ethylene oxide, and γ-irradiation
- Surface disinfectants include sodium hypochlorite, glutaraldehyde, and formaldehyde
- Skin disinfectants include chlorhexidine, 70% ethanol, and iodophors
- Vaccine production may involve the use of formaldehyde, ultraviolet irradiation, or detergents to inactivate the vaccine

Treatment and Prevention of Viral Infections

As bacteria and protozoa do not rely on host cellular machinery for replication, processes specific to these organisms provide ready targets for developing antibacterial and antiprotozoal drugs.

However, because viruses are obligate intracellular parasites, antiviral drugs must be capable of selectively inhibiting viral functions without damaging the host, making the development of such drugs very difficult.

Furthermore an ideal drug would reduce disease symptoms without modifying the viral infection so much as to prevent an immune response in the host.

There is a need for antiviral drugs active against viruses, for which vaccines are not available or are not highly effective.

Anti-viral Development

- Viruses are now becoming better understood and several viral genomes have been properly mapped. Scientists are now looking for the best drug targets

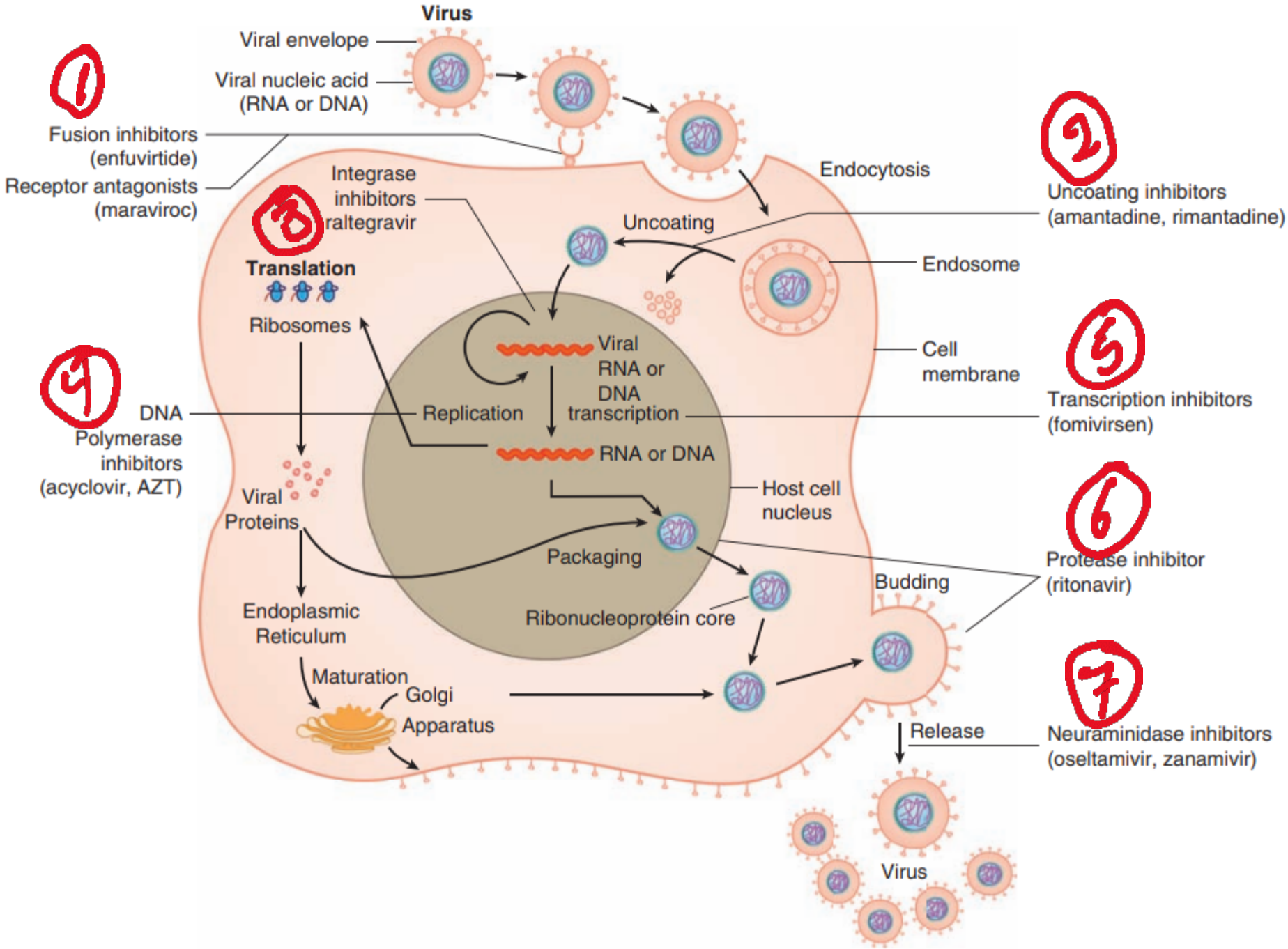
- The main point of interest is any viral protein that the host organism does not normally produce

- Once these viral proteins are identified they are tested using a large-scale screening process to test for effectiveness

Anti-viral Targets

- There are several known methods that the makers of Antiviral drugs are looking at, including:

1. Inhibitors of Attachment
2. Inhibitors of Cell Penetration and Uncoating
3. Neuraminidase Inhibitors
4. Protease Inhibitors
5. Inhibitors of Nucleic Acid Synthesis
6. Nucleotide Analogs
7. Stopping the release of the mature viruses from the host cell


Virus
Viral envelope
Viral nucleic acid
(RNA or DNA)
1
Fusion inhibitors
(enfuvirtide)
Receptor antagonists
(maraviroc)
Endocytosis
2
Uncoating inhibitors
(amantadine, rimantadine)
Uncoating
Endosome
3
Integrase
inhibitors
raltegravir
Translation
Ribosomes
Viral
RNA or
DNA
transcription
Cell
membrane
4
DNA
Polymerase
inhibitors
(acyclovir, AZT)
Replication
RNA or DNA
5
Transcription inhibitors
(fomivirsen)
Host cell
nucleus
Viral
Proteins
Packaging
6
Protease inhibitor
(ritonavir)
Budding
Endoplasmic
Reticulum
Ribonucleoprotein core
Maturation
Golgi
Apparatus
7
Release
Neuraminidase inhibitors
(oseltamivir, zanamivir)
Virus

1. Oseltamivir (Tamiflu)

- Prevents the mature viruses from leaving the cell
- It is a neuraminidase inhibitor, it works on both influenza A and B
- Neuraminidase is an enzyme found on the virus which cleaves sialic acid from cell membrane, leading to a more effective release of viruses
- Used to battle avian flu and influenza

2. Acyclovir (Zovirax)

A widely used antiviral with main implications in the treatment of herpes

Seen as a "new age" in antiviral therapy, Gertrude Elion, its creator, was given the Nobel prize for medicine in 1988

It is a nucleoside analogue and prevents viral replication in infected cells

Inhibits viral DNA polymerase and terminates viral DNA chain growth

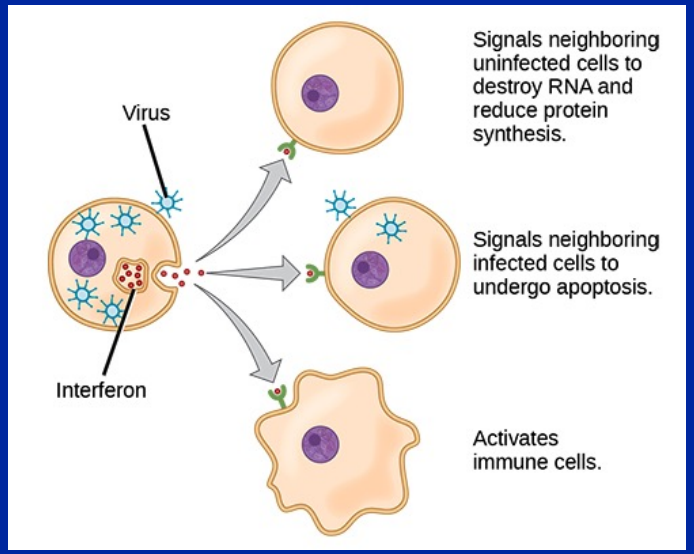
3. Interferons

α and β interferons are produced by all the cells in response to viral infections

γ interferons are produced only by T lymphocyte and NK cells in response to cytokines

The action of interferons leads to an inhibition of translation

Pegylated interferon-α (Peg-IFα) is given for 6 to 12 months to treat chronic hepatitis C disease



Viral Vaccines

- General Principles

Types:

1. Killed-Virus Vaccines
2. Attenuated Live-Virus Vaccines
3. Genetic vaccines

- Proper Use of Vaccines
- Vaccine development and future direction