# LABORATORY DIAGNOSIS OF VIRAL DISEASES

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# VIRUS HOST INTERACTION

- Interaction of virus with host may cause various effect

  ranging from no apparent cellular damage to rapid cell destruction. Considered at three different level :

1) At the cell level – Broad spectrum effect
2) Individual level
3) Community level

# At Cell Level

- Ranging from no apparent cellular damage to rapid cell destruction

  e.g. poliovirus causes cell death (cytocidal) or even cell lysis (cytolysis)

  -cellular proliferation (molluscum contagiosum)

  -Malignant transformation (oncogenic virus)

- Virus infection in tissue culture lead to observable cellular changes—cytopathic effects
- Steady state infection– virus & host cell enter in to a peaceful coexistence but both replicating independently without cellular injury.
- Histological feature in virus infected cells is ‘**inclusion body**’

# Inclusion bodies

- Inclusion bodies are structure with distinct size ,shape, location & staining properties seen under light microscope.
- Situated in cytoplasm e.g.: poxvirus, nucleus herpesvirus.
- Both : measles virus.
- Inclusion bodies are generally acidophilic seen as pink structure when stained with giemsa or eosin methylene blue

# Inclusion bodies cont…

e.g.: Adenovirus form basophilic IB

demonstration of inclusion body useful for diagnosis of viral diseases.

Negri bodies or intracytoplasmic eosinophilic inclusion bodies in brain cell is diagnosis of rabies.

# Pathogenesis of Virus Infection

- Virus infection classified

In apparent (sub clinical ) or apparent ( clinical )

Later may be acute , sub acute or chronic.

Latent infection are different types:

Recurrent herpes simplex & herpes zoster

Clinical manifestation appear after prolonged period of quiescence during virus remain hidden in nerve root ganglion.

- Persistent tolerant infection virus demonstrable in tissue but neither disease nor immune response develops.
- Slowly progressive or slow infection as the incubation period is long : kuru, scrapie, oncogenic virus
- Vertical transmission in some virus from parent to progeny

- Virus enter the body through the respiratory tract , alimentary tract, skin, conjunctiva, genital tract
- Respiratory tract – most important portal of entry for viruses .large no. of viruses are able to infect the cells of respiratory tract.
- Some of them multiply locally to initiate a silent local infection-lymphatic or haematogenous –systemic illness.
- e.g. smallpox & chicken pox

- Influenza & rhinovirus –restricted to RT—multiply & produce local disease—respiratory viruses
- Genital tract– HIV (sexually)
- Alimentary tract

Most important route of entry for virus

Enterovirus, adenovirus, reovirus, hepatitis

Virus causing gastroenteritis set up intestinal infection –confined to gut– local disease

- Poliovirus multiply in gut & transport to target organs
- Skin: produce local lesion –papilloma, vaccinia, cow pox, molluscum contagiosum & orf→ dermal lesion enter thru' the skin abrasions (papilloma virus), insect bites (arbovirus), animal bites (rabies), injection (HBV)
- Conjunctiva: local (adenovirus)

  systemic (measles)

# Spread of virus in the body

- Incubation period

Time taken for virus to spread from the site of entry to the organs of viral multiplication & then to target organs for production of lesions

Site of entry & site of lesions are same –IP short 1-3 days– respiratory infection & gastroenteritis

In systemic disease IP long 10-20 days

e.g. small pox, chicken pox

# Incubation period cont..

- Yellow fever or dengue IP shorter 5-6 days
- IP in HIV infection may be several years
- Papilloma & molluscum contagiosum-long IP
- Host response to virus infection:

Host resistance may be immunological or nonspecific

genetic & physiological factors such as

-interferon production

-body temp.

-nutrition

-hormones

# Immunity in virus infection

Virion –good antigen → AMI
Virion –good antigen → CMI

Surface & internal Ag / non structural Ag as early proteins

In mediating humoral antiviral immunity

Ab IgG, IgM & IgA

IgG & IgM –major role in blood & tissue spaces

IgA – more important in mucosal surfaces

- Ab act in following ways:
- Neutralize the viruses & prevent their attachment, penetration & uncoating
- Attach to viral antigen on infected cells – lysis by complements or destruction by phagocytic/killer cells
- Immune opsonisation of virus & phagocytosis

# Cell mediated immunity

- Primarily mediated by T lymphocytes
- T lymphocytes cytotoxic to virally infected host cell
- T cell produce interferon which interfere with viral multiplication

# Non immunological response

1. Phagocytosis
   - PMN leucocytes do not play imp. role
   - macrophages phagocyte viruses (blood stream)
2. Body temperature
   - inhibits viruses (>$39^0$C)
   - Exception is Herpes simplex virus

   e.g. Herpes febrilis is frequent accompaniment of fever caused by pneumonia, streptococci, influenza & malaria.

3. Hormones

- Corticosteroids ↓ virus infection
- Cortisone ↑ immune response & interferon synthesis

4. Malnutrition

- Measles – higher complications & higher case fatality

5. Age

- common & more dangerous at two extremes of age except influenza

# Interferon

Definition

Antiviral substances produced by cell in response to viral infection & also in response non viral microorganisms.

- Isolated, characterized & named by Isaacs & Linden Mann (1957)

- Not virus specific

- Some have host (species) specificity e.g. mouse interferon are ineffective in humans & a vice versa

-Viruses also varies in capacity to induce interferon - cytocidal & virulent viruses are poor inducers & avirulent viruses are good inducers

- RNA are better inducers than DNA viruses

- production is increased in fever

- inhibited by steroids & increased oxygen tension

Interferon synthesis increase within 1 hrs and reaches maximum in 6-12 hrs

Cellular transcription & protein synthesis are necessary for interferon production

# Interferon cont..

- Interferon have been classified based on antigenic characters, cell origin & other properties-
- 3 types of IFN- alpha, beta & gamma
- Human interferon alpha – HuIFN-α
- **Alpha interferon (IFN-α)** –known as 'leucocyte interferon'

  Produced by leucocytes –induced by suitable viruses

  Nonglycosylated protein

  16 antigenic subtypes identified

- **Beta interferon (IFN-β)** – known as ‘fibroblast interferon’

  Produced by fibroblasts & epithelial cells –induced by viruses or polynucleotides

  Glycoprotein
- **Gamma interferon (IFN-γ)** – known as ‘immune interferon’

  Produced by T lymphocytes stimulation by antigens or mitogens

  Glycoprotein in nature

  Immunomodulatory & antiproliferative action

  Have a separate cell receptor

- Inactivated by proteolytic enzymes  but not by nucleases & lipases
- Resist heating at 56-60$^{o}$C for 30-60 min
- Stable at wide range of pH (2-10) except gamma IFN labile at pH 2
- Molecular wt. 17,000
- Nondialysable & nonsedimentable
- Poorly antigenic – no serological test available
- Potency expressed in IU/ml
- cellular transcription & protein synthesis necessary for interferon production

# Biological effects of interferon

- **Antiviral effects**: induction of resistance to infection
- **Antimicrobial effects**: resistance to intracellular infection e.g.toxoplasma, chlamydia, malaria
- **Cellular effects**: inhibition of cell growth & proliferation, & of DNA & protein synthesis, increased expression of MHC Ags on cell surfaces.
- **Immunoregulatory effects**: enhanced cytotoxic activity of NK, K, T cells, activate macrophage cytocidal activity, modulate Ab formation, activate suppressor T cells, suppression of DTH

# Lab. Diagnosis of viral diseases

Use: prevention of some diseases (screening)

Etiological diagnosis – of rubella

Early specific therapy in herpetic encephalitis & eye lesions

Detection & prediction of epidemics

Identification of antigenic variation of viruses

Discovery of new viruses

# Routinely employed lab methods

- Microscopic demonstration

Virus elementary bodies by examination of stained smear - rarely used

Use of electron microscope –Less commonly

Fluorescent microscope (Ab detection)

- Demonstration of virus antigen:

Virus Ag is abundant in lesions, its demonstration by serological method – precipitation in gel or immunofluorescence

CIEP, RIA & ELISA – wide application in diagnostic virology

- Isolation of virus:

Most viruses are heat labile

Inoculation in animals, eggs or tissue culture

Isolates are identified by neutralization or other suitable serological procedures

Many viruses (adeno, entero) frequently found in normal person

- Serological diagnosis:

Increase of A -b to a virus during the course of a disease is strong evidence

Examination of paired sera the ‘acute’ sample collected early in the course of the disease & the ‘convalescent’ sample collected 10-14 days later

Single sample –IgM detection important

Serological techniques – neutralisation, CFT, ELISA, HAI tests.

# Samples for viral diagnosis

- Respiratory system: throat swab, nasopharyngeal swab
- CNS : Faeces, blood, CSF
- CVS : Faeces
- Skin : macular/papular scraping, faeces
  vesicular/ pustular fluid, ulcer scraping, crust, throat swab
- Eye : conjunctival scrapping or swabs
- Liver : blood for yellow fever
- Congenital infections : throat swab conception products

# Immunoprophylaxis of viral diseases

- Viral vaccine more effective than bacterial vaccine
- May be live or killed
- Live vaccines prepared by plaque selection or from its mutants & development of vaccine strains with desired antigenic characters by recombination e.g. small pox, polio, mumps, yellow fever, influenzae, rubella

- Killed vaccine prepared by inactivating viruses with heat, phenol, formalin or BPL e.g. polio, rabies, mumps, JE