Pox Virus

The small pox virus or the variola virus is representative of the pox viruses a group of agents that infect both humans and lower animals and produce characteristic vesicular skin lesions often called pocks. Pox viruses are the largest of animal viruses. They can be seen with phase optics or in stained preparation with the light microscope. The viral particles originally called elementary bodies are some what rounded brick shaped or ovoid and have a complex structure consisting of an internal central mass, the nucleoid surrounded by two membrane layers. The surface is covered with ridges which may be tubules or threads. Pox viruses contain DNA, protein and lipid. They are relatively resistant to inactivation by common disinfectants and by heat, drying and cold. All pox viruses studied are related immunologically by a common internal antigen extractable from viral particles by 1(N) NaOH. They can be divided into genera on the basis of their more specific antigen, nucleic acid homology, morphology and natural hosts.

Characteristics of Pox viruses :-

1) **Size:**
   250 – 390 nm in length by 200 – 260 nm in breadth.
2) **Morphology:**
   Brick shaped to ovoid. Protein and lipid content.
3) **Stability**:
   Relatively resistant to inactivation by chemicals or by heat, cold or drying. Inactivated by chloroform, variable inactivation by ether.
4) **Nucleic acid**:
   Double stranded DNA. MW and base ratio are - DNA for vaccinia is of 150 x 10^6 daltons MW and size is 231 Kbp. AT:GC ratio is 1.67
5) **Antigenicity**
   Common family and germs antigens.

Pox viruses possess the unusual capacity to reactivate other members of the family previously inactivated by heat or 6 M urea when both infectious and inactivated viruses infect the same cell. The reactivation is accomplished by the infectious viral particles producing the enzyme which uncoates the inactivated viruses and frees its intact DNA from the denatured viral coat permitting it to express its genes and multiply. Genetic recombination may also occur but only between two viruses of the same immunologic group that is known as homoreactivation. Pox viruses vary widely in their ability to cause generalized infection but they share a mode of infection for epidermal cells in which they multiply in the cytoplasm and produce eosinophillic inclusion bodies. Most Pox viruses also multiply readily in epidermal cells of the chorioallantois of chick embryos when they produce characteristic nodular focal lesions termed pocks.

Variola and Vaccinia:-

Variola is small pox viruses and vaccinia is the general pox viruses.

Properties of the viruses:-

The virulence and contagiousness of small pox virus have understandably limited its laboratory investigations. On the other hand the closely related and much less dangerous vaccinia virus is one of the most thoroughly investigated animal viruses. Since the two viruses are very similar in their properties they have been discussed together.

Morphology:-

Pox viruses have a complex architecture without obvious symmetry. Vaccinia virions are brick shaped particles with rounded corners and a central dense region with dense areas on each side. The viral surface layer revealed by negatively stained and freeze etched specimens is composed of thread like double ridged beaded structures that appear to curve around the particle. Thin sections disclose a central nucleoid with the dumbbell shaped dense core composed of regularly arranged DNase sensitive electron dense thread like structures. The nucleoid is surrounded by lipoprotein membranes. Between the nucleoid and the outer viral coat is an ellipsoidal body which causes the central thickening of the virion. Viral particles from small Pox crusts and the vesicle fluids are morphologically indistinguishable from vaccinia particles.

Chemical and physical characteristics:-

Vaccinia virus was the first animal virus to the prepared in sufficient quantity and purified for detailed chemical and physical measurements. The viral particles are composed of about 3.2% DNA, 91.6% protein, 5% lipid including cholesterol, phospholipid and neutral fat and 0.2% non DNA carbohydrate present in membrane glycoproteins.

The viral core contains several enzymes primarily for the transcription of immediate early mRNA, i.e. the DNA dependent RNA polymerase, polyadenylate polymerase, methyl and guanylyl transferase, two nucleoside triphosphatase (NTP) phosphohydrolase, single stranded DNA endonuclease and exonuclease and a protein kinase.

Stability:-

Variola and vaccinia viruses are relatively stable. They resist drying and retain their infectivity for many months at 4°C and for years at -20 to -70°C. the relative resistance of the virus to dilute phenol and other common disinfectants complicates the decontamination of clothing, instruments, furniture etc, however variola and vaccinia viruses are inactivated by apolar lipophilic solvent, i.e. chloroform, by autoclaving__ or by heating at 60°C for 10 mins.

Antigenic structure and immunity:-

The antigenic structure of pox viruses has been examined largely with vaccinia virus but small pox virus is very similar. Viral strains from cases of severe small pox, i.e. variola major and those from cases of variola minor are immunologically indistinguishable.

Antibody response:-

Following infection and immunization antibodies develop for each of the viral antigens. The variations observed in their time of appearance and perristance depend on the nature and quantity of the antigen. Immunity to small pox is long lasting, if not persistant for life, following natural infection. In the rare reinfections that have been reported the disease is usually atypical, very mild and often without skin rash. If infection occurs in artificially immunized individual the clinical disease is also usually milder in unimmunized neighbours. The relative importance of humoral and cellular immunity remains largely unexplored. However immunization with live vaccine has led to disease in persons with congenital defects of tymus derived cells, i.e., T cells.

Host Range

Variola virus has much more limited host range than do vaccinia and other pox virus. Monkeys are the only animals other than man known to be naturally infected. Also when variola virus is placed onto monkeys scarified skin or inoculate intradermally local lesions fever follow. In rabbits variola virus can initiate Keratitis and local skin lesions but it cannot be propagated serially. Many animals including the chick embryo, rabbit, calf and sheep are susceptible to vaccinia virus and have served as convenient host for investigation and production of vaccine. Epidermal cells show the greatest sensitivity. Cultured cells commonly used for propagation of variola virus are human embryonic kidney, monkey kidney and continuous lines of human epithelium like cells. The slowly developing cytopathic effects become maximal in 5-8 days and vaccinia virus propagates in culture more rapidly to a higher and in a wider variety of animal cells than does variola virus.

Multiplication of the viruses

The pox viruses contain DNA. Biosynthesis of viral components and their assembly into viral particles take place entirely within the cytoplasm of the host. However, the biosynthetic reactions can occur in the nucleated cells.

The virus attaches to the host cell receptor whose chemical nature is still unidentified and it enters the cytoplasm by membrane to membrane fusion. After penetration the viral DNA has a two stage uncoating process. The uncoating is initiated almost immediately after penetration. The preexisting host cell enzymes which breakdown the viral membrane phospholipid and part of the protein coat of the viral particle to free the nucleoprotein core. The second stage after a lag of 30-60 min results in breakdown of this core to liberate viral DNA.

At the onset of this second stage when the DNA within the intact core is still protected from DNase activity a DNA dependent RNA polymerase present in the core transcribes about 25% of the viral gemome. The resulting transcript is processed within the core and functional immediate early mRNAs

emerge to code for proteins including one probably a proteolytic enzyme required for the final uncoating events. The liberated viral DNA is stable and can transmit its genetic information for several hours.
As the DNA is released from the core the RNA for a second set of mRNAs known as delayed early mRNAs is transcribed in the cytoplasm. Finally, after viral DNA replication begins late mRNAs appear and atleast some early messengers continue to be made. Synthesis of specific enzymes and of a few viral structural proteins begins early in the biosynthesis process, before replication of viral DNA. The products include second stage uncoating protein, 3 proteins associated with the nucleoprotein core and enzymes related to the DNA biosynthesis, i.e., thymidine kinase, DNA polymerase, Nucleases and polynucleotide ligase. The mode of DNA replication is imposed by the unique covalent crosslinking of the two strands synthesis is initiated at either end of the genome. Large circular and forked replicating forms found indicating that an endonuclease cleaves the single stranded crosslinks at various times during replication. Late viral proteins are first detected about 4hrs after infection and infectious virus is formed about 1hr later by packaging viral DNA randomly selected from the preformed pool. Now posttranslational modifications of several proteins like cleavage, glycosylation, phosphorylation are essential for virion matuaration. Morphogenesis depends particularly upon cleavage of three major proteins which together comprise about 35% of the virion's protein mass.

Pathogenesis by Small pox virus:-

Two basic forms of small pox are recognized,i.e., Variola major which has a case fatality rate of approximately 25% and variola minor are less virulent form wit a mortality rate below 1%. Although a variety of factors may influence the mortality rate in any epidemic. The epidemiologic evidence is convincing that severe and mild small pox exist in distinct entities. Virus multiplies first in the mucosa of the upper respiratory tract and then in the regional lymph nodes. A transient viremia then disseminates virus to internal organs,i.e., liver, spleen.lungs where the virus propagates extensively. A second viral invasion of the blood stream terminates the incubation period,i.e., about 12 days and initiates the toxemic phase characterzed by rashes, fever, generalized itching, headache. Virus spreads to the skin and multiplies in the epidermal cells. The characteristic skin eruption follows in 3-4days. In severe cases the rash may become haemorrhagic. The inclusion bodies surrounded by a clear halo characteristically develop in cells of the skin and mucous membranes infected with variola or vaccinia virus. Each inclusion body consists of an accumulation of viral particles and viral antigens. A hypersensitivity response to viral antigens may contribute to the eruptive lesions of small pox. The toxin like properties of the viral particles may also play a role in the cell necrosis. It is clear however that pustule formation does not result from secondary bacterial infection.

Epidemiology:-

Small pox is confined to man and is spread chiefly by person to person contact. Although Small pox is considered to be highly contagious the spread is slow and the probability of infection from a single exposure appears to be low. Initially the virus is transmitted from the lesions of the upper respiratory tract in droplet secretions or by contamination of drinking or eating utensils and later when pustules ruptures and crusts are formed the lesions also become a source of contagion. Air borne transmission of variola virus is unusual but can occur as has been demonstrated epidemiologically and experimentally. Although any person infected with variola virus is potentially contagious the most dangerous disseminators are persons with unrecognized disease,i.e., partially immune patients with few lesions. Such sources are easily overlooked or misdiagnosed have been primarily responsible for introducing small pox into countries free of the disease.