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POXVIRUSES AND LUMPY SKIN DISEASE VIRUS VACCINE

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Abstract

Poxviruses have very overwhelming effects on the economy. The majority of the poxviruses that cause diseases are animals and birds that contribute their part in the global economy. The very wide host range of poxviruses has played a significant role in disease spread in various species. The Orthopoxviruses are the most widely studied poxviruses because of their dual host range. Different classes of attenuated/inactivated vaccines have been developed to control and eradicate poxvirus diseases. Many vectored vaccines prepared from alternative strains of poxviruses have been licensed to be administered in humans and animals. Different genes of immune-modulation have been discussed that can donate their part of bioterrorism or prevent bioterrorism. In this article, we have reviewed how poxviruses could be of importance in living organisms.

Keywords: *Ascorbic acid,* GC-MS, *IC50, RT*

INTRODUCTION

The poxvirus is a member of the Poxviridea family which consists of 83species and spread across 22 genera and two subfamilies. Their sub families include Chordo poxvirinea which infects vertebrates and Entomopoxvirinea which infects insects (1). The Entomopox viruses (EVs) closely resemble human viruses of Orthopox viruses and Molluscipox viruses. Vago was the first who describe a pox-like virus in insects (2). Recent studies and genomic sequences of EVs have confirmed these viruses as poxviruses. Very well-known viruses of the Chordopoxvirinea that can infect both humans and animals are Variola(Smallpox), Cowpox, Monkey pox, Camel pox, Sheep pox, Goat pox and Lumpy Skin Disease virus (Capripoxvirus) and buffalo pox (3).

HISTORICAL AND CLINICAL BACKGROUND

Poxviruses date back to mummy Ramesses V where lesions of smallpox were found on the body(died 1145 BC) and the black and white documentation of the Eastern Jin dynasty(283-343 China) showed smallpox-like symptoms. Smallpox, in comparison to other infectious diseases, has caused more human deaths (4). Thirty to forty per cent of mortality, the smallpox (variola virus) killed 300 million people in the twentieth century (5). The estimation in endemic was nearly 5 million deaths per year. Another infectious virus of the pox family is molluscum contagiosum which causes benign tumors in healthy individuals and complicated diseases in immune-compromised people (6).

GENOMIC AND MORPHOLOGICAL DISTINCTION

Poxviruses share similar morphological characteristics which may be the reason of sharing immunogenicity among individuals. Virion in non-enveloped stage consists of two forms, the intact M (mulberry) found in vesicular fluid and the deteriorated M form known as C (capsule) form linked with dried scabs. The axial ratios are 1.2 with oval and brick shaped and about 300-400 nm in size (7, 8). The NP antigen of vertebrate poxviruses is assumed to be capable of non-genetic reactivation (9). The core is surrounded by 50-55 nm bilayer lipoproteins (10). The env0elope covered by the membrane is enough in infection. In pigeonpox virus, some virions were observed acquiring envelop in cytoplasmic vacuole (11). The envelop, covered by the membrane, is enough in infection. The linear A+T rich double stranded DNA can have 130-365 kbs and consist of more than 200 genes. The *Chordopoxvirinea* poses 90 gene families and 49 gene families are conserved in overall *Poxviridea* family (12).

LUMPY SKIN DISEASE VIRUS

Lumpy skin disease virus (LSDV) belongs to *Capripoxvirus* genus of *Chordopoxvirinea* (13). It mainly affects cattle that results in skin lesions initially. Signs and symptoms of infected animals are sudden fever, oedema, eye discharge, lymph nodes, weight loss and reduction of milk. As the disease progresses, blisters can be diagnosed in ocular, nasal and oral mucous membrane (14). Disease's severity depends on the breed of animal, age, immunity and virulence factor of the virus. The Lumpy skin disease (LSD) outbreak is associated with blood feeding arthropod. The movement of arthropod vector is decided by wet and warm weather. The female mosquitoes Aedes aegypti have been shown to be the cause of LSDV transmission in experimentally infected animals (15). The presence of Aedes albapictus was anacdotical in LSD pandemics (16). Direct transmission is seen in shared drinking trough, sucking milk and semen (14, 17). Subclinical transmission is observed by contact with intact skin and tick. Though, the viable viral count is very low in intact skin (18).

First country to be affected with LSD was Zambia in 1929 and it spread across the Africa and gradually in other countries (19). Mostly economy affecting viruses are the poxviruses. This is due to vector abundance and susceptible host and frequent social contact. Natural hosts include vertebrates and arthropods. The recent outbreak in Asian countries like China, India, Bangladesh, Iran and Pakistan had very drastic effects (20). The Baluchistan province, where most of the livelihood depends on livestock, was affected badly in comparison to other provinces. Almost 700,000 of livestock and small animals faced severe mortality that caused high inflation (21, 22). Highest rate of mortality was seen in Khyber Pakhtunkhwa and Baluchistan. Economy of Pakistan was shocked with More than 3 million dollars of loss (20). *Capripoxviruses* have been identified as agroterrorism agent because of its spread in cattle (22).

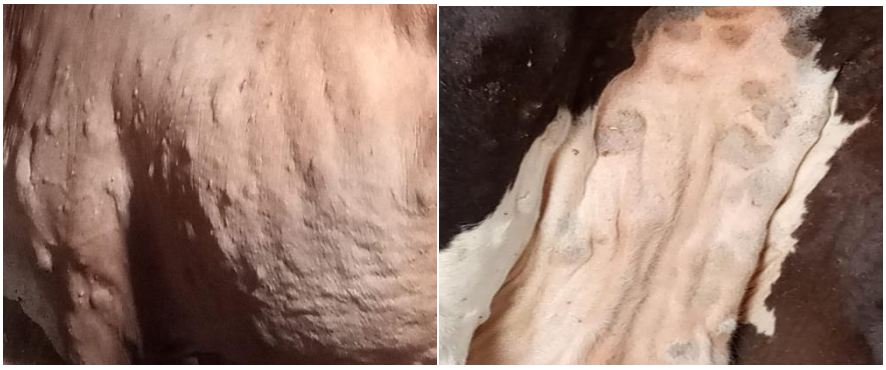

Fig. 1. Clinical presentation of LSD from Balochistan, Pakistan

POXVIRUS TRANSMISSION

One of the main factors of the diseases' spread is the transmission of pathogen and its reservoir. The transmission can either be vertical or horizontal. The transmission also depends on your area of contact, environment and temperature. The poxvirus transmission can be between animal to animal, animal to human, human to human, insect to animal and insect to insect. Transmission in humans may occur by close contact with respiratory droplets, body fluids, skin lesions, and expo-sure to genital and anal lesions of an

infected person (23-24). The reservoir for Cowpox viruses are wild rodents and transmission in human occurs by cats and rodents (25). Monkeypox virus transmission from monkeys to humans and human to humans occurs by direct contact with body fluids, blood, muco- cutaneous lesions of an infected animal or human (26). An arthropod vector has been associated in transmission of camelpox in camels (27). The volepox virus has low trans-mission rate from mice and voles to humans due to low contact (28). Most of the poxviruses are transmitted by vector in animals and in humans the transmission is through contact with infected individual, animal or their fluids.

Fig. 2. Clinical presentation of Pox viruses in various animal population (Bhanuprakash et al., 2012)

IMMUNOLOGICAL IMPORTANCE

The role of immunology is very critical for survival. Initial infection is targeted by a nonspecific immune response (29). The proinflammatory microcircumstances trigger cytokines, chemokines and interferons (IFNs) by the neutrophils, dendritic cells (DCs), macrophages and natural killer cells (NK). The antiviral cytokines such as tumor necrosis factors, interleukin (IL) 1B, IL-18, are produced by these cells. These cytokines induce T helper type 1 (Th1) response, which is critical-ly important for poxvirus clearance (29). However, poxviruses can manage host immune responses through cooperative response of many virulence factors (30, 31). Some viruses when engineered with different immunological genes can have very devastating effects. For example IL4 can turn the vaccinia virus or cowpox virus into a potent pathogen of humans (32-33). The memory immune responses are interfered by the IL4 carrying virus. The Capripoxviruses are observed to carry IL18 binding protein that results in increased virulence (34-35). The monkeypox virus can be turned into more deadly for humans by removing the IL1beta related protein gene (36). By engineering vaccinia virus with IL12 and IL18 gene, the process of virus clearance could be blocked (37).One of the vaccinia virus secreted proteins is involved in inhibition of classical and alternative pathway by binding to the C3b and C4b molecules (38). Various host genetic factors are needed for susceptibility of different hosts specific to Orthopoxvirus. Orthopoxvirus and variola virus target TNF as a viral activity to evade host immune system (39). In addition, it can be used to accept a large amount of DNA and not losing replicative ability and deliver genes or as carrier in various diseases including HIV. Thus it points out to the dual use of pox viruses.

POXVIRUS IMPLICATIONS TO FIGHT OTHER DISEASES

Poxviruses are used as a tool for combat other infections. Its modification into vaccine vector, oncolytic activity for malignancies, delivery of genes, expression of protein and the large genome allows us to insert multiple genes of up to 25 kb through genetic engineering in viral genome (40-41). The advantage

of using poxvirus as an oncolytic agent is due to its ability of replicating in cytoplasm rather than nucleus and it remains apart from integrating into host DNA. The cascade expression (early, intermediate and late classes) of poxvirus genes helps us in understanding and modulating expression of foreign genes (42-43). The first oncolytic virotherapy vaccine is developed from modified vaccinia Copenhagen strain could stimulate antitumor immune responses and lyse malignant cells. The gene for viral thymidine kinase was deleted and a granulocyte monocyte colony stimulating factor gene was inserted (44-45). A number of viruses with diverse capabilities are under developmental oncotherapy. However, poxviruses are the most promising candidates. Poxviruses of alternate genera (*Orthopoxvirus*, Yatapoxvirus and Leporipoxvirus) are focused to be modified. The greater advantage of myxoma virus is found to be non-replicating in normal human cells. However, it can infect and kill the non rabbit cancer cells along with human malignant cells (46). In addition to, the Trovac AI H5 expresses avian influenza H5 antigen, which is a fowlpox virus based influenza vaccine, is used in Central America (47). The canine distemper virus, equine influenza virus and rabies virus are used in ALVAC which is a canarypox virus based vector system to develop veterinary vaccines (48-49).

POXVIRUS DIAGNOSTICS

The type of diagnostic test to be done depends on choice of identifying causative agent, shape, virion demonstration, antigenic proteins, tissues filled with nucleic acid, excretions and secretions and antibodies detection. Seasoned skills are crucial to identify an agent by growing it in embryonated eggs and tissue culture. Chick embryos are used for pox virus culture. Various established cell lines (Vero, Hela, BSC-1, MRc-5 human diploid fibroblast cell, chick embryo kidney cells, permanent quail cell line QT-35, chicken embryo dermal cell, porcine kidney PK15, & rabbit kidney cell RK13) are helpful for many of the poxviruses (50). Electron microscopy is thought to be the first line in poxvirus particles identification, though it cannot distinguish species or genus due to similar morphology. The virus of 105 is the minimum for suitable microscopic examination. The affected tissues are stained with hematoxylin and eosin (HE) alternate (Giemsa or acridine orange stain). Inclusion bodies are in red apparent of 0.2-0.3 um (51). Within a given genus, pox-virus identification and differentiation is insufficient serologicaly because poxviruses share similar antigenicity. The *Orthopoxviruses* and *Capripoxviruses* can easily be detected with antigen capture ELISA. The detection range for Orthopoxvirus is 104-105 TCID50/ml (52). Fast, definitive and sensitive results can be obtained with PCR. Distinction of *Capripoxvirus* (viral attach-ment protein gene and fusion protein gene) can be done with newly introduced PCR assay (53). Authenticity verification of amplicons and target regions of viral genomes can be confirmed with fluorogenic DNA probes in real time PCR. Evolutionary and epidemiological studies are com-pared using sequencing and Bioinformatics. The 52 sequences of poxvirus genome are accessible at www.poxvirus.org representing all the eight genera of *Chordopoxvirinea*. Observed differ-ences of human pathogenecities is now understandable with the help of sequencing (54). For antibody production, enzyme linked immunosorbant assay and neutralization assay is international-ly standardized. The P32 structural protein of *Capripoxvirus* can be detected from biopsy or supernatants of tissue culture by antigen capture ELISA (55). The western blot assay, because of its specific poxvirus protein identification is termed as "Gold standard". The cell lysates of Capri-poxvirus infection are used in western blot assay (56).

ENVIRONMENTAL RESISTANCE of POXVIRUSES

Poxviruses are highly resistant to drying (57). The release method and material (dermal curst, serum, blood and other excretions) of the virus into the environment decides the resistance of the virus (58-59). Particularly bed linen, dust, blanket and personal clothes remain infective with poxviruses and transmissible for months. Virus that is isolated from environment or patient is generally more resistant than the culture isolates (60). Poxviruses possess high temperature endurance in comparison to other enveloped viruses. Cell bound viruses showed 2 log steps titer reduction on heating at 56C for 15 min. Temperature stability within genera and subfamilies is also observed. *Parapoxviruses* are inactivated at 56C for 2.5 h or at 80C for 1 h.

However, *Avipoxviruses* are inactivated at 56C for 1 h. A 4log step reduction can be obtained by treating pure concentrations of virus at 56C for 15 minutes in the presence of 2% fetal calf serum (61).

VACCINE PREPARATION

The very first method used to treat viral infections was variolation. Variolation has proved to be effective protective measure from time to time. Until smallpox has emerged, the death rate in-creased the concerns that if there could be more effective method. The milkmaids, who were re-sistant to smallpox, gave the idea of immunity. Later on, Dr. Jenner confirmed the with his exper-iment of injecting material of the maid's sore into an eight year old boy, and then injecting the boy with smallpox one year later. The boy was protected from smallpox. The procedure was deemed vaccination (from latin vaccinus "from cows") in 1803 (62).

Emerging and re-emerging of different viral infections in humans and animals represent potential health risk in developed and developing countries. The *Poxviridea* family possesses several viruses of medical and veterinary importance. Different types of approaches were applied in production of safe and non infectious vaccine. The first generation vaccines (Dryvax, Lister, temple of heaven and EM-63) were live, animal passaged and virulent. These vaccines were neither highly purified nor cloned and could have microbial contamination. The second generation vaccines were live, tissue culture produced but virulent. The cultured cloned vaccine had the advantage to increase the existing stockpile. ACAM2000 is a cultured cloned of Dryvax and had similar anti-body and vaccination rate (63). The third generation vaccine was live, tissue culture produced and attenuated. The MVA is the attenuated form of VACV grown and passaged consequently in chicken embryo fibroblast. This resulted in loss of replication capacity of the virus in mammalian cells, reduction in transmission and dissemination risk (64). Human epitopes of CD8 and CTL were conserved in MVA after identification (65). This modification provided its safety and abil-ity in several animal models against poxvirus infections (66).

LSDV VACCINES

Immunogenicity refers to provoking one's immune response by non self antigens. Vaccines are potential immunogen that are used to elicit an immune response. Different strains of LSDV are used in disease prevention. There were 5 types of vaccines, used in LSD control previously (67). The Neethling strain is used in South Africa and the Romanian sheeppox and Goatpox were used in Egypt. Because of the severe reaction of sheeppox and goatpox vaccines in the exotic cattles, Neethling strain vaccine is thoroughly adopted. With the advent of new technology, LSDV subunit and recombinant vaccines have been developed. The P32 structural protein was used in combination with Freud incomplete adjuant and proved to be effective by producing antibodies (68). Recombinant vaccines with the *Capripoxvirus* genomes are utilized with the help reverse genetic application. The F and H protein genes have been used in vaccine development (17). Vaccinated cattles with recombinant vaccines provided protection for a period of 3 years against LSDV challenge (69). The caprirab recombinant vaccines provide lifelong immunity against rabies and LSDV (70).

CONCLUSION

The poxviruses pose attentive threat to economy and public health. There is a very momentous roll of the viruses' ecology, transmission, molecular biology and replication rate in taking control over host cells. Understanding these processes will help us manage, diagnose and treat these A cellular organisms to prevent future outbreak. The main reason for the prevalence and stability of poxviruses is lack of knowledge in dairy and poultry workers. The Asian countries which were free of disease previously are now concerned a lot because most of the countries' economy stands on livestock and Agriculture. The Asian outbreak of LSD can be spread to Middle East and Africa. In addition to mammals, wild bird and domestic bird species along with penguins, chickens, turkeys and songbirds can be targeted by avian poxviruses. There is dire need of prevention and eradication of such economy and life threatening diseases by mass vaccination. A

concrete control strategy of vector control, livestock mobility restrictions, immunization and quarantine will be very helpful in shunning disease spread.

Conflict of Interest:

Authors have no conflict of interest.

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