

Geoffrey L. Smith

Genus *Orthopoxvirus*: *Vaccinia virus*

Summary

Vaccinia virus (VACV) and cowpox virus (CPXV) have played seminal roles in human medical and biological science. In 1796 Jenner used CPXV as a human vaccine and, subsequently, widespread immunization with the related orthopoxvirus (OPV), VACV, led to the eradication of smallpox in 1980. VACV was the first animal virus to be purified and chemically analyzed. It was also the first virus to be genetically engineered and the recombinant viruses applied as a vaccine against other infectious diseases. Here the structure, genes and replication of VACV are reviewed and its phylogenetic relationship to other OPVs is described.

Inger K. Damon

Genus *Orthopoxvirus*: *Variola virus*

Summary

Variola major virus caused the human disease smallpox; interpretations of the historic record indicate that the initial introduction of disease in a naïve population had profound effects on its demographics. Smallpox was declared eradicated by the World Health Organization (WHO) in 1980. This chapter reviews epidemiological, clinical and pathophysiological observations of disease, and review some of the more recent observations on the microbiology of variola virus.

Sandra Essbauer and Hermann Meyer

Genus *Orthopoxvirus*: *Monkeypox virus*

Summary

Monkeypox virus is an orthopoxvirus that is genetically distinct from other members of the genus, including variola, vaccinia, ectromelia, camelpox, and cowpox virus. It was first identified as the cause of a pox-like illness in captive monkeys in 1958. In the 1970s, human infections occurred in Central and Western Africa clinically indistinguishable from smallpox. By contrast with variola virus, however, monkeypox virus has a wide range of hosts, which has allowed it to maintain a reservoir in wild animals. Human monkeypox was first recognized outside Africa in 2003 during an outbreak in the US that was traced to monkeypox virus-infected rodents imported from West Africa. Today, monkeypox is regarded as the most important orthopoxvirus infection in human beings since the eradication of smallpox. There is currently no proven treatment for human monkeypox, and its potential as an agent of bioterrorism is discussed.

Sandra Essbauer and Hermann Meyer

Genus *Orthopoxvirus*: *Cowpox virus*

Summary

Cowpox virus (CPXV) is distinguished from other orthopoxvirus (OPV) species by producing cytoplasmic A-type inclusion bodies and flattened pocks with a hemorrhagic center on the chorioallantoic membrane. CPXV is endemic to Western Eurasia and naturally infects a broad range of host species including domestic animals, and zoo animals, as well as humans.

Infections in humans seem to increase in importance due to a changed epidemiology in the rodent reservoir hosts or in the biotype of the virus. Genetic characterization of CPXV isolates revealed differences, which do not correlate with either host species or geographic origin. Phylogenetic analyses suggested a rodent-transmitted CPXV as an ancestor of all other OPV species. So far, only two strains from the UK and Russia are entirely sequenced. However, more knowledge on other strains from the geographic center in Germany and Scandinavia are of evolutionary, epidemiological and taxonomic importance, and may contribute to clarifying differences in virulence and severity of infection.

Joachim J. Bugert

Genus *Molluscipoxvirus*

Summary

Molluscum contagiosum (MC) is a common wart-like skin infection mainly seen in children and caused by molluscum contagiosum virus (MCV). The typical poxvirus particle morphology and genome organization of MCV led to its classification as a member of the family Poxviridae where it is the sole member of the genus molluscipoxvirus. The genome of MCV type 1 (MCV 1/80) has been completely sequenced (GenBank accession U60315). Of 182 hypothetical MCV open reading frames (>45 amino acids) only 35 have a significant homology to coding sequences of other poxviruses. Unique MCV genes include mc159, an apoptosis inhibitor (vFLIP), mc054, a viral IL-18 binding protein, mc148, a soluble IL-8 antagonist, and mc162, a Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate) binding protein. MCV does not encode an epidermal growth factor (EGF) homolog. MCV shares a number of genes only with para- and avipoxviruses and stands out as phylogenetically distinct from all other poxviruses. This is reflected in a number of unique biological characteristics that set MCV apart from other poxviruses: MCV replication in vivo is limited to differentiating keratinocytes of the spinous layer of the human epidermis. MCV induces an enhanced rate of mitosis in keratinocytes, possibly by way of EGF receptor up-regulation, and interferes with the normal epidermal cell differentiation program. The lack of local inflammation gives typical MCV lesions a pearly bland appearance. MC infection can persist in human skin for years. An inflammatory reaction, spontaneous or induced by trauma, frequently leads to the sudden and complete disappearance of MCV lesions. The local, subacute and proliferative nature of the MC infection puts MCV close to a group of animal poxviruses causing slow growing skin tumors. MCV replicates inefficiently in skin xenotransplants to immunodeficient mice. There is currently no cell- or tissue culture system that supports replication of MCV in vitro.

Geoffrey L. Smith

Genus *Yatapoxvirus*

Summary

Yatapoxviruses are a small group of Chordopoxviruses that infect humans and primates. There are two viruses in this genus, Yaba monkey tumour virus (YMTV) and Tanapox virus (TANV), hence the name Yatapox. A third virus called Yaba-like disease virus (YLDV) is very closely related to TANV so that YLDV and TANV are considered strains of the same species. TANV and YMTV infect primates in equatorial Africa and these infections may be transmitted to man by biting insects as zoonoses. Notable feature of yatapoxviruses are their slow growth in cell culture and the ability of YMTV to induce tumours (histiocytomas) in primates. Here the properties of the Yatapoxvirus genus are described.

Stephen Fleming and Andrew A. Mercer

Genus *Parapoxvirus*

Summary

Highly contagious pustular skin lesions of sheep, goats and cattle that were unwittingly transmitted to humans from close contact with infected animals, have been the scourge of shepherds, herdsman and dairy farmers for centuries. In more recent times we recognise that these proliferative pustular lesions were probably caused by a group of zoonotic viruses classified as parapoxviruses. In addition to infecting the above ungulates, parapoxviruses have more recently been isolated from seals, camels, red deer and reindeer. The central core of the 140 kb parapoxvirus genomes encodes factors for virus transcription, replication, and structural proteins whilst the terminal regions encode accessory factors that give parapoxviruses many of their unique features. Several genes of parapoxviruses are unique to this genus and encode factors that target inflammation, the innate immune responses and the development of acquired immunity. These factors include a homolog of mammalian interleukin-10, a chemokine binding protein and a GM-CSF/IL-2 binding protein. The ability of this group to reinfect their hosts, even though a cell mediated memory response is induced during primary infection, may be related to their epitheliotropic niche and the immunomodulators they produce. The discovery of genes encoding variants of vascular endothelial growth factor probably explains the highly vascular nature of parapoxvirus lesions. Many parapoxvirus genes do not show significant matches in public databases, separating this genus from most other mammalian poxviruses. These genes appear to be involved in processes such as inhibiting apoptosis, manipulating cell cycle progression and degradation of cellular proteins that may be involved in the stress response. Parapoxviruses in common with *M. contagiosum* virus lack a number of genes that are highly conserved in other poxviruses including factors for nucleotide metabolism, serine protease inhibitors and keltch-like proteins. Parapoxviruses have evolved a unique repertoire of genes to allow adaptation to the highly specialised environment of the epidermis.

Adama Diallo and Gerrit J. Viljoen

Genus *Capripoxvirus*

Summary

The Capripox genus is composed of three closely related viruses: goatpox, sheeppox and lumpy skin disease (LSD) viruses. The natural hosts from which these were isolated include goats, sheep and cattle, respectively, although domestic buffaloes are also susceptible to LSDV. Cross protection can be induced by all three viruses. Unfortunately, serological distinction between these viruses is not possible. Previous classification was based only on animal host origins, but today differentiation is possible using genomic DNA restriction digestion patterns or comparisons of gene sequences. Although most strains grow readily in goat, sheep or cattle, their pathogenicities may differ according to the animal origin. The diseases they cause are characterized by fever, papules, and nodular and sometimes pustular lesions on the skin. The nodules can be also found in internal organs, particularly the lungs. They induce immune depression in infected hosts, thereby favoring secondary bacterial infections with an associated increase in the mortality rate. A high morbidity is, however, usually observed with economic implications in the case of LSD, such as loss of milk production in cows, the infertility in bulls following orchitis and damage caused to hides. Capripoxvirus diseases are of a transboundary nature and are on the World Organization for

Animal Health (OIE: Office International des Epizooties) list of important animal diseases that need to be notified. The geographical distributions of these three viruses differ: whereas sheeppox and goatpox viruses are endemic to Asia, the Middle East and Africa south of the equator, LSDV is mainly confined to sub-Saharan Africa. These differences in geographical distribution may be an indication that goatpox and sheeppox viruses evolved separately from LSDV.

John W. Barrett and Grant McFadden

Genus *Leporipoxvirus*

Summary

Leporipoxvirus infection is restricted to lagomorphs (rabbits and hares) and gray squirrels. The genus is composed of four recognized members including myxoma virus (MV), the type species, rabbit fibroma virus (RFV), (also called Shope fibroma virus, SFV), hare fibroma virus (HFV) and squirrel fibroma virus (SqFV). The genus has traditionally been found in the Americas (MV, RFV/SFV and SqFV) and Europe (HFV). However, since the early 1900s MV has been employed in several countries to control the spread of feral European rabbits and can now be found enzootically in Australia and Europe. Based on sequencing data, the generic leporipoxvirus genome is approximately 160 kb and encodes between 165 genes (RFV/SFV) and 171 genes (MV). The best characterized Leporipoxvirus is MV. MV infection of its evolutionary host, *Sylvilagus brasiliensis*, results in a cutaneous fibroma at the site of infection. This tumor resolves but clearance takes over a month. In contrast, MV infection of its pathological host, *Oryctolagus cuniculus*, results in a lethal disease called myxomatosis. This is a devastating infection that produces numerous tumors on the skin, ears, face and genital regions of the infected animal. Full-blown myxomatosis is most often fatal and is accompanied by the collapse of the host immune system. It is this close interaction between virus and host that has allowed researchers to identify a wide range of immune evasion molecules directed at numerous host immune pathways. To date, MV immunomodulators have been identified that target a variety of host cytokines, host cell signaling cascades, apoptosis and numerous sentinel immune molecules.

Gustavo A. Delhon, Edan R. Tulman, Claudio L. Afonso and Daniel L. Rock

Genus *Suipoxvirus*

Summary

Swinepox virus (SWPV) has been classified as the sole member of the genus Suipoxvirus in the subfamily Chordopoxvirinae. Swine represent the only known host of SWPV; in adult animals the virus usually causes a mild, self-limiting disease. Infection occurs via skin abrasions, and the virus replicates in epidermal keratinocytes of the stratum spinosum. Tissues other than the skin are rarely affected. The SWPV infection induces protective immunity. The complete genomic sequence of SWPV (strain 17077-99) is known. The genome contains a central coding region and two identical inverted terminal repeat regions. Four of 150 putative genes seem unique for this virus. A number of SWPV proteins are likely involved in the disruption or modulation of host immune responses as indicated by their similarity to other viral immunomodulators and by the presence of predicted sequences. The distinct nature of the SWPV multigene family gene complement suggests that it contributes to SWPV host specificity. Due to its restricted host range, use of SWPV as a vaccine expression vector has been proposed.

David B. Boyle
Genus *Avipoxvirus*
Summary

Poxviruses identified in skin lesions of domestic, pet or wild birds are assigned largely by default to the Avipoxvirus genus within the subfamily Chordopoxvirinae of the family Poxviridae. Avipoxviruses have been identified as the causative agent of disease in at least 232 species in 23 orders of birds. Vaccines based upon attenuated avipoxvirus strains provide good disease control in production poultry although there are risks of emergence of strains against which current vaccines might be ineffective. Genome sequence analysis has revealed overall genome structure and function resemblance to the Chordopoxvirinae, however avipoxvirus genomes exhibit large-scale genomic rearrangements with more extensive gene families and novel host range genes in comparison with the other Chordopoxvirinae. Phylogenetic analysis places the avipoxviruses externally to the Chordopoxvirinae to such an extent that considering Avipoxviruses as a separate subfamily within the Poxviridae might be appropriate. A unique relationship exists between fowlpox (FWPV) and reticuloendotheliosis (REV) viruses. All FWPV strains carry a remnant REV long terminal repeat whilst field strains carry a near full length REV provirus integrated at the same location in the FWPV genome. With the development of techniques to construct poxviruses expressing foreign vaccine antigens, the avipoxviruses have become important vaccine vectors in the past 20 years. The seminal observation of their utility for delivery of vaccine antigens to non-avian species has driven much of the interest in this group of viruses. In the veterinary area, several recombinant avipoxviruses are commercially licensed vaccines. The most successful have been those expressing glycoprotein antigens of enveloped viruses, e.g. avian influenza, Newcastle disease and West Nile virus. Several recombinants have undergone extensive human clinical trials as experimental vaccines against HIV/AIDS and malaria or as treatment regimens in cancer patients. The safety profile of avipoxvirus recombinants for use as veterinary and human vaccines or therapeutics is now well established.

Marie N. Becker and Richard W. Moyer
Subfamily *Entomopoxvirinae*
Summary

The subfamily Entomopoxvirinae is a related but distinct member of the family Poxviridae. These viruses share many biological features of the poxviruses of chordates, but instead infect the larvae of a number of insect families. The three genera that comprise the entomopoxviruses are the genus Alphaentomopoxvirus, infecting beetles; genus Betaentomopoxvirus, infecting butterflies, moths, grasshoppers, and locusts, and the genus Gammaentomopoxvirus infecting flies and mosquitoes. The entomopoxviruses, like their vertebrate counterparts, have a double-stranded linear DNA genome that is transcribed in a temporal fashion. Entomopoxviruses are occluded in a paracrystalline protein matrix, forming spheroids that protect the virus from environmental conditions. A number of genes are conserved between the entomopoxviruses and chordopoxviruses defining a minimal complement of poxvirus genes. The entomopoxviruses have some unique molecular features. This review covers pathogenesis, transcription, and molecular analysis of the entomopoxviruses.

Steven H. Nazarian and Grant McFadden

Immunomodulation by poxviruses

Summary

Large DNA viruses, such as poxviruses, encode an array of gene products, both secreted and intracellular, that systematically debilitate the various host responses to virus infection. The primary targets of the secreted gene products are members of the inflammatory innate immune system, such as the interferons, tumor necrosis factors, diverse interleukins, complement and the chemokine pathways. Poxvirus-infected cells also maintain a low profile to escape the cell-mediated arm of the adaptive immune (CMI) response. Virulence factors that mediate this 'virostealth' are generally expressed intracellularly and interfere with host signaling processes or antigen presentation. Poxviruses also interfere with the cellular apoptotic response by regulating several key checkpoints within the cell. While many poxvirus virulence factors exhibit some sequence relationship with host proteins, suggesting that these genes may have been acquired from an ancestral host, others show no obvious similarity to any known host genes. Due to the intimate nature of the co-evolution with their hosts, poxviral immunomodulators have proved useful in examining diverse aspects of immunology, virology and cell biology.

Olaf Weber, Percy Knolle and Hans-Dieter Volk

Immunomodulation by inactivated *Orf virus* (ORFV) – therapeutic potential

Summary

Viruses manipulate the immune system either by bypassing or suppressing the immune reaction or by activation of the immune system.

Parapox ovis (ORFV) is an epitheliotropic DNA virus that belongs to the genus Parapoxvirus of the Poxviridae family. ORFV can repeatedly infect its host in spite of a vigorous inflammatory and complex host immune response. The viral genome encodes for several immunomodulating genes, including orthologues of IL-10, and mammalian vascular endothelial growth factor (VEGF).

Novel immunomodulating agents that are based on active or inactivated poxviruses might have therapeutic potential in various diseases where the immune system is out of its balance; ORFV-based drugs are already used in veterinary medicine for prophylactic and therapeutic uses.

Inactivated ORFV showed strong effects on cytokine secretion by human immune cells which involved up-regulation of inflammatory and Th1-related cytokines as well as anti-inflammatory and Th2-related cytokines. This combination of suppressive and stimulating mechanisms could be exploited as a novel principle of therapeutic immunomodulation.

Current preclinical data, together with a favourable side effect profile, call for further investigation of ORFV for its potential use as a novel immunomodulatory agent.

Barbara S. Schnierle, Yasemin Suezter and Gerd Sutter

Recombinant poxvirus vaccines in biomedical research

Summary

In biomedical research recombinant poxviruses are investigated as important candidate medicines to derive advanced options for prevention and/or treatment of infectious diseases or cancer. Genetically engineered viruses can readily synthesize biologically active heterologous proteins, serve to determine relevant targets of cell-mediated and humoral immunity, and

identify types of immune responses needed for protection against a multitude of different specific diseases. Substantial progress in vaccine development is based on the availability of exceptionally safe but efficient carrier viruses, on increasingly versatile vector technologies and on the feasibility of large scale manufacturing. Moreover, advances in deciphering the molecular pathways regulating poxvirus-host interactions will provide additional means to potentially activate innate immune stimulation upon vaccination and to derive vectors with specifically targeted replicative capacity for experimental tumor therapy.

Lauren M. Handley, J. Paige Mackey, R. Mark Buller and Clifford J. Bellone
Orthopoxvirus vaccines and vaccination

Summary

Immunization procedures against variola virus, from the historical perspective most often first credited to Edward Jenner in the late 18th century, helped finally to eradicate smallpox from the world. Since its eradication, the study of this disease and its pathology has been given little attention; however, with the emergence of monkeypox virus into the human population and the potential use of smallpox as a bioterrorist weapon, the need for an option to vaccinate the world's population is once again a reality. The vaccines used during the eradication program were live, attenuated vaccinia virus preparations of varying virulence that caused a significant number of adverse reactions in naïve subjects. Currently, immunosuppressed individuals, persons with certain skin diseases, and people with cardiovascular complications are contraindicated against receiving this type of vaccine. A new vaccine is needed. Until now, the only known correlate of immunity to the smallpox vaccine conveying protection has been the development of a scar at the site of vaccination. Characterizing the protective immune response established upon vaccination with Dryvax®, at both the innate and adaptive levels, would greatly enhance our understanding of the human immune response to the vaccine, and thus generate information for the production and evaluation of new and safer third- and fourth-generation vaccines.

Martin Pfeffer and Hermann Meyer
Poxvirus diagnostics

Summary

Members of the family Poxviridae form a large group of viruses that can infect humans as well as animals including the major domestic animal species (cattle, sheep, goat, swine, dog, cat and chicken). Poxviruses can be highly pathogenic for men (i.e., variola virus), are of zoonotic importance (e.g., monkeypox) or are highly contagious among animal populations (e.g., sheeppox). Therefore, laboratory confirmation of the specific poxvirus involved is, indeed, essential. This is especially true for the most notorious member, smallpox virus, which might reemerge as a weapon, and also for those “exotic” poxviruses, which are absent in many countries but still enzootic in other parts of the world. Today, poxvirus diagnostics covers the entire spectrum of either traditional (such as inoculation of embryonated eggs) or more advanced laboratory tests (such as genome sequencing or microarray assays). This chapter presents methods of sample collection and handling, and reviews techniques used in the diagnosis of poxvirus infections by briefly describing the principle and procedure of the method, and critically weighting the pros and cons as well as providing some examples of application for each method.

Robert Snoeck, Graciela Andrei and Erik De Clercq

Therapy of poxvirus infections

Summary

Poxviruses have been recognized already for centuries as a threat for human health. The most dreadful representative of the family, variola virus, responsible for smallpox, was eradicated last century, after a wide and intensive campaign of vaccination. Meanwhile, the importance of other poxviruses has been recognized in human pathology, as well as the possible use of microbial agents, including smallpox, by bioterrorists. This, together with the development of safer vaccination approaches, has strongly stimulated research on antivirals, which has already led to the discovery of several families of active therapeutical compounds. The increased understanding of the viral replication and pathogenicity, as well as the improvement in pharmacokinetics has led to the development of new and promising classes of compounds. These new molecules are either prodrugs given a better bioavailability, or compounds interfering with new molecular target, both viral and cellular. Over the last few years, these latter developments have opened new perspectives for the treatment of poxvirus infections, and are discussed in this chapter.

Friedrich v. Rheinbaben, Jürgen Gebel, M. Exner and Axel Schmidt

Environmental resistance, disinfection, and sterilization of poxviruses

Summary

The virion of the poxviruses is an enveloped particle that differs significantly from other enveloped viruses. Apart from DNA, proteins and phospholipids, poxviruses also contain carbohydrates. They show a high environmental stability and stay contagious over a period of several months in an ambient environment. Poxviruses show an extraordinary high resistance to drying, which is even enhanced by materials in which they are released into the environment (e.g., dermal crusts, serum, blood residues and other excretions). Dried vaccinia virus can be stored at 4°C over a period of more than 35 weeks without any loss of infectivity. Frozen in buffer at –20°C, a titer reduction of only 3 log-steps is observed within 15 years. In general, virus isolated from patients and/or environment is more resistant to environmental conditions than virus deriving from cell cultures. In addition, poxviruses show a high stability towards different pH values. Due to their low lipid content, they are less sensitive to organic solvents/disinfectants compared to other enveloped viruses. This is the reason for the considerably higher resistance of poxviruses to diethylether in comparison to other enveloped viruses. Despite all of these aspects, poxviruses are highly sensitive against all common approved disinfection regimens. Cell-bound poxvirus may show a higher stability than cell-free virus. This phenomenon is not observed if quaternary ammonium compounds are used. Due to the possible importance of smallpox, e.g., in case of abuse in biological warfare, but also because of impact of poxviruses in veterinary medicine, representatives of the poxvirus families have been chosen to test the efficacy of common disinfectants. The common sterilization procedures – thermal, chemical, an/or radiation – are usually effective against poxviruses.

Andrea Ammon, Julia Sasse and Klaus Riedmann

Early disease management strategies in case of a smallpox outbreak

Summary

As a consequence of the potential of smallpox as a means of bioterrorism, many countries have developed preparedness plans for smallpox. This chapter summarizes some of the most important issues for the management of smallpox.

The strategy for the management of clinical cases of poxviruses includes the early detection of cases, rapid laboratory diagnosis, an assessment of the risk of further spread and containment measures. For the detection of cases, special training for clinicians and practitioners is necessary. If a suspected case has been identified, rapid diagnostic tests are required. In addition to the national and international notifications based on given case definitions, an initial risk assessment of the epidemic development is needed. Further decisions have to be taken on the basis of a continuous risk assessment. Medical and non-medical countermeasures and their logistic aspects have to be considered in preparedness planning, e.g. resources necessary for the implementation of mass vaccinations, and the prioritisation of groups to be vaccinated.

Axel Schmidt

Historic aspects and early smallpox management approaches in the New World

Summary

Smallpox are an ancient burden of mankind. They are a typical disease of the Old World brought into the New World in the post-Columbian era, although preventive efforts seem to have been made in non-European countries long before this time. After smallpox rapidly spread throughout the New World, outstanding achievements in early disease management were made there. During the Boston smallpox endemia in 1721, a clergyman, Cotton Mather, and a physician, Dr. Zabdiel Boylston, introduced the immunization against smallpox into the New World against tremendous opposition. Boylston communicated his immunization experiences to the Royal College of Physicians and the Royal Society of London in 1726. The state of Massachusetts subsequently released a public health law, the “Act to Prevent the Spreading of Contagious Sickness”. All of these efforts are not sufficiently recognized, in contrast to the unanimous fame and glory of Sir Edward Jenner, who introduced the immunization against smallpox in England in 1796, 70 years after Boylston's presentation at the Royal College of Physicians and the Royal Academy of London.

Poxviruses

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