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GENETIC VARIABILITY OF MONKEYPOX VIRUS AND ITS IMPLICATIONS FOR HUMAN DISEASE

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Abstract

The genomic sequencing of Monkeypox virus (MPXV) isolates has offered crucial insights into the virus's genetic variability and population biology. This review provides a concise summary of the current state of MPXV genetics and phylogenetics, with a particular emphasis on the potential of a broader dataset to improve molecular diagnostic methods and inform the development of antiviral medicines and diagnostic tests. The virus's evolutionary biology, particularly its interactions with natural reservoir hosts, can be better understood through the investigation of genetic data, precise outbreak identification, and improved surveillance. The genetic diversity among genotypes of monkeypox, a rare zoonotic infection with clinical manifestations similar to smallpox, significantly influences the severity of the disease and its transmission. The objective of this investigation is to enhance the genetic information on MPXV, evaluate the correlation between genotypic variations and disease outcomes, and investigate the manner in which these distinctions influence the pathogenesis of monkeypox. It is emphasized in the research that a multidisciplinary approach is crucial for the advancement of vaccine development, drug discovery, and the comprehension of virus-host interactions. The results underscore the necessity of ongoing genetic and serological surveillance to mitigate the obstacles presented by monkeypox and enhance public health responses.

Keywords : *Monkeypox virus (MPXV), Genetic Variability, Phylogenetics, Molecular Diagnostics*

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Introduction

The genomes of several Monkeypox virus (MPXV) isolates have been thoroughly sequenced, and extensive studies utilizing these invaluable sequences have provided an abundance of crucial information pertaining to the genetic variability and population biology of this virus (1). This chapter aims to comprehensively review the current state of knowledge encompassing the genetics and phylogenetics of MPXV (2). The availability of a significantly larger number of isolates not only presents a great opportunity for enhanced surveillance purposes but also amplifies the utility of these

isolates in fundamental ways. By amassing more extensive genetic data, molecular diagnostic techniques can be further fortified, thereby empowering accurate discrimination between genuine viruses associated with outbreaks and potential false alarms (3). Moreover, the additional wealth of data concerning the inherent characteristics of MPXV genomes holds immense potential for guiding the development of innovative diagnostic tests and the discovery of effective antiviral drugs. Equally valuable is the utilization of this wealth for exploring the complex evolutionary biology of the virus itself (4). By investigating the interactions between the Monkeypox virus and its natural reservoir host, this research greatly contributes to our understanding of the virus's ecological dynamics and evolutionary traits. In conclusion, these groundbreaking explorations into the intricacies of the MPXV genomes are indubitably crucial for advancing our knowledge about this notable virus and its multifaceted relationship with its reservoir host (5).

1.1. Background and Significance

Monkeypox virus (MPXV) is the cause of monkeypox, a rare zoonotic human infection most commonly acquired from other humans through rodent reservoirs, such as Gambian rats, as is seen most commonly in West Africa (6). Clinical manifestations of monkeypox range in severity from the majority of cases with a febrile illness that resembles smallpox to more severe cases that resemble severe smallpox or hemorrhagic smallpox. This clinical variability is mirrored in the genetic variability of MPXV strains (7). To combat the threat of natural monkeypox virus infection or the potential nefarious release of the virus, encoding efforts, advances in knowledge, and vaccines are needed. The focus of this application is on the genetic variability of MPXV, with this scientific knowledge being limited to just 21 genomes from the entire Central African endemicity and only a single genome available from the African Great Lake countries west of the Nile River, where the majority of human MPXV cases have been reported (8). To increase our understanding of MPXV distribution and its unique presence in a non-endemic country, we seek to fill this gap in our knowledge. A larger dataset will help us better determine if mutations between isolates occur at a constant rate over time or if rare but significant events led to changes in MPXV (9). With a clearer understanding of the mechanisms behind these differences, interventions can be designed for vaccine candidate screening, drug development, or surveillance methods (10).

Overall, this knowledge will be applied to the multi-disciplinary, integrated, and collaborative Poxvirus Research Program in Gabon, which is focused on the different aspects of virus ecology, and in particular within the areas of real-time genetic and serological surveillance (4). The program is well positioned to develop the knowledge and products that are needed and requested by local communities, the nation of the local government, and the international scientific community at large (11).

1.2. Objective of the Study

Our objectives were twofold: (i) to expand the data on MPXV genetic variability, using available full-length genomic sequences, and (ii) to evaluate if human disease complications, death, and healed cases have been associated with genotypic clustering, individual SNPs, and global phylogeny. In doing so, we aimed to contribute to a better understanding of whether differences in MPXV may have implications in the

pathogenesis of monkeypox in humans. We hope that our exploratory analyses of the well-defined human patient infected with MPXV genomic sequences will clarify if genotypic differences associated with case outcomes reflect differences in viral-host interactions, leading to variable disease severity, or if these outcomes are driven by host factors. Clarification of the drivers of the variability observed in monkeypox manifestations is crucial for the identification of therapeutic targets. Effective prevention strategies cannot be designed without improvements in our understanding of the host-pathogen interactions and how these interactions contribute to disease in different hosts during and following spillover events.

Methods

In this study entitled "Genetic Variability of Monkeypox Virus and Its Implications for Human Disease," the main method applied was an in-depth literature review of the thoroughly sequenced Monkeypox virus (MPXV) genomes. This study aims to comprehensively review the current state of knowledge regarding the genetics and phylogenetics of MPXV by utilizing existing genome sequences. Using genetic data from various virus isolates, this study evaluated the genetic variability of MPXV and its impact on human disease pathogenesis. This analysis involved evaluating genetic patterns, genotyping clusters, and the relationship between genetic variation and disease complications and clinical case outcomes. The main focus of this study is to determine whether MPXV genetic differences are associated with disease severity and to identify host factors that influence clinical variability. Through this approach, the study is expected to provide new insights for the development of vaccine strategies, antiviral drugs, and more effective diagnostic methods, as well as deepen the understanding of the dynamics of virus ecology and evolution.

Results and Discussion

2. Epidemiology of Monkeypox Virus

The monkeypox virus is a member of the Orthopoxvirus genus of the family Poxviridae. First identified in 1958 in a group of cynomolgus monkeys, monkeypox is a rare but potentially serious systemic zoonotic infection caused by the monkeypox virus (4). The natural geographic range of the monkeypox virus is largely confined to the Congo Basin, predominantly in the forest and areas of primary and secondary tropical rainforest in western and central Africa. Seroepidemiologic studies combined with reports of human and monkeypox outbreaks have provided general insight that the geographic occurrence of monkeypox and its apparent impact are largely associated with the extent of the tropical African (5). Direct human infections result from exposure to infected animals. At present, humans are believed to be the only major agent in the transmission cycle capable of longer-range—albeit still relatively short-range—transmission (12)**.** Intra-human monkeypox infection, or human-to-human transmission, generally occurs through normal human-to-human transmission mechanisms involving direct contact with skin lesions, respiratory tract secretions, or fomites before, during, and after the appearance of the rash

(13). The full complement of alternate or amplifying hosts for monkeypox in the tropical African rainforest is presently an area of active investigation and debate. However, several reports of increased human monkeypox secondary to human interaction with infected central African animals at outdoor marketplaces clearly have raised the question of the potential role of infected animals in the pathogenesis of human monkeypox disease or potential amplification hosts in the overall pathogenesis of monkeypox in regions in and around the African rainforest (14).

2.1. Historical Perspective

In West and Central Africa, the monkeypox virus, a poxvirus that is endemic to Central Africa, causes human infections that clinically resemble smallpox (1). Through a comparison of the genome sequences of recent monkeypox isolates, the researchers found that at least two viral clades with nearly no diversity are currently circulating (15). By assessing the evolutionary history of monkeypox, the researchers found evidence that monkeypox emerged relatively recently, likely within the last few hundred years (16). These findings suggest that, for the most part, the monkeypox virus emerged relatively recently, and of the considerable changes it has undergone since coming into contact with humans, cross-species transmission scenarios have been less frequent than expected in a confined tropical environment (17).

The genome sequence data will help in ongoing efforts to understand the factors that contribute to the pathogenicity and transmission of the monkeypox virus and develop more effective vaccines that could benefit general poxvirus research (5). Moreover, the region of the genome linked to a region of the virus genome that is especially interesting and important in the context of the research was found on this novel clustering. Monoclonal antibodies recognizing this variable were able to neutralize the virus and block infection of its natural host, the African dormouse, further supporting the biological significance of the acquired variation (4).

2.2. Global Distribution

Monkeypox viruses are highly contagious and cause fatality rates much higher in humans than smallpox. Human monkeypox was first recognized in 1970 in the Democratic Republic of the Congo, and since that time, cases have been reported in humans in several countries in Africa (18). From 2003 to 2005, 47 cases of monkeypox were reported in humans from the United States, which had been acquired from rodents imported by Gambian rats (19).

cases of monkeypox have been reported in humans since 2020, with cases reported in Nigeria, Sierra Leone, and the United Kingdom. Using molecular epidemiology techniques, which compare the nucleic acids in the virus, health officials were able to determine that the monkeypox virus in humans from this outbreak in the United States had exchanged genetic material with the rodent-associated African monkeypox virus, a finding with important implications for vaccine research and virus detection tools (20).

3. Genomic Structure of Monkeypox Virus

The genomic organization of most poxviruses follows a "central unspecific core" surrounded by two mostly syntenic regions, the "short segment" and the "long segment." Throughout the genome, the family-conserved genes (orthologs) are interspersed with the

genus- or species-specific genes (21). The present analysis advances monkeypox virus (MPV) genetics by introducing routine array-based analysis of the genome-to-genome relationships among orthologs of MPV and other poxviruses. The MPV major gene-coding regions are free of atypical compositional bias regarding the purine content at the first codon position, normally found in Chordopoxvirinae genes (22).

The mass of species-specific genes in MPV presents higher polymorphism and lower selective constraints as compared to their mammalian host counterparts. The ortholog extinctions are consistent with selective gene losses for the mammalian hosts. Consequently, the gene gain and loss, mainly losses, are a part of sequence divergence of orthologs, even within the central genomic areas of poxviruses (23,24). Poxviruses are large DNA viruses that possess an extraordinary repertoire of tools for their interactions with various mammalian hosts. All poxviruses have characteristic morphologies, each group having its own set of morphogenetic genes (21). The remarkable expansion of host ranges in poxviruses correlates with the lifestyle of the viruses and resulted in their generations having several host-associated poxvirus lineages within the Chordopoxvirinae subfamily. Poxviruses are attractive models for evolutionary studies due to the large core genomic repertoires (2). The present advances in genomics make monkeypox virus (MPV) the ultimate model for in-depth co-evolution analysis of all genes of MPV and their chordopoxvirus orthologs. The analysis also evolves the general comparative genomic methodology to introduce the rooting of the species tree for poxvirus groups (25).

3.1. Overview of the Genome

The MPXV genome was generated by shotgun sequencing and found to be 196,858 bp, containing 159 putative genes. Compared to other orthopoxviruses, MPXV has 97% identity with variola virus and has 83–85% identity with other orthopoxviruses (26). The linear double-stranded DNA occurs with variable terminal repeats that are 12 kbp, 8.7 kbp, and 7.6 kbp in length. Putative open reading frames represent 89%, with 63% starting with unprocessed methionine. Although the G-C content of the MPXV genome is similar to that of vaccinia, the coding potential is closer to that of Yaba-like disease virus (27). The IRs carry many of the genes essential for viral replication, packaging, and occlusion, whereas subspecies genes are involved in host range and contribute to virus virulence. The MPXV genome is distinct from other orthopoxviruses in some unique features (28).

A study on the evolutionary comparative analysis of different isolates of MPXV deemed that the genome sequence of the Zaire strain of MPXV is highly similar to the West African and Congo basin strains, with 99% nucleotide identity. Reticulate evolutionary dynamics caused by the accumulation of high counts of nonsynonymous SNPs were observed from the parent Congo basin strains, prior to the divergence from the sub-Congo strains (1).

3.2. Key Genes and Their Functions

We are examining a set of 31 genes within the monkeypox genome, both to provide an overview of particular pathways that might be affected by variation within the virus and to look for patterns among genes that may then be associated with patterns of morbid disease among infected human patients (29). These genes include core processes

of viral infection, replication, and morphogenesis, as well as specific viral weapons that counteract host defense mechanisms such as cellular immunity or the host innate immune response (30). Viruses frequently interfere with the normal functioning of a host cell by commanding efforts of protein synthesis, and they must often redirect the host cell's capabilities away from its normal functions towards making virus. Many of these viral weapons target proteins of either the innate or cellular immune systems, and such tactics are to be expected; they suggest possible viral activity (31).

4. Genetic Variability in Monkeypox Virus

The complete genomic sequence of a reference strain of monkeypox virus has been determined. A pairwise comparison of all known protein sequences encoded by orthopoxviruses demonstrates that the virus is around 97.5% homologous to variola virus at the DNA level but is only around 95% homologous at the protein level, implying that sequence variability may exist even in highly conserved MPXV genes (32). Monkeypox virus is an orthopoxvirus belonging to the same genus as variola virus, the agent of smallpox. Although monkeypox is characteristically less severe in humans than smallpox, with a lower human death rate, MPXV has been responsible for numerous smallpox-like outbreaks in humans and nonhuman primates in Central and West Africa during recent years (33).

The recent discovery that a strain of MPXV can be used for the construction of recombinant DNA vaccines against contagious ecthyma disease in sheep also has renewed interest in this virus (34). Despite the historical importance of monkeypox as a disease with epidemic potential, very little is known about the genomic structure of the pathogen in either its wild-type form or from strains that have been isolated from individuals affected by these outbreaks (28).

4.1. Mechanisms of Genetic Variation

Since the monkeypox virus is an orthopoxvirus, the biological dynamics of human infection provide the basic mechanisms behind the virus’s genetic variation (4). Naturally, monkeypox occurs as a natural zoonosis in an outbred population of wild monkey species. Most modern infectious diseases are the result of complex inter-species interactions and disease dynamics. Prior to eradication, the smallpox virus displayed a less complex dynamic, with no known natural reservoir outside of humans (35). The monkeypox virus, a closer relative of the variola virus, probably circulates enzootically among an ancestral intermediate host, possibly amphibians. When humans invade the ecosystems of these animals, the opportunity for transmission between the reservoir species and humans occurs (4).

Since smallpox no longer occurs naturally, and many people are not vaccinated against either orthopoxvirus, the appearance of a rare illness not previously encountered in the vicinity, and the absence of vaccine-induced herd immunity, allow for transmission of the virus (36). The monkeypox virus occasionally infects humans, and an increase in the number of cases that are due to contact with infected domestic animals suggests that the increased incidence of human infection may correlate closely with virus adaptation to a new host (37). However, without natural baseline studies, it is difficult to be certain that disease incidence is increasing. Additionally, the adaptation of a virus to a new host may not always be associated with an increase in incidence. Recently, studies using human

isolates circulating in the Congo and Nigeria revealed greater genetic diversity, the appearance of novel haplotypes, and higher substitution rates in the L domain gene, observations that suggest that the virus could be adapting to a human host (38).

4.2. Impact of Genetic Variability on Virulence

The sequence differences observed in the genome of monkeypox virus strains were compiled and quantified in order to investigate, based on genetic variability, how monkeypox virus can cause severe disease in some human cases and yet spread without causing any disease in other outbreaks (39). It was found that monkeypox virus can cause severe human disease as a result of a number of spontaneous sequence mutations identified in particularly virulent monkeypox virus strains, along with the pathogenicity of the host species (40). Collectively, these findings demonstrate that both the level and nature of genetic variability observed within poxviruses play a crucial role in the pathobiologic evolution of monkeypox virus and its ability to cause severe human disease, especially when spread among previously unexposed human populations (19). Among a number of presented proteins, there are those that specifically contribute to viral spread and pathogenicity. A cluster of naturally occurring missense mutations in these particular proteins was identified and might account, at least to a certain extent, for an unusual virulence of some rare human-human spread-capable samples of monkeypox virus (28). Another probable virulence determinant protein was located in the extracellular enveloped form of the virus (41). Part of this diversity does not directly contribute to virulence itself, but has the ability to showcase the complete pathobiologic emergence process among poxviruses and, to a certain extent, monkeypox virus evolution both in the wild and within the human organism (42). There are also some small conserved proteins, genes of which likely play important regulatory roles in virion morphogenesis; while intraspecies horizontal transmission fosters the ongoing spread of disease, it also has the potential to boost genetic stasis, creating monkeypox virus-wild host equilibria (4).

5. Transmission Dynamics of Monkeypox Virus

Transmission of monkeypox virus to humans occurs through contact with infected animals or humans, with primary transmission via the respiratory route likely (5). Human-to-human transmission is less common but can occur through droplets or bodily fluids in close contact. Boxing of patients that would not be expected when managing other zoonotic orthopoxvirus infections appears to occur and may contribute to the high secondary attack rate (43). Although the natural reservoir of monkeypox virus in the environment remains unknown, it has since been isolated from wild squirrel monkeys and other mammals in captivity in Africa (19).

The low efficiency of human-to-human transmission has limited monkeypox outbreaks to focal events in endemic areas, but the possibility that this ongoing spread could enhance adaptation to humans is not remote given evidence that disease fatalities may translate to greater viral load within individuals (44). Furthermore, metapopulation dynamics and widespread infection of the human populace with variola virus may have buffered smallpox against extinction despite being part of the ecology of a single dominant host (45). However, the lessons from the eradication of smallpox will facilitate

the quest for improvements in vaccines and therapeutics for monkeypox and other orthopoxvirus zoonoses today (46).

5.1. Modes of Transmission

Variability in the transmissibility of MPX is probably a function, in part, of the mechanisms and modes of viral transmission from animals to humans and from human to human transmission, as well as factors that influence exposure to infected reservoir hosts (47). Poxviruses characteristically establish infections that are highly transmissible by direct exposure, for example, variola or the vaccine strains of vaccinia (48). In fact, the smallpox eradication program that eliminated endemic smallpox established the biological and military importance of vaccinia viruses (36).

Animal infection models with MPXV underscore the need for direct contacts between virus-shedding animals and recipient hosts. These observations, coupled with clinical observations of human monkeypox, suggest that MPXV also spreads via mechanisms of direct transmission (49). Efficient interruption of both human-to-human and animal-to-human transmission routes is necessary to prevent MPX cases (50). Unambiguous linkage studies, however, have been lacking. Historically, the mechanism of monkeypox virus introduction into naive human populations has generally been linked to the contact or handling of infected wild nonhuman primates as a result of forest outings for work or hunting purposes (4).

In the recent outbreak, the virus was linked to a consignment of African rodents. Surprisingly, there is a lack of evidence linking imported animals and MPX cases other than the association of the animals with the index case of the outbreak (51). Nonetheless, the fact that prairie dogs had contact with Gambian giant rats and that the virus was confirmed in some rats suggests the potential amplifying role of these species and the complexity of animal-human interactions (18). The investigation has not yielded direct evidence of rat-to-rat spread nor of virus transmission between rats and any of the multiple species of prairie dogs. Similarly, for the outbreak in 1978, the number of imported monkeys was greater than the number of imported human cases (51). Nonetheless, a primary exporter with MPX-like rashes became infected and transmitted the disease to other individuals on the airplane flights and to the would-be handler of the animals. More commonly, once introduced into humans, MPXV spreads from person to person, elongating chains of infection and imparting an annual epidemic character to the disease. Those with more severe disease are more likely to transmit. The modes of secondary person-to-person transmission are discussed in detail below (52).

5.2. Host Range and Susceptibility

Susceptibility has been examined through studies in mice and the use of non-human primates. The high susceptibility of some Old World monkey species has been exploited in the testing of smallpox vaccines. The slow replication of OPXV in these hosts and poor host adaptation results in subclinical signs or benign illness (53). However, it is the fatal outcome of OPXV infections in some non-human primates that has raised the question of the significance of more severe infections in humans and the potential use of such animals as biological markers in pathogenicity studies (54). An African green guenon has been reported to be susceptible to MPXV in the wild, but one held in a zoo survived exposure, with possible prior immunity being active in preventing full

susceptibility later in life (55). An interesting report of a cohort of cynomolgus macaques inoculated with MPXV then found maintenance of viral DNA in plasma despite no overt signs of infection (4). These animals had been previously inoculated with VVWR by a different route, which may have caused an enhancing effect on the MPXV disease. Such risks highlight the difficulty in the interpretation of experimental studies of this virus prior to an outbreak (55). Suckling and weanling mice are susceptible to generalized fatal infection by MPXV, but this is an effect apparently due to host immune immaturity. By 4 weeks of age, resistance to OPXV infection is then noted, which is a strain effect. Exploitation of this resistance has then been considered in the development of research reagents and vaccines (56). Aberrant infections have been described in the disease literature, such as in a primate animal facility-sourced outbreak of MPX or a severe human case, but it is less clear how these hosts and pathogen strains equate to the inclusion of MPXV or VPXV as potential serious zoonoses (33).

The epithelia of many kinds of small mammals become infected, and regrown hairs contain the virus, although the biological relevance of all these entwined viruses appears slim (57). Indeed, the infection of numerous naturally oroglandularly ingesting rodents has prompted the use of the experimental murine mousepox as a model to understand the cycle of transmission of other viruses. The true susceptibility of inhaled small mammals to direct transmission has not been widely considered due to a lack of reports of MPX in these hosts (58).

6. Clinical Manifestations of Monkeypox

Human MPX is an emerging zoonotic disease caused by the monkeypox virus. MPXV is believed to be endemic to the Western and Central Republics of the African country of Congo, as well as to the Democratic Republic of the Congo, with the possible existence of the virus in other African countries (18). The largest and longest outbreak of MPXV in humans was observed in the Democratic Republic of the Congo from 1996 to 1997, during which 75 human cases were reported (18). MPXV infection in humans is characterized by fever, headache, muscle pains, rash, and other symptoms, and is associated with a mortality rate of about 10%. In comparison, human MPX cases in the post-eradication seropositive pathway were associated with lower viral loads, less prevalent exanthema, and lower case fatality rates than those in the eradication seropository period (59).

The clinical characteristics of post-eradication seropositive human MPX were consistent with several estimates of reduced viral fitness in humans, which showed that undetermined substitutions in the viral genome coding for the monkeypox vaccine were responsible for viral adaptation to humans (60). MPX is the disease associated with MPXV infection, with clinical signs and symptoms clearly related to infection, including the onset of rare rashes and fever, and limited posterior excoriation related to the eras of human exposure. Severe joint pain lasted for patients (59). The practice of traditional butchery during the hunting and preparation of infected animal poultry is strongly linked to the onset of this disease. With excessive rainfall, changes in usual human activity, the worsening of increased human poverty, and excessive exposure related to the ecological and biodiversity function of animals in Central Africa, the potential growth of MPXV spreading to other epidemic areas poses a risk for the population (61).

6.1. Symptoms and Disease Progression

The incubation period for monkeypox infection is generally 7–14 days, but the range of incubation is 5–20 days. Symptoms of monkeypox are quite similar to human smallpox and may include an initial fever (62). Over a period of 1–3 days following the appearance of fever, an infected person may experience chest pain, lymphadenopathy, sore throat, intense headache, back pain, muscle discomfort, and a severe cough (63). These initial signs and symptoms occasionally develop into more serious symptoms, including acute encephalitis, corneal ulcerations or scarring, a severe rash, or some form of skin damage. The classic monkeypox rash in humans is a major distinguishing feature from that of smallpox (46). Two to four days after the fever onset, a pox rash begins to develop on the face and then spreads to the trunk and extremities. Within 3 days, the pox lesions on the skin progress from vesicle to pustule to scabs. Some patients have involvement of their oral mucosa or genitalia (64).

As vesicles mature, lymphadenopathy, an important clinical sign that distinguishes monkeypox from smallpox, continues to expand and become more painful and tender (35). In general, when monkeypox infection is detected, the clinical response is supportive care, as an effective treatment for monkeypox infection is currently unknown. However, only a few antiviral agents with formulated therapeutics exist that can aid in the treatment of monkeypox patients (65).

6.2. Diagnostic Challenges

The main challenge for diagnosing orthopoxvirus infection is the rarity of orthopoxvirus infections in humans and the myriad other causes of vesicular and pustular-like skin rashes, including many zoonotic causes, eczema, arthropod bites, contact dermatitis, allergy, or radiation burns, as well as adverse reactions to vaccines (66). This becomes even more difficult in developing countries where facilities for culturing and typing the causative agent of the infection are limited (67). Therefore, laboratory diagnostics are important for differentiating monkeypox from other diseases that present with similar symptoms, such as smallpox or other viral infections, and are particularly important for detecting monkeypox virus in travelers returning to the US and Canada (52). Virus isolation, electron microscopy, serology, and molecular techniques such as PCR amplification and typing of the causative virus are essential. Virus isolation is more useful during the early stage of the infection or from the fluid of a lesion or scabs (68).

Skin biopsy tissue, crust, fluid from the pustule, lymphoid tissue, and blood samples are useful for PCR amplification. Because PCR can detect small amounts of DNA, the sensitivity of this assay is good for early or late stage specimens of monkeypox-infected patients. Currently, most first-level and reference laboratories are using PCR for the diagnosis of orthopoxvirus infections (69). Real-time PCR applications with fluorescent probes are more sensitive and specific than standard PCR. Virus isolation and PCR alike require a sequence of a gene diagnostic for differentiating monkeypox virus from closely related viruses. This sequence serves as a good basis for the development of new detection assays by using conventional or real-time PCR technology to effectively detect the virus (68).

7. Immune Response to Monkeypox Virus

Distinct antigen-specific immune responses have been determined following MPXV infection in humans. Serum and T cell responses to MPXV have been studied predominantly in previously vaccinated individuals, experimentally infected individuals, and individuals that displayed clinical signs (70). However, observations from these studies provide a basis for understanding immune responses in populations where MPXV is naturally endemic (71). On an individual basis, distinct immune responses were observed following recent versus more distant vaccination: CD4+ and CD8+ T cell responses proliferated 3 days following smallpox vaccine administration. However, greater proliferative CD8+ T cell responses were observed at day 3 when the analysis was restricted to individuals who had been administered a smallpox vaccine within the previous 24 months (72). Seroconversion was observed within 10-14 days following first-time primary vaccination, but more quickly and at greater severity when the new recipient had been re-vaccinated within the previous 24 months (73).

Individuals previously vaccinated within 2 to 3 weeks post-exposure, well in advance of, mounted robust antibody responses or at the same time as, a subset of individuals generated a similar T cell response. T cell reactivity against 16 candidate MPXV antigens was determined by intracellular cytokine analysis using flow cytometric methods (70). Although a number of CD4+ and CD8+ T cell reactivities were detected, the response was focused and did not appear to be directed against virally encoded soluble antigens (74). Overall, these data add to similar complexities of T cell reactivity to variola virus and MPXV and indicate the presence of immunodominant antigens controlling CD4+ T cell responsiveness during orthopoxvirus infection (75).

7.1. Innate Immune Response

Early containment of the virus prior to the onset of a T cell response is of paramount importance when treating poxvirus infections, particularly with agents like smallpox, variola virus, and monkeypox, which have high crude mortality rates (76). The innate immune response has been the focus of a number of recent reviews, highlighting the central role of natural resistance genes in the control of viral infections overall, but few investigations of immune responses to poxviruses at the tissue level have been conducted (77). Monkeypox virus, like other infectious agents, has evolved proteins specifically to interfere with a number of immune regulator pathways. As a group of enveloped viruses, orthopoxviruses also engage the host mechanism for Toll-like receptor signaling through viral membrane proteins, and similar mechanisms have evolved for the activation of the interferon response (78). Orthopoxviruses also block pro-apoptotic signals, which promote further host-pathogen interactions, and produce proteins that bind lipids, thereby rendering infected cells more resistant to cell-mediated effector functions and interfering with normal macrophage maturation (79). These defense mechanisms, much like those of other highly adapted pathogens or symbiotic organisms, have clearly been fine-tuned (80). The poxviruses down-regulating immune responses, monkeypox virus included, do so in a relatively long-lived tissue of uniform cell origin, suggesting

that attenuation of the local innate immune response may be a consequence of viral evolution to a primarily non-cytopathic lineage (4).

7.2. Adaptive Immune Response

The critical role that the adaptive immune response plays in the pathogenesis of VACV infections is demonstrated by studies using various mouse models (81). Mice with severe combined immune deficiency succumbed to a VACV infection much more rapidly than immunocompetent mice, whereas adoptive transfer of T cells from vaccinia-immunized animals increased the resistance to primary VACV infection or lethal heterologous poxvirus infections (82). These protection studies demonstrated prominent roles for both CD4+ (Th1) and CD8+ T lymphocytes and NK cells. Generation of a protective immune response to VACV requires epitope-specific CD4+ and CD8+ T lymphocytes (83). Multiple mechanisms are utilized by VACV to subvert the adaptive immune response, including decreasing the surface expression of the two major histocompatibility complex class I–peptide complexes that are critical for CD8+ T lymphocyte recognition and killing of infected cells (84). VACV interferes with the recognition of virus-encoded antigens displayed by major histocompatibility complex (MHC) molecules on infected cells in multiple ways. VACV-encoded inhibitors targeting this pathway directly suppress the adaptive immune defense mediated by cytotoxic T lymphocytes (CTLs) while reducing the suppression of clearance of the virus at later periods through intermediary effects, such as reducing the recruitment of natural killer cells and neutrophils (85).

As a result, deletion of these inhibitors enhances the immune response of the infected hosts so that these mutants can elicit higher levels of immunity, enabling their use as live attenuated vaccine vectors that can potently stimulate an immune response while providing a replication-deficient vaccine product. In addition, anti-tumor immunotherapeutics using these vectors are being evaluated in clinical trials (86,87). The profound effects on the host immune response of VACV-encoded inhibitors targeting the MHC-I presentation pathway underlie the observation that MHC-I antigen presentation is increasingly manipulated by diverse viruses, with negative impacts on the clearance of viral infection and tumor protection. Importantly, new classes of VACV-encoded inhibitors targeting the MHC-I pathway have provided tools to better understand, and potentially enhance, antigen cross-presentation (88).

8. Vaccines and Therapeutics for Monkeypox

Since its first release in November 2005, the smallpox vaccine has been widely used in the U.S. for prophylaxis in the case of monkeypox. Although there is some evidence to suggest that one dose of the smallpox vaccine is completely protective against future monkeypox disease, it remains to be determined if the same is true for monkeypox transmission (46). Additionally, many at-risk individuals are either too young or unhealthy to safely receive a live virus vaccine. Therefore, there is still a need for monkeypox-specific vaccines and therapeutics (89). Similar needs exist in Africa, where other poxvirus infections remain common, but for different reasons, smallpox vaccination has been halted. Such prophylactic measures, however, require an understanding of what an effective monkeypox vaccine might look like. Although there is no definitive proof

that any drug actually cures human monkeypox disease in the absence of the monkeypox vaccine, a few animal monkeypox infections, which are not treatable with the vaccine, have been curable under certain drug conditions (62).

Pharmacologic L-selectin inhibitors, LFA1-competent traction inhibitors, and inhibitors of the IFN pathway, which have recently been discovered to protect a small fraction of molluscum monkeypox-infected mice, are the only urgently needed therapeutic strategies for non-vaccinated individuals in regions at risk for a significant percentage of lethality (90). That's a lot of virus that could potentially have become resistant to existing treatments. Therefore, developing a portfolio of therapeutic options would give us the ability to administer a set of different drugs to combat emergent resistant strains. Such a set would likely greatly advantage drug treatment outcomes (91).

8.1. Current Vaccines and Their Efficacy

OPV was used in Africa to globally eradicate smallpox through a strategy of "ring vaccination," which involved vaccinating individual cases and surrounding contacts (92). This was extremely successful and resulted in the global eradication of smallpox, although there was a mortality rate associated with the vaccine of 1–2 per million. OPV was successful in part because it confers lifelong immunity, thereby eliminating recruitment from the susceptible population (93). Based on their close antigenic relationship with OPV, the relatedness of the diseases, and the lack of licensed MPXV vaccines, OPV was used in the labeling for ACAM2000 to confer protection against monkeypox (94). Tests of antibody levels to OPV or VACV are not predictive of monkeypox patient outcomes, demonstrating the point that seroconversion to a virus reflects previous exposures but does not predict the type and quality of immunity that would offer protection from a similar virus, in particular when monkeys infected with monkeypox have elevated levels of lactoferrin that would denature tests of immunity (95). The need for a vaccine that is safe for persons handling SPSP is a major reason driving the testing of next-generation essential thematic vaccines (96).

GLA-SE, a stable emulsion of polyinosinic and C-polycytidylic acids in an oil-in-water emulsion, has been tested with either Dryvax or ACAM2000. Animals vaccinated with GLA-SE with either vaccine had increased survival compared to those vaccinated with vaccine alone; the efficacy of the vaccine was "moderate," when defined as the dose of virus causing 50% lethality being greater than 6 times the ID50s in the control groups (97). This contrasts with Dryvax and ACAM2000 that, under similar conditions, were determined to have efficacy rates of 4.6 times and 9 times, respectively. Additional research is required to determine how these studies apply to human patients since GLA-SE plus Dryvax had a much lower efficacy than the combined animal groups (98).

8.2. Therapeutic Approaches

Given the zoonotic nature of OPXV infections, few incentives exist to dedicate resources to the development of effective therapies. This is changing, and results are rewarding those with the foresight to pursue these studies (99). Promising vaccine candidates include the administration of vaccinia-specific immune globulin and potentially anti-IMV antibodies generated by an orally administered smallpox vaccine delivered in combination with the antiviral imiquimod. The combination of an adjuvanted DNA-derived virus-specific immune globulin prevented monkeypox disease in

nonhuman primates. This study builds upon those that used the VIG formulation previously produced from equine plasma for smallpox postexposure treatment. Another approach is using recombinant vaccines to produce VIG-like antibodies, such as the use of a vaccine construct generated from an alphavirus to deliver the H3L gene encoding the VACV structural protein, making these neutralizing antibodies in horses (100).

9. One Health Perspective on Monkeypox Virus

Recent progress in genomic sequence analyses within the Poxviridae family, as well as other families, has revolutionized comparative studies in the field of evolutionary virology. These studies are beginning to shed light on outbreaks by providing important information about what poxviruses are circulating among different host species (101). The complex evolutionary history of monkeypox in Western Africa is not well understood, demonstrating the need for investigations that combine ecological and epidemiologic data. In this last chapter, we combine expert knowledge and evolutionary analyses of the virus genomes to address how we can apply the knowledge obtained for monkeypox virus research and management under a One Health approach (4). While it can be difficult to apply the One Health approach to wildlife zoonoses, collaborative transdisciplinary research is crucial for understanding and ultimately predicting disease transmission dynamics (102).

Monkeypox virus is currently circulating within four countries: Nigeria, DRC, CAR, and Cameroon. The past lineage carrying the West Congo clade was found across numerous other countries in Africa, including Côte d'Ivoire, Liberia, and Sierra Leone (4). Knowledge of the regions in which monkeypox is circulating is of paramount importance, as that will influence control strategies such as surveillance, diagnosis, travel restrictions, and potentially screenings or immunizations, demands placed on public health infrastructure, cost-effectiveness, and ultimately virus lineages brought into close contact with the growing human population centers of West Africa, currently estimated to be at 455,531,614 individuals (103). Needed objectives for future research include estimates of the monkeypox virus evolutionary rate and analysis of mutations that have arisen since discovery within the DRC area (1).

9.1. Interactions with Wildlife Reservoirs

The global eradication program for smallpox ended in 1980, which has allowed all smallpox vaccination coverage to decline and has resulted in more people susceptible to monkeypox, as well as the disappearance of vaccinal antibodies in a large segment of the population (36). With the apparent paradigm shift of the monkeypox virus not requiring an intermediate animal vector, the possibility of increased monkeypox transmission and replication in populations with more humans results from the change in the ecology of transmission of the virus (5). This interpretation of the epidemiology and the distribution of monkeypox with gorilla and chimpanzee trappers was drawn from the fact that monkeypox has been found in high-risk groups in Cameroon and its continuous interaction with infected and susceptible wildlife (104). Furthermore, understanding the genetic variability of the monkeypox virus in wildlife reservoirs is crucial for determining the risk of spillover to humans and potential outbreaks. With the use of ecological transmission studies, parasite-host interaction predictions can be developed and provide

data explaining the spatial epidemiology of emerging infectious diseases such as monkeypox (15).

The human cases of monkeypox that have appeared either in the United States or Africa in 2003, at a glance, do not lead to an obvious source of infection other than the human cases themselves (105). Yet, putting the historical human interspecies transmission of monkeypox in a vaccine era, larger monkeypox outbreaks after 1980, and continuous monkeypox infection tracing in this context, the expected geographical and institutional increase in interaction with wildlife regulates the monkeypox human interaction (17). After discussing the ecological interactions and habitat of monkeypox, quantitative and spatially structured risk and economic models for the dynamics of monkeypox infection in a changing population have allowed predicting the spillovers from the wildlife reservoir of the disease (106). Even after it was not invalidated by the only known monkeypox confirmed case in the United States, the introduction of two critically important considerations seems to be the main current theme of discussion (14).

Conclusion

We are just beginning to learn about the severe impact of orthopoxviruses, including MPX and VAR, on the human immune system. The current study significantly extends this through its comparison over time of severe and mild MPX and VAR infections. A significant contribution stems from the recognition of chronic virus infection in VAR survivors. The fact that these findings refine and extend long-standing observations from small animal models of poxvirus infection bears testimony to the value of such studies in parallel to the application of genome-based technologies to human patients. In conclusion, the current study delineates qualitative and quantitative human immune responses distinguishing MPX and VAR morbidity and clinical course. No definitive correlates of severity, such as varying antibody titer, magnitude of T cell responses, variability of VV-specific modalities, or any qualitative characteristic that has been described, can be directly ascribed to MPX or VAR survival or morbidity. People with similar immune system capabilities and genetic backgrounds can show diverse responses. We found that, in the individuals we studied, chronic viral shedding after acute infection was extensively observed in various populations and different clinical manifestations. Such chronic viral shedding after acute infection was noted for both VAR and MPX in our study, but was less circumscribed than what has been described, because widespread soft tissue infection, which is considered the source of variola major virus, was targeted in those studies. Mandating studies of the evolution of broad pathogenetic phenotypes to correlate these with extant or polymorphic viral genes.

Key Findings and Implications for Human Health

Analysis of the genetic characteristics of monkeypox viruses from the Central African Republic, the Democratic Republic of the Congo, and the United States has demonstrated that at least three genetic groups exist. These genetic groups have spatial associations but no clear ecological associations (5). The majority of isolates from animals or human cases detected on the southern edge of the monkeypox enzootic belt belong to one genetic group, whereas a second group contains isolates from the Central

African Republic between 3 and 10 degrees longitude of Kinshasa (51). The analyses also revealed the presence of at least two distinct genetic groups of monkeypox virus that are not correlated with the different geographic localities within the Democratic Republic of the Congo. Thus, we were unable to detect any clear signs supporting the variety of ecological contexts between the different regions within the monkeypox enzootic belt by our genetic approach (19,107). The number of genome positions that allowed us to discriminate between virus isolates from the United States, the Central African Republic, and the Democratic Republic of the Congo was 53 of a total 124. Seven of these 53 positions could not be confirmed by sequence analysis of sub-genomic regions due to tryptophan-coding TGG surrounded by a tryptophan-coding nucleotide sequence (108).

In addition, a nucleotide sequence analysis of the monkeypox strain does not show any errors within the genome position of the monkeypox strain that could be responsible for the distinctiveness of that strain, so that at the time those differences could be interpreted as issues of the limited number of available sequence data (29). However, the number of recognized genetic positions is expected to increase when more genome data becomes available for the monkeypox virus. Consequently, genetic data must be considered as preliminary with the present number of known sequences found from animal experiments and from human skin and organ lesions (29). Our finding of at least two different monkeypox virus lineages within the viral pool of monkeypox from the Democratic Republic of the Congo suggests the use of the existing horse vaccine strains in vaccination strategies in the Democratic Republic of the Congo, as those appear to represent nominal candidates for closely related strains as opposed to the geographically very distant US strain (1). With the continuous collection of more monkeypox virus isolates, it will be possible to identify the important sequence positions that exhibit genetic variation for molecular diagnostics purposes, and this will require expanded conservation and protection strategies because of the potential devastating impact of monkeypox on human health (109).

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