The land-scape of immune response to monkeypox virus

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Summary

Human monkeypox is a viral zoonotic smallpox-like disease caused by the monkeypox virus (MPXV) and has become the greatest public health threat in the genus Orthopoxvirus after smallpox was eradicated. The host immune response to MPXV plays an essential role in disease pathogenesis and clinical manifestations. MPXV infection leads to skin lesions with the genital area as the main feature in the current outbreak and triggers a strong immune response that results in sepsis, deep tissue abscess, severe respiratory disease, and injuries to multiple immune organs. Emerging evidence shows that the immunopathogenesis of MPXV infection is closely associated with impaired NK-cell function, lymphopenia, immune evasion, increased antibodies, increased blood monocytes and granulocytes, cytokine storm, inhibition of the host complement system, and antibody-dependent enhancement. In this overview, we discuss the immunopathology and immunopathogenesis of monkeypox to aid the development of novel immunotherapeutic strategies against monkeypox.

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Introduction

Monkeypox is a zoonotic disease caused by the monkeypox virus (MPXV), a member of the orthopoxvirus genus that was previously primarily endemic to central and western Africa.[1,2] Beginning in May 2022, human monkeypox cases have been reported from many countries in non-endemic regions. The disease has attracted worldwide attention due to an increasing number of confirmed cases and unusual reports of human-to-human and community transmission. Currently, monkeypox has spread rapidly in more than 100 countries and caused >79,000 confirmed cases worldwide. Considering the global threat, the outbreak was declared as a Public Health Emergency of International Concern (PHEIC) by the World Health Organization (WHO) on July 23, 2022.

MPXV causes a human disease similar to smallpox and has been identified as the most common orthopoxvirus affecting humans since the abolition of smallpox in 1980. Although the virus was identified decades ago, the clinical presentation and course of the current monkeypox outbreak differ from previous outbreaks. Historically, human monkeypox presented as monomorphic pustular rashes, with genital lesions being rare.[3,4] In contrast, the current outbreak of monkeypox is distinguished by genital rashes. Also, in non-endemic areas outside of Africa, the genital rash frequently precedes the generalized pustular rash.[5–8] The genital area is the site of a primary infection that results in a localized rash and, in some cases, a secondary disseminated infection. In addition, there is no significant association between prodromal symptoms and skin lesions, and only about half of cases have systemic symptoms.[9] Skin rashes are commonly asynchronous. Therefore, clinical physicians and scientists should be aware of this new situation that presents a different scenario from prior outbreaks.

A growing number of studies demonstrate that immune characteristics are closely related to the pathogenesis and disease progression of patients infected with viruses. Immune escape has been widely

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found in orthopoxvirus infection and plays an important role in its pathogenesis.[10,11] Decreased T lymphocyte subsets in peripheral blood are a distinct feature of patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.[12,13] Besides, the number of natural killer (NK) cells was found to be lower in Ebola patients compared to healthy individuals.[14,15] As the relationship between immune response and severe monkeypox disease is revealed, immune patterns may be potential biomarkers of disease progression and potential therapeutic targets for monkeypox. In this review, we focus on and discuss MPXV-induced immunopathological changes and their potential immunopathogenesis and attempt to provide a crucial framework for guiding future research into monkeypox therapy.

Structure and genome annotation of MPXV

The MPXV particles were relatively large, rounded brick or oval in shape, and 200–250 nm in size, as observed under an electron microscope.[16] The virion consists of five distinct structures (core, membrane, lateral body, surface tubules, and nucleocapsid). Its core is double-concave dumbbell-shaped, surrounded by an outer membrane with double-layered lipoproteins (Fig. 1A). Virions encapsulate numerous enzymes associated with the RNA polymerase system, which are essential for the primary transcription of viral and structural genes.[17] Their proteins are usually found in the M (mulberry) form, showing regular 10 nm long protrusions (tubules) on the surface, and are less frequently found in the C (capsule) form, containing a thick membrane and a smooth uniform surface. However, the key receptor for the entry of MPXV into host cells has not yet been discovered.

MPXV genomes sequenced to date confirm the existence of two distinct monkeypox virus clades, namely the Central and West African clades, with case fatality rates of 10.6% and 3.6%, respectively.[18] Mortality is higher in immunocompromised individuals, children, and young adults. As shown in Fig. 1B, the MPXV genome is a double-stranded linear DNA molecule of approximately 197 kb in length. The genome has a large conserved central region (approximately 101 kb in size) and two terminal variable regions.[19] Besides, each end of the genome contains an inverted terminal repeat (ITR) of 6.4 kb, and the ITR is made up of some ORFs, hairpin loops, and short tandem repeats.[20,21] The central genomic regions of MPXV encode structural proteins and essential enzymes and are delimited by ORFs, A25R, and C10L, which share a 96.3% identity with the corresponding parts of the smallpox virus.[21,22] In contrast, two-terminal variable regions encode the

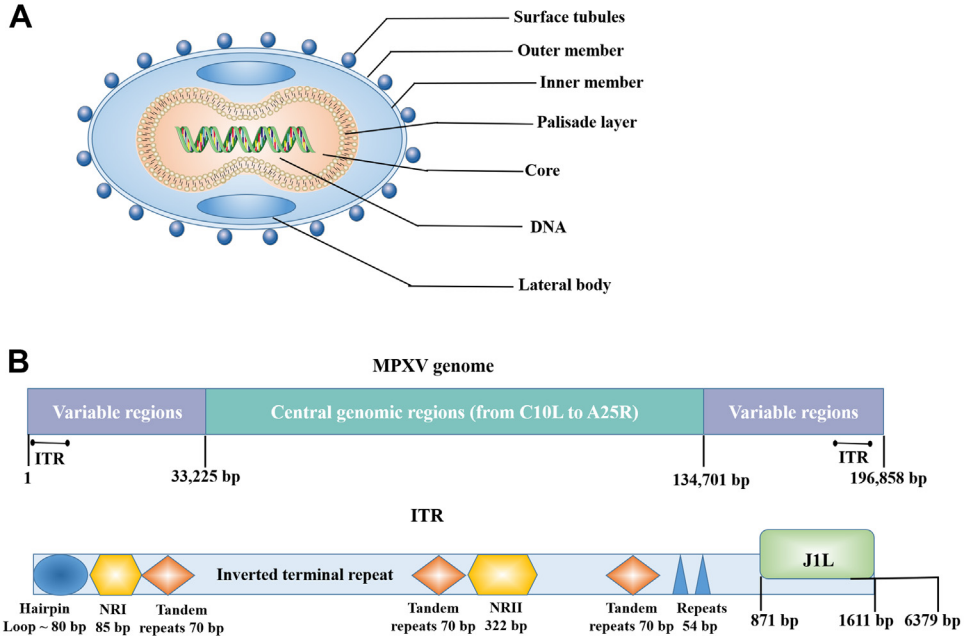

Fig. 1: **Structure and genome of monkeypox virus (MPXV).** (A) MPXV contains five distinct structures, including the core and palisade layer, outer and inner members, surface tubules, and nucleic acids (DNA). (B) The MPXV genome is comprised of two variable regions and a large conserved central genomic region (from C10L to A25R). An inverted terminal repeat (ITR) is located in the variable region, which consists of some ORFs, hairpin loops, and short tandem repeats. The terminal hairpin is adjacent to the tandem repeat region, the latter including NR II (322 bp) and NR I (85 bp), separated by two 70 bp repeats. Two elements of 54 bp and one tandem repeat of 70 bp are located between the ITR coding sequence (J1L) and NR II.

majority of host-range and virulence genes, which is substantially different from the variola virus.[23] Notably, genetic variation is essential for the survival of MPXV, promoting adaptation to hosts and ever-changing environments. The two major African clades contain several lineages, and each lineage has multiple mutations. Genomic variation of the MPXV was reported in samples from the Democratic Republic of the Congo, which is related to the disease's transmissibility and severity.[20] More recently, it was found that the apolipoprotein B mRNA-editing catalytic polypeptide-like 3 (APOBEC3) enzyme is important for MPXV evolution and potential human adaptation.[24] Genome sequencing may help track the spread of the virus in real-time globally and reveal potential mutations that could make it more transmissible or pathogenic.

Pathogenesis of monkeypox

The four stages of MPXV infection are viral particle entry, fusion, replication, and release. There are two distinct types of infectious virions termed intracellular mature virions and extracellular enveloped virions (Fig. 2). Enveloped virions are bound by antigenically distinct triple membranes, specialized for detachment from intact cells and diffusion within the host. In contrast, mature virions are comprised of a single membrane and mediate transmission between hosts.[25,26] Remarkably, antibodies and vaccines that target or produce both mature virion and enveloped virion antigens are significantly more protective than anti-mature virion or enveloped virion antibodies alone.[27,28] Smaller viruses subvert the host's defenses via rapidly replicating or slipping through gaps, while larger viruses, such as MPXV, are more likely to mount an immune response and require more comprehensive strategies to survive within the host.[29,30] It has been shown that two multi-subunit tethering complexes, conserved oligomeric Golgi (COG) and Golgi-associated retrograde protein (GARP), are essential for MPXV infection.[25,31] The COG complex is made up of eight proteins (COG1-8) and plays a key role in the regulation of glycosylation enzymes, membrane trafficking, and the maintenance of Golgi structure.[32,33] Notably, knockout of cellular COG7 and COG4 significantly reduces MPXV entry, fusion, and spread,[25] suggesting that loss of individual COG complex proteins results in an impairment of virus-cell interactions required for virus egress and entry. Similar to the COG complex, the GARP complex is also an evolutionary conserved multi-subunit tethering complex essential for the maintenance of Golgi apparatus functions. The GARP complex consists of four vacuolar protein sorting (VPS) proteins (VPS51-54).[34] VPS54 and VPS52 have been identified as the most important subunits for the formation of extracellular enveloped virions and MPXV infection.[31] In addition, two distinct regions of a Central African MPXV Congo strain, R1 (ORF 17-31) and R2 (181–192), have been reported to be associated with the virulence of MPXV.[35] Knockout of dual (ΔR1/R2) or individual (ΔR2 and ΔR1) target genomic regions in MPXV-Congo results in decreased mice morbidity and mortality and attenuated MPXV virulence.[35] Further studies showed that deletion of R2 mainly affects viral pathogenicity, whereas loss of R1 has a significant effect on viral replication.[35] However, the exact mechanisms by which multiple proteins or genes coordinately regulate MPXV-induced host pathogenesis and immunity remain largely unknown.

The immunopathology and immunopathogenesis of severe monkeypox

The MPXV-mediated immune injury leads to poor clinical outcomes in patients with monkeypox. MPXV infection not only causes skin lesions with the genital rashes as emerging clinical symptoms but also triggers a strong immune response that results in sepsis, deep tissue abscess, and severe respiratory disease.[3,36–38] Besides, MPXV infection also causes damage to multiple immune organs, including diffuse myeloid hyperplasia, thymitis, tonsillitis, splenic injury, and lymphadenopathy[39–43] (Fig. 3). A growing number of studies show that the immunopathogenesis of severe MPXV infection is related to impaired NK-cell function, lymphopenia, increased antibodies, increased blood monocytes and granulocytes, immune evasion, cytokine storm, inhibition of the host complement system, and antibody-dependent enhancement. These mechanisms are outlined in detail in the following sections (Fig. 4).

Lymphopenia and impaired NK-cell function

Lymphopenia usually represents a reliable indicator for disease severity. Lymphopenia, and elevated C-reactive protein were observed in monkeypox patients, as reveled by clinical chemistry tests.[44] A prospective observational cohort study of confirmed cases of MPXV infection revealed that 11% patients had a decreased CD4 cell count (less than 500 cells/μL).[42] This may at least be attributed to MPXV-induced immune organ damage, including the thymus, tonsils, spleen, and lymph nodes.

Natural killer (NK) cells are key elements of innate immunity. The suppression or activation of NK cells is triggered via interactions between their inhibitory or activating receptors and their ligands, such as major histocompatibility complex 1 (MHC-1) molecules. The killing function of NK cells is mediated through the secretion of granules (which contain granzymes and perforin) and cell–cell interactions. Besides, tumor necrosis factor α (TNF-α) and interferon α (IFN-α), which are produced by NK cells early in the infection, mediate inflammatory responses in inflamed tissues, and these cytokines also coordinate dendritic cells to promote T-helper type 1 (Th1) cell polarization.[45,46]

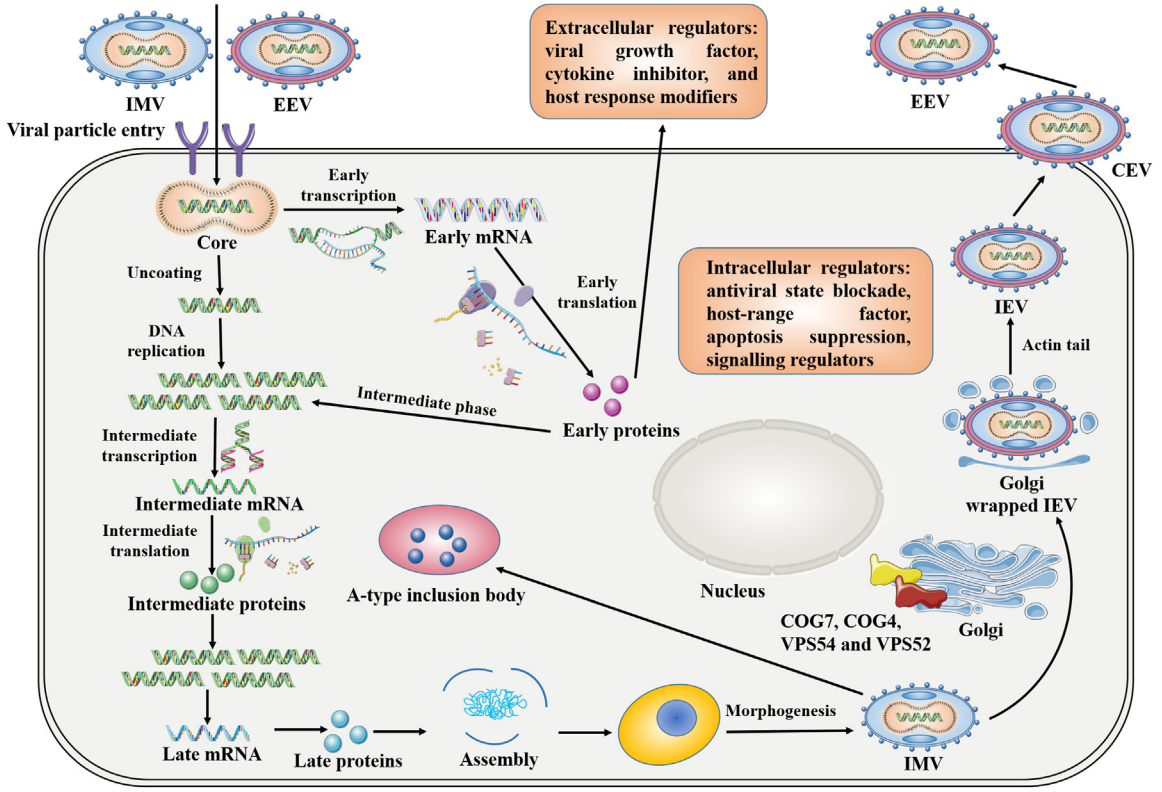

Fig. 2: **Schematic diagram of the mechanism of MPXV infecting host cells.** MPXV infects host cells through a complex but largely conserved morphogenetic pathway. There are two distinct types of infectious virions: intracellular mature virions (IMV) and extracellular enveloped virions (EEV). The number of enveloping membranes and surface glycoproteins of EEV and IMV particles are different. The virions bind to the surface of the host cell via unknown receptors or extracellular matrix components. MPXV replication involves three stages, including early, intermediate, and late viral mRNA and protein synthesis, followed by the assembly and morphogenesis of infectious virions. Then, IMV is wrapped by a double membrane derived from the Golgi to form an intracellular enveloped virus (IEV). Of note, golgi-associated retrograde protein (GARP) and conserved oligomeric Golgi (COG), two multi-subunit tethering complexes, are essential for MPXV infection. COG7 and COG4 play important roles in virus-cell interactions required for virus egress and entry, while VPS54 and VPS52 are responsible for the formation of extracellular enveloped virions and MPXV infection. Golgi-wrapped IEVs lose their outer membrane wrappings by Actin-tall and subsequently fuse with the cell membrane to form cell-associated enveloped viruses (CEVs). The CEVs are eventually released to form EEVs. In addition, MPXV also expresses an array of intracellular and extracellular modulatory proteins, which are essential for completion of the viral replication cycle.

MPXV infection induces lymphadenopathy and lymphoid depletion, and the changed number of lymphocytes, especially NK cells, is an important result of MPXV infection.[37,47,48] NK cells purified and expanded *in vitro* or *in vivo* with interleukin 15 (IL-15) overcome the inherent susceptibility of CAST mice to lethal infection with MPXV.[49] It has been reported that the NK cell number is significantly increased in both the lymphoid tissues and the blood of rhesus macaques during MPXV infection.[47] MPXV may express specific peptides or proteins that decrease inhibitory signals via reducing/altering the binding of NK cell inhibitory receptors to their ligands, such as by downregulating MHC-I expression, or directly interact with NK cell activating receptors to initiate activation signals. However, although MPXV induces increases in NK cell numbers, the cells display compromised degranulation capacity and reduced migration potential. The expression of chemokine receptors, such as CCR5, CXCR3, and CCR6, are strikingly reduced following MPXV infection, losing their capacity to secrete both TNF-α and IFN-α.[47] Compromised cytokine production by NK cells during MPXV infection can cause serious consequences. Firstly, reduced IFN-γ expression and impaired degranulation may directly dampen the capacity of NK cells to clear MPXV-infected cells as both perforin and IFN-γ are essential for NK cell-mediated antiviral activity.[50,51] Secondly, to deal with enormous viral loads, the immune system needs the collaboration of several arms of immunity, such as virus-specific antibodies and T cell responses. NK cells play an important role in initiating and directing adaptive immune responses, particularly T cell responses, via secreting cytokines and working in partnership with dendritic

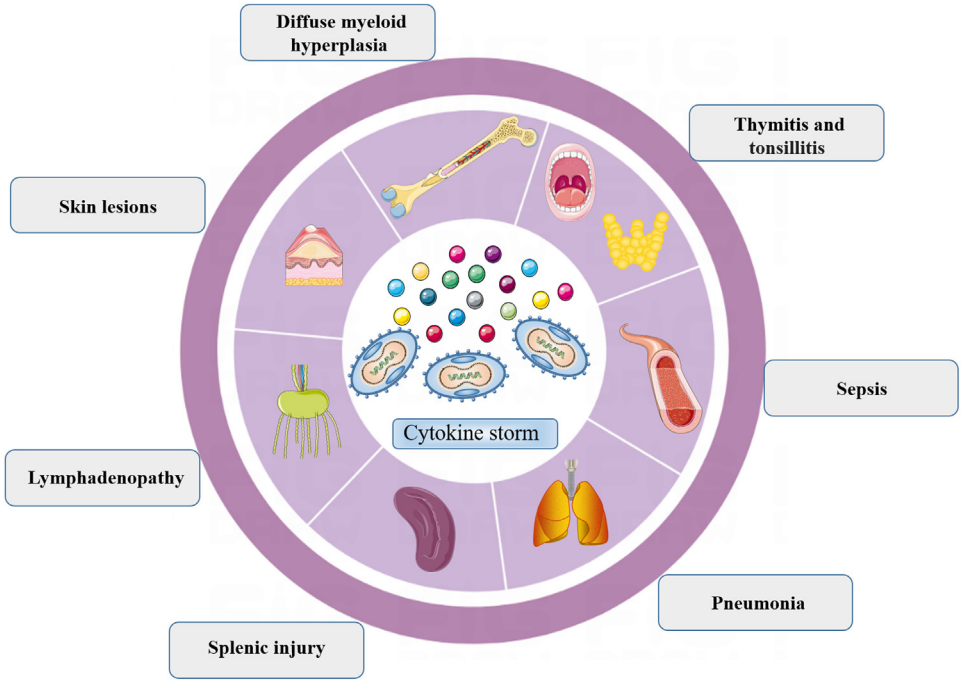

***Fig. 3:* Clinical implications of monkeypox virus-induced immunopathology.** Patients with monkeypox and presenting with lymphopenia have decreased immunity and are more prone to be infected with the microbe, which results in disease progression and increased severity. Besides, cytokine storms can initiate inflammatory-induced immune injury in multiple organs and consequently related diseases, including diffuse myeloid hyperplasia, thymitis, tonsillitis, sepsis, pneumonia, splenic injury, lymphadenopathy, and skin lesions.

cells.[52,53] Impaired cytokine secretion by NK cells may lead to decreased/delayed T cell activation and subsequent B cell activation.

Immune evasion

Innate immunity cells are activated by the binding of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) via their pattern-recognition receptors (PRRs). The most common PRRs are melanoma differentiation associated protein 5 (MDA5), C-type lectin-like receptors (CLRs), cytosolic protein kinase R (PKR), RIG-1 like receptor (RLR), NOD like receptor (NLR), and toll-like receptors (TLRs).[54,55] Recognition of viral genes induces association of the kinases TANK-Binding Kinase 1 (TBK1) and IκB Kinase Epsilon (IKK-ε), which collectively phosphorylate the interferon regulatory factor 7 (IRF-7), nuclear factor kappa B (NF-κB), and IRF-3.[56] Generally, activation of RIG/MDA5 stimulates nuclear translocation of IRF-3. The type 1 IFN responses serve as the first line of immune defense against viral infection by inducing the expression of many IFN-stimulated genes via activating the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway.[57,58] The innate immune response is largely dependent on the type I IFN responses, and the primary mechanism of viral immune evasion is also to target against the type I IFN responses.

MPXV evades the host antiviral innate immunity via inhibiting the type I IFN responses (Fig. 5). Song et al. reported that MPXV prevents IFN-γ production via impairing NK-cell function.[47] Another study by Arndt et al. showed that MPXV can replicate in the presence of IFN and antagonize the IFN-mediated antiviral immune response.[59] Furthermore, MPXV encodes the F3 protein, a homologue of the vaccinia virus E3 protein, which is essential for the IFN-resistant phenotype of MPXV. It has been reported that MPXV infection induces some accumulation of double-stranded RNA (dsRNA).[60] The MPXV F3 protein can bind dsRNA and sequester it away from known PRRs (e.g., MDA-5, RIG-I, and PKR), thereby blocking their activation.[59] For example, the MPXV F3 protein inhibits phosphorylation of PKR and eukaryotic translation initiation factor 2 (eIF2α) via a dsRNA-dependent manner, resulting in translational shutdown and reduced IFN production.[59,60] The production of secreted IFN decoy receptors, such as the IFN-γ receptor and IFN-α/β-binding protein (IFN-α/βBP), which bind IFNs with high affinity and block their interaction with cellular receptors, is also an important strategy for IFN evasion.[61,62] It was found that the highly virulent MPXV can encode secreted IFN-α/βBPs and inhibits type I IFN-induced immune

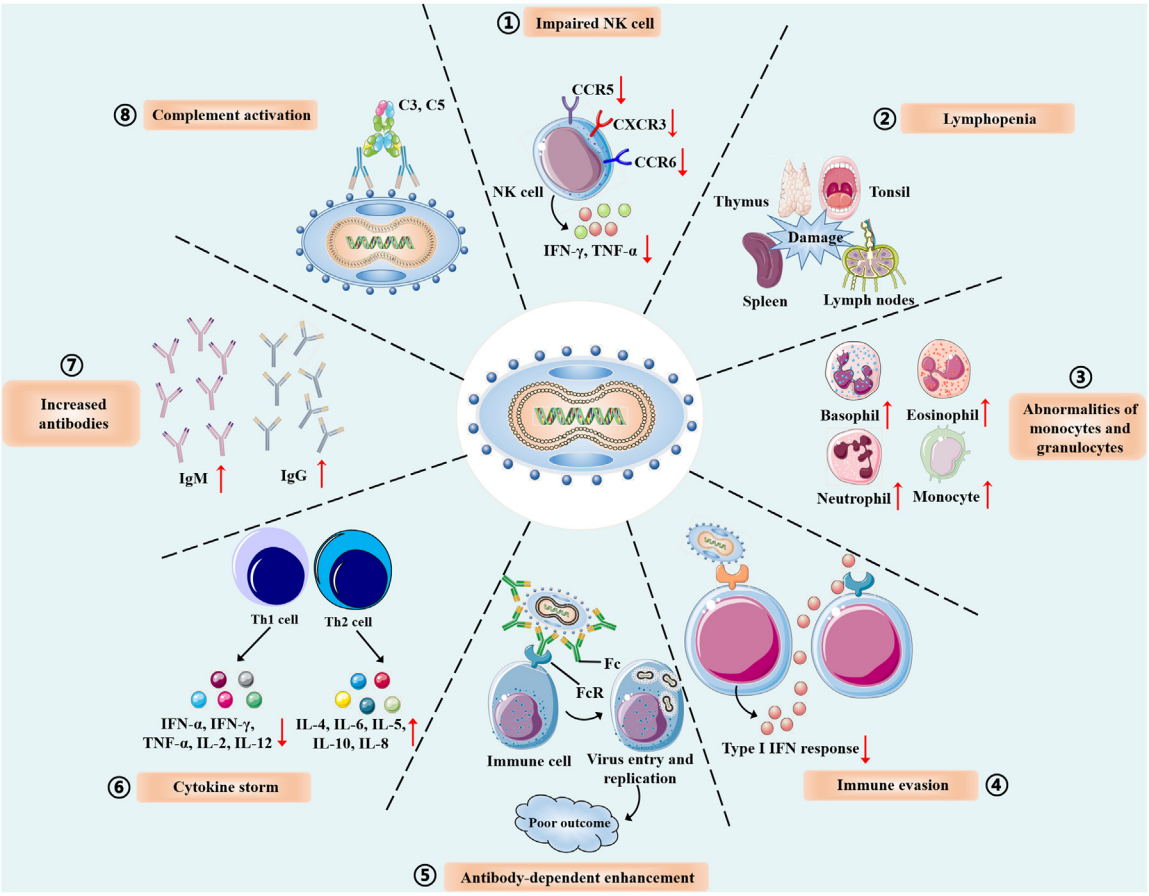

Fig. 4: **The immunopathology and immunopathogenesis of monkeypox.** 1) Monkeypox virus impairs NK cell function and reduces secretion of IFN-γ and TNF-α via inhibiting the expression of chemokines (CCR5, CXCR3, and CCR6). 2) Monkeypox virus infection directly damages immune organs, including the thymus, tonsils, spleen, and lymph nodes, thereby leading to lymphopenia. 3) Monkeypox virus induces abnormalities of granulocytes and monocytes, including basophil, eosinophil, neutrophil, and monocyte. 4) Monkeypox virus evades the innate immune response via suppressing the antiviral type 1 IFN responses. 5) A neutralizing monoclonal antibody targeting the virus can promote virus entry into cells by the Fc region of the antibody bound to the Fc receptor (FcR) on cells; this is correlated with disease progression and poor outcomes of patients with monkeypox. 6) The cytokine storm caused by monkeypox virus infection is associated with a prominent T helper 2 (Th2) immune response characterized by elevated serum levels of IL-4, IL-6, IL-5, IL-8, and IL-10 and attenuation of Th1-associated cytokines, such as (IFN-α, IFN-γ, TNF-α, IL-2, and IL-12.7) IgG and IgM levels are also elevated, and there is a higher titer of total antibodies. 8) Inhibition of the host complement system was found to be responsible for monkeypox virus-induced immunopathology.

response.[63] In addition, MPXV is capable of evading antiviral CD8+ and CD4+ T cell responses through preventing T cell receptor-mediated T cell activation via alternative antigen presentation.[64] Taken together, these studies suggest that MPXV facilitates mechanisms of immune subversion and evasion that allow its continued and persistent spread, which may lead to more serious clinical disease.

Cytokine storm

The cytokine storm, also known as hypertyrosinemia, is a group of disorders featured by the uncontrolled production of pro-inflammatory cytokines and is an important cause of multiple organ failure.[65,66] Severe MPXV infection induces a sustained cytokine storm with an overproduction of pro-inflammatory mediators and cytokines in cynomolgus monkey.[37] A study of nineteen confirmed cases of MPXV infection revealed that a cytokine storm occurs during human monkeypox disease and is positively associated with disease severity.[67] The cytokine storm caused by MPXV infection is associated with a prominent Th2 immune response characterized by elevated serum levels of IL-4, IL-6, IL-5, and IL-10 and attenuation of Th1-associated cytokines, such as IFN-α, IFN-γ, TNF-α, IL-2, and IL-12.[67] Consistently, MPXV infection induces significant increases in NK cell numbers but impairs NK-cell

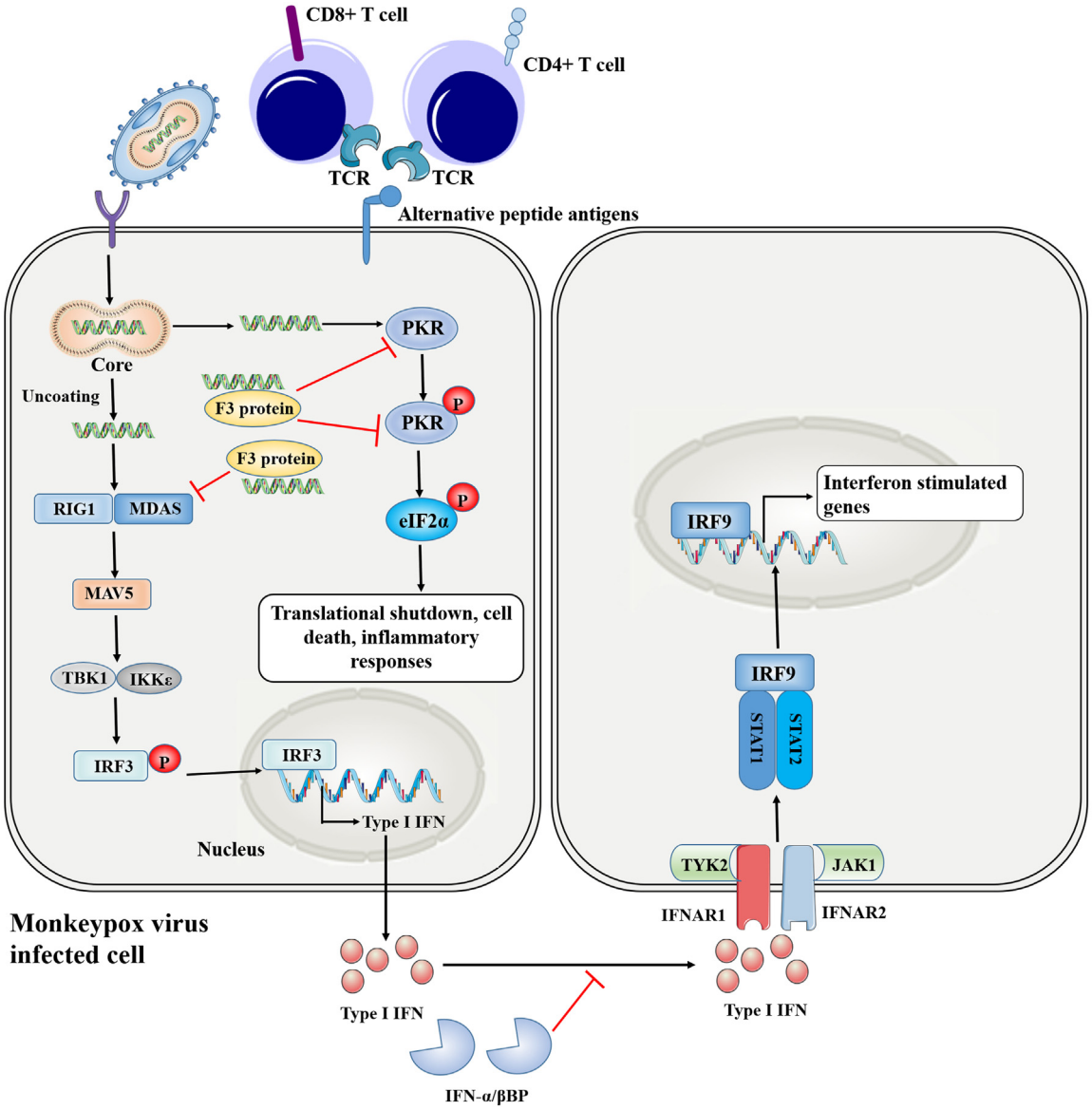

Fig. 5: **Immune evasion by monkeypox virus.** Monkeypox virus is sensed via various pattern-recognition receptors (PRRs) present in the host cell like melanoma differentiation associated protein 5 (MDA5) and retinoic acid inducible gene (RIG)-1. Recognition of monkeypox virus via PRRs stimulates the production of type I interferons (IFNs). Type I IFNs are then secreted in an autocrine and paracrine manner and stimulate the expression of interferon stimulated genes via the JAK-STAT signaling pathway, leading to the activation of antiviral response. The F3 protein encoded by monkeypox virus antagonizes multiple steps of the IFN signaling and escapes the immune response. Moreover, monkeypox virus also blocks the binding of IFNs to cellular receptors by secreting IFN-α/βBPs. In addition, monkeypox virus also evades antiviral CD4+ and CD8+ T cell responses through inhibiting T cell receptor-mediated T cell activation via alternative antigen presentation. JAK1: Janus Kinase 1, IKKε: IκB Kinase Epsilon, TBK1: TANK-Binding Kinase 1, IRF: Interferon Regulatory Factor, P: phosphorylation, TYK2: Tyrosine Kinase, STAT: Signal Transducer and Activator of Transcription, IFNAR: Interferon- Alpha/Beta Receptor, MAVS: Mitochondrial Antiviral-Signaling, eIF2α: eukaryotic translation initiation factor 2.

functions and suppresses the secretion of TNF-α and IFN-γ.[47] Therefore, cytokine storm may be a crucial immunopathogenesis of severe monkeypox, which may eventually result in viraemia, sepsis, deep tissue abscess, and severe respiratory disease.

Increased antibodies

Nucleic acid-based assays combined with the detection of MPXV-specific antibodies, including immunoglobulin G (IgG) and IgM, serve as the basis of monkeypox diagnosis. IgG, together with IgM, represent the acute-

phase humoral response following MPXV infection. It has been reported that both IgG and IgM levels are elevated in older patients, while younger individuals showed higher IgG levels only, perhaps being due to exposure to other orthopoxviruses and/or smallpox vaccination.[68] The IgG antibody is used to detect prolonged exposure after the onset of the rash, while the IgM is helpful for providing evidence of recent infection with and exposure to MPXV and defining the acute-phase humoral response.[69] Thus, the presence of both IgG and IgM e is strong evidence for recent exposure to orthopoxviruses in individuals who have been previously exposed to natural infection or vaccinated. However, although the increased IgG and/or IgM levels are detected in patients with MPXV infection, it remains unclear whether these antibodies are related to the disease severity and prognosis.

Antibody-dependent enhancement

Antibody-dependent enhancement is referred to a phenomenon of the virus infection that preexisting subneutralizing antibodies or non-neutralizing antibodies facilitate virus entry and replication, which has been found in a range of different viruses, such as the SARS-CoV-2, Dengue virus, Zika virus, and Ebola virus.[70–72] Antibodies elicited via vaccination may trigger antibody-dependent enhancement, leading to a more severe disease. For example, vaccine-elicited high-titer IgG antibodies have been suggested to induce antibody-dependent enhancement via binding to Fc receptors (FcRs) on the surface of immune cells, thereby increasing the risk of exacerbating COVID-19 severity.[71,73] Although antibody-dependent enhancement has been well established in many viruses, it has not been reported in MPXV-infected cases. However, in cases where antibodies to MPXV or other natural orthopoxviruses already exist in wildlife populations, domesticated animals, and humans, natural superinfection with orthopoxviruses or exposure to smallpox vaccine may enhance infectivity and alter the tissue tropism and host range of the MPXV. Therefore, investigation of antibody-dependent enhancement during experimental and natural infections will undoubtedly contribute to our understanding of the risks related to the use of next-generation vaccines, antiviral immunoglobulins, and vaccine-induced antibodies as monkeypox therapy.

Increased blood monocytes and granulocytes

Elevated monocytes and granulocytes (eosinophils, basophils, and neutrophils) in the blood have been reported in patients with severe monkeypox.[40,74] In non-human primate models of monkeypox, increased granulocytes and monocytes are also observed.[75,76] The high ratio of neutrophil-to-lymphocyte usually indicates a poor clinical outcome and a worsening of the disease.[77,78] Consistently, several lines of evidence also show that blood granulocytes and monocytes are the major cells to be positive for poxvirus antigen in non-human primates with severe MPXV infection.[79,80] By contrast, some non-human primates that were infected with a lethal dose of MPXV and treated with cidofovir were negative for pox-antigen and all survived.[79] This result suggests that detection of virus antigen in granulocytes and monocytes may be important predictors of disease progression and outcome.

Inhibition of host complement system

The complement system is a crucial component of the innate immunity, performing a variety of functions, including the removal of cellular debris, the initiation of an inflammatory response, the activation of adaptive immunity, and the recognition of virus-infected cells.[81,82] The complement system can be activated by three different routes: the lectin, alternative, and classical pathways. All of these pathways converge on the C3 convertase complex, which consists of either C3b and/or C4b proteins. The complement system plays a crucial role in the neutralization of viruses because it coats the outside of viral particles with C3b, allowing dendritic cells and macrophages to engulf them, and thereby promoting the antigen-presentation to the adaptive immune system.[83,84]

Monkeypox Inhibitor of Complement Enzymes (MOPICE), a 24 kDa secretory protein that is a homologue of the complement control protein of the vaccinia virus, is encoded by the more virulent Central African strain of MPXV.[85] The West African strain of MPXV lacks this gene. It was found that MOPICE can bind to C4b and C3b and has cofactor activity to enable the proteolytic cleavage of C4b and C3b by the plasma serine protease factor I.[86] MOPICE also directly cleaves C3b to iC3b2, thereby preventing the formation of a C3 convertase complex.[85] Deletion of MOPICE in the Central African strain markedly decreases the mortality and morbidity of monkeypox disease in prairie dogs.[87] Moreover, loss of MOPICE leads to a delayed and reduced adaptive immune response against MPXV in a non-human primate model of infection.[88] Conversely, the inclusion of recombinant MOPICE in the West African clade MPXV does not significantly aggravate the clinical disease course and affect disease mortality.[87] These data indicate that MOPICE may not be the sole factor responsible for the increased virulence in the Central African clade of MPXV. Several homologues of known virulence factors, such as IL-1 binding protein, B14R, D14L, and the myxoma virus M-T4, are also absent in the West African strain genomes and are thought to be important in determining virulence.[86,89]

Taken together, these studies suggest the important role of MOPICE in severe MPXV infection, and targeting MOPICE may be a promising immunotherapeutic strategy for severe monkeypox disease.

Immunotherapies for monkeypox

As discussed above, MPXV-induced immunopathology is closely associated with the severity of monkeypox disease. Immunotherapy has shown remarkable results in the treatment of a variety of diseases, including inflammatory diseases and viral infections. Targeting the specific immune profiles of monkeypox may be a promising therapeutic strategy for severe cases.

Vaccines

It has been reported that smallpox vaccination provides 85% cross-protection against MPXV infection.[90,91] The current resurgence of monkeypox may be partly due to the discontinuation of smallpox vaccinations since the eradication of smallpox in 1980. There are currently two approved vaccines available to prevent smallpox in the USA, including ACAM2000 (a purified clone isolated from the Dryvax vaccine) and JYNNEOS (modified vaccinia Ankara; Bavarian Nordic). The JYNNEOS in the USA is also referred to as IMVAMUNE in Canada and IMVANEX in Europe. The Advisory Committee on Immunization Practices (ACIP) recommends both the ACAM2000 and JYNNEOSTM vaccines for orthopoxvirus prophylaxis. Currently, the only vaccine approved by the US Food and Drug Administration (FDA) and the European Medicine Agency (EMA) for use in the prevention of monkeypox is JYNNEOS/IMVANEXTM.[92,93] Of note, individuals with immunodeficiencies such as atopic dermatitis and acquired immunodeficiency syndrome (AIDS) should not be vaccinated with ACAM2000 (the second-generation vaccine) but can receive JYNNEOS/IMVAMUNE/IMVANEX (the third-generation vaccine). Another third-generation vaccine is LC16 m8, an attenuated vaccinia virus derived from the Lister (Elstree) strain that has been licensed for active smallpox immunization in Japan since 1975. This vaccine was currently expanded by Japan to include protection against monkeypox.[94] These third-generation vaccines have a higher safety profile due to their attenuated phenotype and can be given to immunocompromised people. Besides, it is essential to determine the protective efficiency of these vaccines in areas currently endemic to monkeypox. Before exposure, the smallpox vaccine is approximately 85% effective in preventing monkeypox. However, the vaccine provides less protection when given after exposure. Therefore, when tracing and vaccinating contacts of cases, the vaccine effectiveness in practice will depend on how many at-risk groups can be vaccinated before infection.

Intravenous immunoglobulin (IVIG)

Intravenous immunoglobulin (IVIG) contains the pooled polyclonal immunoglobulins that have been purified from the plasma of thousands of healthy donors. IVIG has been employed in the treatment of various immune-related, rheumatological, dermatological, and neurological disorders.[95,96] The underlying mechanisms by which IVIG improves immune response has been partly attributed to decreased macrophage activity, reduced endogenous antibody production, inhibited auto-reactive T cells, and a balanced cytokine profile.[97] IVIG has previously been used to treat smallpox, which may be considered for the treatment of MPXV infection. IVIG is recommended for monkeypox patients with severe complications, or as a prophylactic measure for T-cell immunodeficiency exposed patients for whom smallpox vaccination is contraindicated.[98,99] In addition, vaccina immune globulin, a medical product recently licensed by the FDA, has shown efficacy against complications following variola virus immunization and may play a role against MPXV.[9,100] Taken together, these studies suggest that IVIG is an important therapeutic strategy for improving the outcomes of patients with severe MPXV infection.

Antiviral drugs

Antiviral drugs are important therapeutic tools for MPXV infection. Young children under 8 years of age, patients with severe disease or immunodeficiency, and pregnant individuals should be considered for antiviral therapy.[101] There are two FDA-approved antiviral drugs for the treatment of smallpox, which may also be adapted to treat monkeypox: brincidofovir and tecovirimat. Antiviral therapy was reported to be more effective than smallpox vaccination for lethal MPXV infection in a cynomolgus monkey model.[102] Tecovirimat (ST-246) is the first FDA-approved drug available for the treatment of smallpox, which has specific efficacy against several orthopoxviruses including monkeypox.[103–105] Tecovirimat inhibits viral envelope formation via targeting the viral p37 protein, a highly conserved protein in all orthopoxviruses.[103,106] The therapeutic effects of tecovirimat on monkeypox disease have been well established in animal studies.[107,108] Of note, tecovirimat treatment significantly attenuates lethal MPXV infection-induced inflammatory effects in cynomolgus macaques.[104] Several clinical studies suggest that tecovirimat is safe and well tolerated in healthy human volunteers.[109,110] More importantly, there were few side effects observed in 369 patients among 99.8% of 549 MPXV-infected patients who received tecovirimat orally during outpatient visits, supporting the continued treatment with tecovirimat for patients with clinically diagnosed or laboratory-confirmed MPXV in the ongoing monkeypox outbreak.[111] Although there is limited clinical experience

using the drug in human outbreaks, tecovirimat was licensed by the FDA and EMA for the treatment of monkeypox in 2022.

Brincidofovir (CMX001) is a lipid conjugate of cidofovir with broad antiviral activity against double-stranded DNA viruses by inhibiting DNA polymerase.[112,113] *In vivo* and *in vitro* experiments have demonstrated that brincidofovir has antiviral activities against a variety of orthopoxvirus species.[114–116] In a murine model of lethal MPXV infection, treatment with brincidofovir markedly improves MPXV-induced immunological and pathological responses.[117] Of note, a recent retrospective observational study showed that three patients treated with brincidofovir did not show any clear benefit.[38] In contrast, patients treated with tecovirimat show reduced virus shedding and symptom duration without any side effects.[38] However, the relatively small number of patients included in this study makes it difficult to evaluate the relationship between brincidofovir treatment and disease course.

Other potential immunotherapeutic strategies

Other potential immunotherapies include immunomodulator agents, monoclonal antibodies, and NK cell-based therapy. Immunomodulator agents, which have an impact on immune system function, may be a potential therapeutic strategy for monkeypox. For example, recombinant IFNs are important immunomodulator agents that are approved for the treatment of SARS-CoV-2 and hepatitis viruses, which may be considered a candidate drug for severe monkeypox therapy. The *in vitro* experiment showed that IFN-β markedly inhibits the production and spread of MPXV.[118] Recombinant IFN-β has the potential to be a novel and safe agent for the treatment of monkeypox disease. Monoclonal antibodies have been widely suggested for the treatment of orthopoxvirus infections,[119–121] which may be a potential therapeutic strategy for severe MPXV infection. In addition, NK cells-mediated antibody-dependent cellular cytotoxicity (ADCC) is specific to almost all virus-infected cells, including human immunodeficiency virus (HIV), cytomegalovirus, orthopoxvirus, and SARS-CoV-2.[122–125] The activating NK cell receptor NKG2D is essential for NK cell-mediated killing of orthopoxvirus-infected cells.[125] Further studies are necessary to investigate the role of NK cell-based therapy in monkeypox.

Conclusion

Human monkeypox, a close relative of smallpox, has long been a neglected zoonotic disease. Over the past decades, considerable progress has been made in unraveling the immunopathology and immunopathogenesis of monkeypox. Severe MPXV infection impairs the immune system through multiple mechanisms, including impaired NK-cell function, lymphopenia, increased antibodies, increased blood monocytes and granulocytes, immune evasion, cytokine storm, inhibition of the host complement system, and antibody-dependent enhancement. An outline of the relationship between immune profiles and monkeypox provides novel insights into the pathogenesis of monkeypox and the treatment of severe cases.

Outstanding questions

In the future, ongoing studies on monkeypox need to address the following issues. Firstly, it should be noted that men who are living with HIV are overrepresented in the current multinational monkeypox outbreak, with up to about 50% of MPXV-infected patients with known HIV status being HIV positive.[126] Monkeypox was diagnosed at the same time as acute HIV infection, emphasizing the significance of testing for both infections when monkeypox is suspected or diagnosed.[127,128] Compared with other MPXV-infected patients, those with HIV infection have compromised immune systems and are more likely to develop prolonged or severe monkeypox disease after infection.[129,130] Several interventions have been proposed to reduce the risk of serious illness in patients infected with HIV and monkeypox. For example, tecovirimat is a first-line medication that can be used in combination with antiretroviral therapy (ART) to treat patients with HIV infection and monkeypox.[111,130] Besides, the JYNNEOS vaccine, which is licensed for the prevention of monkeypox and smallpox, is considered safe for HIV-infected individuals and can be used as pre- and post-exposure prophylaxis.[131–133] Secondly, more research is needed for treatments targeting the immunopathology of monkeypox infection, in addition to vaccines and antiviral drugs that directly target the virus or prevent viral entry. The combined use of antiviral drugs and immunotherapies may be more effective than using either modality alone. Thirdly, antibody-dependent enhancement has been observed in various viruses, which may be a major concern for antibody-based therapies and next-generation vaccine development. Additional investigations, especially large prospective cohort studies, are required to dismiss or confirm this possibility. Fourthly, although there have been case reports of antiviral drugs being used to treat patients infected with the MPXV, these data are insufficient to demonstrate efficacy. Therefore, it is necessary and feasible to carry out clinical trials on humans suffering from monkeypox. Fifthly, disorders of the neuroendocrine-immune crosstalk induced by viral stress may be an important causative factor that influences treatment outcomes. It is well known that viral infection-induced release of cytokines can trigger the neuroendocrine system to produce some peptides and

glucocorticoids, impairing immune functions.[134,135] Of note, the neuroinvasive potential of MPXV infection has also been reported.[136–138] Further studies are necessary to investigate whether MPXV infection impairs immune responses via inducing disorders of the neuroendocrine-immune crosstalk. Sixthly, whether prior infection with MPXV or vaccination against smallpox triggers any form of mucosal immunity is currently unknown. Given that MPXV has been identified and detected in the upper respiratory tract and semen.[38,139] Therefore, it is important to characterize MPXV-induced mucosal immune responses in the respiratory tract and prepuce. Understanding the roles of tissue-resident memory T cells and IgA in MPXV infection will provide greater insight into MPXV-induced mucosal immune responses.[140–142] Finally, it is also important to implement a standardized successful treatment protocol globally and identify prognostic biomarkers for severe patients, which may contribute to prevent the monkeypox epidemic. Collectively, addressing these sorts of questions in the future will undoubtedly offer greater insight into the immunopathology and immunotherapies of severe monkeypox.

Contributors

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Search strategy and selection criteria

We searched PubMed from Jan 1 2000 to Nov 11 2022 with the following search terms, alone or in combination: monkeypox, immune response, immune evasion, Orthopoxvirus, monkeypox virus, immune cells, clinical manifestations, pathogenesis, immune organ, and therapy. Our search was performed between Aug 10 and Nov 11th, 2022.

Declaration of interests

The authors declare they do not have anything to disclose regarding conflicts of interest with respect to this manuscript.

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