Monkeypox is a zoonotic viral disease caused by the monkeypox virus (MPXV), an orthopoxvirus belonging to the Poxviridae family of viruses. MPXV was first identified in 1958 in research monkeys that were shipped from Singapore, which is the likely reason for the disease being called ‘monkeypox’. However, the natural hosts of MPXV are more likely to be rodents and other small mammals. The Orthopoxvirus genus also includes variola virus (VARV), the causative agent of lethal smallpox disease. The symptoms of monkeypox in humans are relatively similar to those of smallpox, but with a lower mortality rate.

Sporadic cases of MPXV in humans were first identified in the 1970s in several African countries, but the virus became more widespread within the African continent over the past 20 years (see BOX 1 for details). Since May 2022, there has been a drastic increase in the number of MPXV cases worldwide (FIG. 1), leading the World Health Organization (WHO) to declare the monkeypox outbreak a global health emergency.

Possible reasons for this current outbreak could be attributed to waning smallpox immunity in the general population and termination of the smallpox vaccination regime. Vaccination against smallpox has been shown to offer protection against monkeypox. An early study from Zaire in 1988 reported that individuals vaccinated against smallpox (during a national smallpox vaccination campaign beginning 12 years before the start of data collection) were approximately 85% less likely to contract monkeypox than those who were not vaccinated. In another study, severe complications and long-term effects of MPXV infection were found to be less common (39.5% versus 74%) with lower death rates in patients vaccinated against smallpox. Recently, a study on 328 infections diagnosed during this current outbreak reported that only 9% of the individuals who were infected received prior smallpox vaccination. Interestingly, one characteristic of the recent outbreaks is a disproportionate number of infections in men who have sex with men (MSM).

Phylogenetically, MPXV can be divided into two distinct clades — Central African (also commonly known as Congo Basin) and West African. Depending on the source of the West African MPXV, sequence similarity of ~95% or >99% between the two clades was reported. The Central African clade is generally considered to be more virulent, with an average fatality rate of 10.6% (95% CI 8.4–13.3%) compared with the 3.6% (95% CI 1.7–6.8%) reported for the West African clade. MPXV of the Central African clade has been identified in cases appearing in the Democratic Republic of the Congo (DRC) and South Sudan, whereas cases in Nigeria between 2010 and 2019 appeared to be due to the West African clade. Cases reported outside Africa, including those currently in circulation, have all been caused by the West African clade. Whether genetic changes in the MPXV genome could be responsible for this current outbreak is currently being investigated (see BOX 2 for details).

Here, we discuss our current understanding of the transmission and immunopathogenesis of MPXV and the unique epidemiological and pathological characteristics observed in this outbreak. Specifically, we examine the potential mechanisms of host immunity against MPXV, drawing parallels from other poxviruses where necessary. We also discuss vaccines and therapeutics, and highlight remaining critical gaps in our knowledge.
Of note, nomenclature for orthopoxviruses gene/protein orthologues is very complex. In this Review, we refer to orthopoxvirus genes and proteins according to publicly available reference genomes: NC_063383 for the West African MPXV strain, NC_003310.1 for the Central African MPXV Zaire strain and NC_006998 for the vaccinia virus (VACV) Western Reserve strain. MPXV genes/proteins are referenced to the Central African MPXV Zaire strain, unless otherwise stated.

MPXV genome and phylogeny

Computational analysis of the Central African MPXV Zaire strain revealed the presence of at least 190 open reading frames (ORFs)\(^\text{15/17}\). Genes known to be important for orthopoxviruses are present within the central region of the MPXV genome (between ORFs C10L and A25R)\(^\text{16/17}\). However, a subset of ORFs are either missing or truncated in the MPXV genome compared with the genomes of other orthopoxviruses\(^\text{11,16,18}\). Disruptions to several ORFs that code for genes involved in immune evasion have been reported in the West African clade, and these may be responsible for this clade’s lower virulence relative to the Central African clade\(^\text{11,14,18}\).

The virus exists in two distinct infectious forms, the intracellular mature virus and the extracellular enveloped virus, which differ in their surface glycoproteins and infect cells via different mechanisms\(^\text{11}\). MPXV replication is a complex process but is generally thought to be identical to other orthopoxviruses\(^\text{11}\). Entry receptors for MPXV have not been clearly identified, although it was suggested that viral entry is dependent on the viral strain and host cell type and involves multiple surface receptors, including chondroitin sulfate\(^\text{20}\) or heparan sulfate\(^\text{14/21,22}\). In VACV, surface proteins H3, A27 and D8 have been associated with viral binding\(^\text{20–22}\). Following binding, VACV gains entry into the cell through 11 conserved proteins, which form a complex known as the entry fusion complex\(^\text{23}\).

Monkeypox pathology

MPXV infection has an incubation period of 5–21 days, and common symptoms include fever (between 38.5 °C and 40.5 °C), headache and myalgia. A distinguishing feature of MPXV infection is the presence of swellings at the axillary, cervical or inguinal lymph nodes (lymphadenopathy)\(^\text{11/12}\). In the recent outbreak, a report from Portugal suggested that inguinal lymphadenopathy was more common than cervical and axillary lymphadenopathy\(^\text{23/25}\). Rashes appear following the onset of fever, beginning on the face, tongue and oral cavity (enanthem) before spreading across the body. In the later stages of infection, lesions in the oral cavity can cause difficulties in drinking and eating\(^\text{4}\). However, in the recent outbreaks, several atypical clinical observations have been reported. In patients who are MSM, these include the presence of genital lesions that subsequently spread to other sites in the body, as well as anal ulcers, and it appears that skin lesions may be more limited in distribution than reported in previous outbreaks\(^\text{15/26}\).

Disease severity can be classified using the lesion count, as higher lesion counts correlate with increased risk of severe complications\(^\text{1}\). Patients with severe complications may experience respiratory and gastrointestinal issues\(^\text{27}\), encephalitis\(^\text{1}\), septicaemia\(^\text{27,28}\) and ocular infections leading to permanent vision loss\(^\text{29}\). Skin lesions also increase the likelihood of dermal bacterial infections, especially in patients who are not vaccinated against smallpox\(^\text{30}\).

Lesions typically progress through four stages — macular, papular, vesicular and pustular — before falling off as scabs\(^\text{3}\). Patients are typically considered non-contagious once lesions have crusted over. However, scabs have been reported to contain significant quantities of MPXV DNA even after falling off\(^\text{23}\), which may indicate the presence of infectious viral material. Of note, viable VARV has been isolated from scabs of patients with smallpox\(^\text{3}\).

During pregnancy, MPXV can be vertically transmitted from the mother to the fetus\(^\text{3}\). In one study involving four pregnant women who were infected with MPXV in the DRC, only one gave birth to a healthy infant. Two women experienced miscarriage in the first trimester and one had a stillbirth. In the stillborn, skin lesions were observed across the body\(^\text{31}\). In another study, four out of five women who were infected with MPXV in the DRC had fetal demise and lesions were observed on the maternal surface of the placenta\(^\text{32}\). The studies did not report which clade of MPXV these patients were infected with, although given the location of the studies it is very likely the Central African clade\(^\text{4}\).

A study conducted in Zaire during 1980–1985 reported a higher incidence of fatal disease in young children infected with MPXV, with a case fatality rate of 14.9% in children aged between 0 and 4 years compared with a rate of 0% in individuals aged 10 years or older\(^\text{3}\). This disparity could potentially be due to differences in their immune responses\(^\text{3}\). Currently, data on severity of infection in children infected with the West African clade are lacking\(^\text{1}\). Nevertheless, the severe outcomes of monkeypox in pregnant women and in young children highlight the importance of future public health efforts to limit the spread of MPXV and minimize the risk of adverse events.

MPXV pathogenesis

The clinical outcome of orthopoxviral infection in a vertebrate host is strongly dependent on the entry route used by the virus to establish the primary infection\(^\text{3}\) (FIG. 2). For several orthopoxviruses, such as the highly
Box 1 | History of monkeypox

Epidemiological surveys to identify residual clusters of smallpox in the 1970s also led to the identification of the first cases of monkeypox virus (MPXV) in humans. A total of 48 confirmed and/or suspected cases were reported across six African countries: the Democratic Republic of the Congo (DRC), Cameroon, Liberia, Nigeria, Sierra Leone, and Côte d’Ivoire. Between 1980 and 2000, MPXV was confined to the African continent, with no reports of infection or transmission in other parts of the world. The number of MPXV cases in Africa continued to climb, mainly in the DRC (>800 confirmed and/or suspected cases), with sporadic cases reported in Gabon, Cameroon, Côte d’Ivoire and the Central African Republic (CAR).

During this period, Nigeria reported 181 confirmed and/or suspected cases, and the DRC reported more than 20,000 suspected cases. The first report of MPXV infection outside Africa was in 2003, when an outbreak of 47 confirmed and/or suspected cases was reported in the United States. This outbreak was caused by human exposure to prairie dogs that had been infected by imported Gambian pouched rats. Other countries with reported MPXV infections were Israel (2018; one case), the United Kingdom (2018–2021; seven cases), Singapore (2019; one case), and the United States (2021; two cases) (reviewed in Ref. 1).

As described above, MPXV was largely restricted to endemic regions prior to the current outbreak. Particularly, it is noteworthy to highlight that between 2017 and 2022, Nigeria reported more than 650 confirmed cases, where nine patients eventually succumbed to the infection. The majority of the infected patients were male and aged between 21 and 40 years.

Since May 2022 there has been a drastic increase in the number of MPXV cases reported from Europe, the Americas, the Middle East and Australia (see Fig. 1). The current spread of MPXV is unprecedented, with outbreak clusters expanding each day. By 28 August 2022, close to 50,000 confirmed and suspected cases were reported from more than 100 countries, with 13 deaths. Recently, Singapore confirmed several imported and local cases of monkeypox, which are the first reported in Southeast Asia.

In VACV infection, it is believed that lymphoid organs such as the spleen and bone marrow support virus replication, but there is less evidence for hepatic involvement. The presence of orthopoxviruses in the small dermal vessels marks the start of skin infection and development of skin lesions. However, how the virus reaches the upper skin layers, which lack blood and lymphatic vessels, is not well understood. It is possible that infected migratory skin dendritic cells such as Langerhans cells might be responsible, as they are known to be susceptible to VACV infection. Infiltration of macrophages, dendritic cells and CD3+ T cells has been observed around the infective pustule. The role of skin-infiltrating CD3+ T cells in the context of MPXV infection requires characterization; this is particularly true for cytotoxic T lymphocyte responses, which have been associated with better virus control in vaccinated rhesus macaques. Importantly, epithelial lesions (enanthema) also appear during MPXV infection in the oropharyngeal mucosa, tongue, pharynx, larynx, trachea and oesophagus, eventually evolving into ulcers which release infectious viral particles into the saliva.

Infection can also occur via the skin. It has been postulated that infection of the dermal keratinocytes, fibroblasts and tissue-resident antigen-presenting cells such as monocytes, macrophages, Langerhans cells and dendritic cells might occur and migratory antigen-presenting cells could contribute to virus dissemination through the lymphatics. Nonetheless, recent evidence from a mouse model of orthopoxvirus
skin infection suggested that dendritic cell migration from the skin to draining lymph nodes is impaired on VACV\(^6\). The relocation of the virus from the skin to the lymphatics might also be supported by other mechanisms such as direct lymphatic vessel access, as observed in skin infection models of Zika virus\(^5\).

Sexual transmission of monkeypox has been speculated\(^5\), and MPXV was recently identified in the semen of three male patients in Italy\(^26\). Cases of monkeypox with exclusive genital lesions have also been reported\(^5\), which might indicate preferential MPXV tropism into the testes. Being an immune-privileged tissue\(^5\), the testes could act as a site for latent MPXV infection, but this requires further investigation. Nonetheless, recent animal studies showed that the related VACV exhibits tropism for testicular and ovarian tissues\(^6,51\). Viral shedding was also reported in faeces and contact with the rectal mucosa might increase the likelihood of MPXV transmission\(^69\). It was previously noted in patients with human immunodeficiency virus 1 (HIV-1) that these tissues could act as a reservoir for the virus\(^6\). A recent study\(^6\) determined that the rectal mucosa immune environment in MSM was significantly different compared with individuals who are heterosexual, with a higher presence of immune activities indicative of mucosal injuries. This condition could lead to the recruitment of immune cells, which could then be easily targeted by infectious agents, such as observed with HIV-1\(^6\). This could similarly apply to MPXV transmission and infection in MSM. However, this does not indicate that monkeypox has become a sexually transmitted disease, as MPXV can spread through any form of close contact with the infectious pustules that are symptomatic of monkeypox.

**Immunity to MPXV**

Even though the virus was identified decades ago, human immunity to MPXV infection has not been extensively characterized. As such, inferences on MPXV interaction with the host immune system are often drawn from studies performed with VACV and related orthopoxviruses. In the following sections, we highlight the potential mechanisms of host immunity against MPXV and discuss the immune evasion strategies utilized by MPXV during active infection.

**Innate immune responses to MPXV.** Innate immune cells typically act as the first line of defence following active viral infection, but these cells also serve as targets for some viruses. Numerous in vitro and in vivo studies have demonstrated that monocytes are the initial targets of poxviruses\(^36,65–68\). Early detection of poxvirus antigen in both monocytes and neutrophils has been suggested to be a strong predictor of MPXV lethality\(^69\). Susceptible monocytes are actively recruited to sites of infection, with marked expansion of CD14\(^+\) monocytes in the lungs of cynomolgus macaques experiencing viral pneumonia following MPXV infection\(^69\). Mouse CD45\(^+\)CD11b\(^+\)GR-1\(^+\) inflammatory monocytes have also been shown to be permissive to VACV replication and may be plausible vehicles for virus dissemination\(^6\). It was also reported that human primary M2-like macrophages allowed VACV replication and dissemination\(^6\). Following VACV infection, these primary macrophages formed actin tails,
Genetic accordions
Multistep events involving gene application and mutations that allow poxviruses to subvert host antiviral responses.

Box 2 | Genetic changes in current circulating MPXV

Understanding whether the recent monkeypox outbreaks are a result of genetic changes in monkeypox virus (MPXV) remains a research challenge. The 2022 outbreak is caused by lineage B.1 of the West African clade (MPXV Clade 3)\(^\text{173}\). At least 46 single-nucleotide polymorphisms have been found to be specific for this lineage, including 24 non-synonymous single-nucleotide polymorphisms\(^\text{173}\). Recent molecular epidemiology studies have shown a higher than expected rate of genomic variance among the outbreak sequences, suggesting accelerated evolution\(^\text{173}\) and, potentially, APobec3 editing\(^\text{174}\). One preprint publication (not peer reviewed) reports the presence of insertions and deletions in the DNA of the MPXV strain that is circulating in the 2022 outbreak compared with MPXV strains that were circulating prior to 2017 and suggests that these changes may be responsible for the current outbreak\(^\text{175}\). However, it remains unclear whether and how these genetic differences drive the epidemiological phenotype. A leading hypothesis proposes that the three non-synonymous single-nucleotide polymorphisms (D209N, P722S and M1741I), which are found in the surface glycoprotein B21R, a key antibody target, are enhancing the transmissibility of the virus\(^\text{176,177}\). However, MPXV is a complex virus, with a DNA-based genome that is approximately six times as large (~197 kb) compared with the RNA-based genome of SARS-CoV-2. It encodes more than 190 open reading frames (orFs), many of which are needed to investigate whether such a phenomenon could be a plausible mechanism for MPXV evolution and spread.

In unvaccinated cynomolgus macaques, significant changes in host gene expression were detected following infection with VARV\(^\text{165}\). In particular, transcription of a cluster of interferon-associated genes was upregulated; this cluster was enriched for genes regulated by both type I and type II interferons, including PKR, STAT1, STAT2, MX1, MX2, IP10, OAS1, OAS2 and OAS3\(^\text{165}\). The animals that succumbed to the infection (two out of seven) had minimal interferon responses, indicating that this early interferon response protects...
against fatality. Human IFNβ has been demonstrated to inhibit MPXV replication and spread. However, MPXV does not robustly activate TNF-regulated and NF-κB-regulated genes, especially in animals that succumb to infection. This is not surprising, given that VARV and other orthopoxviruses harbour genes that can modulate TNF and NF-κB pathways. Although host immunity is required to combat infections, aberrant immune signalling can adversely affect infection outcomes. In another study of VARV-infected...
Monocytosis
An elevated number of infection-fighting monocytes in the blood circulation.

Cytokine storm
A sudden increase in levels of circulating cytokines leading to an over-activated immune response, which can be life-threatening.

**B cell and antibody protection.** The importance of B cells and immunoglobulins against poxviruses was first demonstrated with the successful global vaccination campaign that eradicated smallpox, which used a live VACV vaccine. 

It was further demonstrated that treatment with vaccinia immune globulin (VIG) isolated from the serum of vaccinees successfully protected close contacts of patients with smallpox from infection. In rhesus macaques, VACV-specific B cell responses were instrumental in protecting against a lethal MPXV infection. Importantly, epidemiological studies have further demonstrated that the VACV vaccine confers protection against other poxviruses, including MPXV. The VACV-specific memory B cells and antibody levels induced by vaccination were exceptional, in some cases lasting longer than 50 years. However, only ~50% of vaccinated individuals >20 years after vaccination had neutralizing antibody titres greater than 1:32, a correlate of protection suggested to confer protective immunity against smallpox. It is likely that cross-protective immunity against monkeypox may similarly wane over time.

Fourteen MPXV proteins have been shown to be recognized by cross-reactive VACV-induced immunoglobulins from human vaccinees. Three proteins in particular — MPXV (Zaire-1979_005) proteins D8, H3 and A26 — were targeted by neutralizing antibodies against MPXV in infected macaques. In VACV, orthologue D8 and H3 proteins are involved in the attachment of mature virions, whereas A26 associates with A27 to bind surface laminin. MPXV (Zaire-1979_005) proteins C19, A33 and A44 were also prominent antigens for IgM isolated from MPXV-infected macaques during the acute phase of infection — these proteins could thus be further developed as antigen-based serological diagnostic tools. In another study, prophylactic treatment with a cocktail of two mAbs — c7D11 and c8A — successfully protected marmosets against lethal MPXV infection. c7D11 and c8A target the VACV proteins L1 and B5, respectively, and have been recently formulated as a potential mRNA vaccine encapsulated in lipid nanoparticles. Gilchuk et al. showed that a mixture of human-derived mAbs targeting the VACV proteins D8, H3, A33, A27, B5 and L1 effectively cross-neutralized four clinically relevant orthopoxviruses, including MPXV and live VARV. However, despite knowledge of the MPXV proteins that are recognized by neutralizing antibodies, MPXV-specific epitopes (both conformational and linear) have not been extensively characterized.

The isotype composition of the anti-MPXV response may provide an important clue to pre-existing immunity and protection, as IgM antibodies typically dominate in primary immune responses, whereas IgG antibodies dominate in secondary immune responses. In a cohort of 200 patients infected with MPXV who were recruited between March 2007 and August 2011 in the DRC, individuals with both IgM and IgG responses were 5.09 times more likely to develop severe lesions compared with individuals who had IgG-only responses. Similarly, in a cohort of infected individuals from the 2003 MPXV outbreak in the United States, patients with moderate/severe disease had an overall higher titre of anti-orthopoxvirus
IgM compared with those with mild disease, and anti-orthopoxvirus IgG responses were much reduced and less frequent in patients with moderate/severe disease. It is plausible that an IgG-only response reflects robust levels of cross-protective IgG+ memory B cells that produce a dominant secondary antibody response, whereas the lack of such memory necessitates an IgM-dominated primary response that is less effective at preventing disease. Thus, IgM responses may be a biomarker for disease severity. This also emphasizes the critical and immediate need to extensively characterize the antibody profile of patients with MPXV in different cohorts. Likewise, VACV vaccine correlates of protection must be determined to explain why certain vaccinated patients experience breakthrough infections.

**T cell immunity.** CD4+ T cells, particularly T follicular helper cells, play a role in enhancing recall and differentiation of memory B cells into antibody-secreting cells. Memory CD4+ T cells were found to persist for up to 50 years or longer following VACV vaccine, with an estimated half-life of 8–15 years. These VACV-specific CD4+ T cells were capable of producing IFNγ and TNF following stimulation. However, no direct correlation was reported between numbers of virus-specific CD4+ T cells and anti-VACV antibody titres. By contrast, the number of CD4+ T cells was shown to be critically important in inducing a protective antibody response against lethal MPXV infection in VACV-vaccinated rhesus macaques. Simian immunodeficiency virus (SIV)-infected macaques with CD4+ T cell counts <300 cells mm−3 were not able to produce VACV-specific IgG following vaccination and died when challenged with MPXV. This observation is of high concern to both VACV-vaccinated and unvaccinated individuals with uncontrolled HIV-1 infection, as their CD4+ T cell counts are typically very low. This group of individuals is therefore at high risk of developing severe MPXV infection if exposed. They might also experience a more complicated pathology and provide the virus with an opportunity to acquire mutations that result in higher virulence or transmission potential. By contrast, a recent patient infected with MPXV who was on antiretroviral treatment for HIV-1 had a CD4+ T cell count >700 cells mm−3 and did not experience a severe disease outcome. This may suggest an important role for CD4+ T cells in regulating monkeypox severity. Nevertheless, more studies will need to be carried out to fully understand the role of CD4+ T cells during MPXV infection.

In addition to supporting antibody development, T cells can play direct antiviral roles. Given that orthopoxviruses, including MPXV, infect and disseminate in macrophages, cytolytic T cells can be instrumental in killing infected macrophages to prevent viral spread. CD8+ T cells have been shown to eradicate virus-infected monocytes and minimize virus dissemination in a mouse model of VACV infection. In fact, activation of CD8+ T cells in response to VACV infection has been shown to be dependent on γδT cells, which present VACV peptides via MHC class I molecules. Moreover, γδT cells also upregulate co-stimulatory molecules CD80 and CD86 and secrete IL-1 and IFNα for activation of CD8+ T cells. In a mouse model of VACV respiratory infection, IFNγ secretion by primary activated effector CD8+ T cells was shown to protect against lethality. Indeed, CD8+ T cell-derived IFNγ was sufficient for protection even in the absence of CD4+ T cells and B cells, highlighting the possibility that CD8+ T cells also confer protection against infection with other orthopoxviruses. Likewise, memory CD8+ T cells, which are induced following VACV immunization, were also demonstrated to protect against lethal ECTV infection in mice. These memory CD8+ T cells execute their protective effects via a combination of IFNγ and perforin secretion, and work concomitantly with primary effector CD8+ T cells to achieve optimal protection. In humans, standard smallpox vaccine administered by scarification was also able to induce primary cytotoxic CD8+ T cells and IFNγ-producing T cells. This observation was supported by another study, in which participants received live vaccinia smallpox vaccine (Dryvax). High levels of IFNγ-producing CD8+ and CD4+ T cells were detected following immunization.

In humans infected with VACV, activated CD4+ T cells were shown to upregulate genes related to cytolytic activities. Interestingly, MHC class II-restricted cytotoxic CD4+ T cells have also been reported in individuals immunized with VACV. These cells could be responsible for virus clearance in vaccinees with reduced or missing memory CD8+ T cell responses. In the experimental mousepox model, perforin-dependent cytolytic CD4+ T cells have been reported. Taken together, these observations highlight the importance of T lymphocytes in controlling orthopoxviral infections.

Across the orthopoxvirus proteome, numerous CD4+ and CD8+ T cell epitopes have been identified in humans, mice and NHPs. Many are conserved among the major orthopoxviruses and bind to human MHC class I and class II molecules. In particular, CD8+ T cells specific for two identified epitopes (MHC class II-restricted GRFVKDADGSKRDA and MHC class I-restricted NPVTYINELY) in the immediate-early E3 protein of VACV were capable of killing infected cells and halting the spread of VACV. Both epitopes are conserved in the MPXV homologue, which is encoded by the MPXV F3L gene. An earlier study showed that VACV missing the E3 protein did not protect cynomolgus macaques from subsequent MPXV infection. As E3 protein is detected within 30 min of VACV infection, it should be readily processed and presented by infected cells, allowing T cell-mediated lysis at an early infection stage before virion production and release. These properties make E3 an excellent possible candidate for future vaccine designs targeting all major orthopoxviruses.

Despite the potential importance of T cells in disease protection, smallpox vaccination does not necessarily provide robust T cell-mediated immunity against MPXV. In two out of five vaccinated individuals who subsequently contracted MPXV, orthopoxvirus-specific CD4+ and CD8+ T cells were not detectable throughout convalescence. Furthermore, similar levels of orthopoxvirus-specific CD4+ T cell reactivity were observed in vaccinated and unvaccinated patients, and orthopoxvirus-specific CD8+ T cell responses were in...
interferon regulatory factor 3 (IRF3) and NF-κB disrupting key transcription factors for the expression of inflammatory genes, such as involved in interfering with the signalling cascade of pathogen recognition receptors, numerous viral proteins that are involved in evading the host immunity. These can be

**Fig. 3 | Immune evasion by MPXV.** Monkeypox virus (MPXV) is known to encode numerous viral proteins that are involved in evading the host immunity. These can be involved in interfering with the signalling cascade of pathogen recognition receptors, disrupting key transcription factors for the expression of inflammatory genes, such as interferon regulatory factor 3 (IRF3) and NF-κB. MPXV can also interfere with interferon signalling by blocking IFNα/β binding or suppressing IFNα/β production and by blocking protein kinase R (PKR)-mediated pathways. In addition, MPXV secretes proteins that can target key inflammatory molecules such as TNF, IFNγ, IL-1β, IL-18 and IL-6. Moreover, MPXV can prevent apoptosis in infected cells by expressing numerous viral proteins that target the apoptotic pathways. The Central African MPXV Zaire strain also expresses D14 which blocks the activation of the complement cascade. However, this viral protein is not expressed in the West African MPXV strain. Lastly, MPXV can also downregulate the activities of natural killer cells and T cells by interfering with their activation processes (see also [TABLE 1](#tab1)). PRR, pattern recognition receptor.

One of the most important transcription factors downstream of PRR binding is IRF3, which controls the expression of the crucial antiviral molecules IFNα and IFNβ. The VACV B19 protein directly interacts with soluble interferons and inhibits their binding to receptors. Although deletion of the B19R gene in VACV did not affect its virulence, deletion of B19 in ECTV did severely attenuate its ability to establish infection. The MPXV orthologue B16 can inhibit IFNβ signalling. Interestingly, the interferon response is known to be stronger in children and has been shown to protect against severe SARS-CoV-2 infections, but the reverse was observed in children infected with MPXV, which emphasizes the need to better understand host–pathogen interactions and further characterize the mechanisms of MPXV pathogenesis.

The activation of NF-κB is controlled by proteins of the IκB family, which contain ankyrin repeats. The association of CD4+ and CD8+ T cell responses with the severity of MPXV infection remains inconclusive in human studies.

**MPXV immune evasion**

Orthopoxviruses have accrued an arsenal of genes that encode proteins which interfere with host cell signalling pathways that are involved in the recognition, apoptosis and immune regulation ([FIG. 3](#fig3)). Here, we discuss some of the evasion mechanisms used by MPXV during active infection ([TABLE 1](#tab1)). For a more in-depth review of orthopoxvirus immune evasion strategies, see [REFS. 85,133–136](#refs). Preventing cellular signalling. Mammalian cells can detect the presence of microbial infection through pattern recognition receptors (PRRs). These can trigger intracellular signalling cascades involving numerous host cofactors such as MYD88, TRAM, TIRAP and TRIF, eventually activating important immune transcription factors such as NF-κB and interferon regulatory factors (IRFs). Numerous orthopoxviral proteins can antagonize these signalling processes ([Table 1](#tab1)). For example, the VACV genome encodes numerous B cell lymphoma 2 (BCL-2)-like proteins that inhibit NF-κB and IRF3 activation by interacting with cofactors that are recruited following PRR binding. BCL-2-like proteins are generally conserved across orthopoxviruses — A47, B13, P1, C6 and D11 are orthologues of BCL-2-like proteins in MPXV. VACV also encodes the E3 protein, which binds to double-stranded RNA (dsRNA) that is produced late in its replication cycle, and prevents the dsRNA from being detected by host intracellular PRRs. This leads to complete inhibition of the protein kinase R (PKR)-mediated pathway, which can otherwise block protein synthesis by phosphorylating the eukaryotic translation initiation factor subunit 2α (eIF2α). The F3 protein of MPXV is a homologue of the VACV E3 protein with a 37 amino acid truncation at the amino terminus. Unlike a similarly truncated recombinant VACV (VACVA37N), MPXV can still inhibit host immune responses. Interestingly, another recombinant VACV expressing the MPXV F3L gene (VACV-F3L) did not inhibit host PKR activation, suggesting that MPXV has evolved to encode for yet undiscovered proteins that compensate for the missing N-terminal amino acids of F3 in limiting host antiviral activities.

Regulation of apoptosis. Another mechanism of orthopoxvirus immune evasion involves regulating apoptosis. The BCL-2-like proteins encoded by orthopoxviruses may interfere with the BCL-2-mediated...
regulation of the intrinsic apoptotic pathway. Numerous orthopoxvirus-encoded serine protease inhibitors (SPIs; serpins) have also been reported, such as CrmA in cowpox virus (CPXV)\cite{159}; its homologue SPI-2 (B13) in VACV is considered the virus’s most potent inhibitor of apoptosis\cite{160}. CrmA interferes with granzyme B\cite{161}, which is secreted by cytotoxic T cells to initiate cell death in the virus-infected target cells\cite{162}. It also inhibits caspase 1

| Protein function                                                                 | VACV (Western Reserve) | MPXV (Central African) | MPXV (Western African) |
|---------------------------------------------------------------------------------|------------------------|-------------------------|-------------------------|
|                                                                                | Gene\*                | Accession No. NC_006998 | Gene\*                | Accession No. NC_003310 | Gene\*                | Accession No. NC_063383 |
|                                                                                | Size, amino acids     |                         | Size, amino acids     |                         | Size, amino acids     |
| Inhibitor of NF-κB activation; BCL-2-like protein                               | A46R                  | 240                     | A47R                  | 240                     | OPG176               | 240                     |
| Inhibitor of NF-κB activation; BCL-2-like protein                               | B15R                  | 149                     | B13R                  | 149                     | OPG200               | 149                     |
| Inhibitor of IRF3 and NF-κB activation, apoptosis inhibitor; BCL-2-like protein | N1L                   | 117                     | P1L                   | 117                     | OPG035               | 117                     |
| Inhibitor of IRF3 NF-κB activation; BCL-2-like protein                          | K7R                   | 149                     | C6R                   | 149                     | OPG044               | 149                     |
| Inhibitor of IRF3 and IRF7 activation; BCL-2-like protein                       | C6L                   | 151                     | D11L                  | 153                     | OPG029               | 155                     |
| Double-stranded RNA-binding protein, inhibitor of interferon signalling, apoptosis inhibitor | E3L                   | 190                     | F3L                   | 153                     | OPG065               | 153                     |
| IFNα/β binding proteins                                                         | B19R                  | 351                     | B16R                  | 352                     | OPG204               | 351                     |
| IFNγ binding proteins                                                           | B8R                   | 272                     | B9R                   | 267                     | OPG193               | 267                     |
| Dephosphorylation of STAT1; phosphatase                                          | H1L                   | 171                     | H1L                   | 171                     | OPG106               | 171                     |
| Ankyrin-like protein                                                            | C19L                  | 112                     | J3L                   | 587                     | OPG003               | 588                     |
| Ankyrin-like protein                                                            | –                     | –                       | D1L                   | 437                     | OPG015               | 437                     |
| Ankyrin-like protein                                                            | –                     | –                       | –                     | –                       | –                     | –                       |
| Ankyrin-like protein                                                            | C9L                   | 634                     | D9L                   | 630                     | OPG025               | 630                     |
| Ankyrin-like protein                                                            | M1L                   | 472                     | O1L                   | 442                     | OPG037               | 442                     |
| Ankyrin-like protein                                                            | K1L                   | 284                     | C1L                   | 284                     | OPG039               | 284                     |
| Ankyrin-like protein                                                            | B4R                   | 558                     | B5R                   | 561                     | OPG189               | 561                     |
| Ankyrin-like protein                                                            | –                     | –                       | –                     | –                       | B17R                 | 793                     |
| Ankyrin-like protein                                                            | –                     | –                       | N4R                   | 437                     | OPG015               | 437                     |
| Ankyrin-like protein                                                            | B25R                  | 112                     | J1R                   | 587                     | OPG003               | 588                     |
| Apoptosis inhibitor, caspase 1 and caspase 8 inhibitor, SPI-2                   | B13R                  | 345                     | B12R                  | 344                     | OPG199               | 344                     |
| Apoptosis inhibitor, SPI-1                                                      | C12L                  | 353                     | B19R                  | 357                     | OPG208               | 357                     |
| Apoptosis inhibitor                                                             | F1L                   | 226                     | C7L                   | 219                     | OPG045               | 219                     |
| Apoptosis inhibitor                                                             | VACWR013              | 181                     | D5R                   | 242                     | OPG201               | 242                     |
| TNF and chemokine binding protein, CrmB                                           | C22L                  | 122                     | J2R                   | 348                     | OPG002               | 349                     |
| IL-1β binding protein                                                           | VACWR197              | 326                     | B14R                  | 326                     | –                    | –                       |
| IL-18 binding protein                                                           | VACWR013              | 126                     | D6L                   | 126                     | OPG022               | 126                     |
| Inhibitor of complement enzyme                                                  | C3L                   | 263                     | D14L                  | 216                     | –                    | –                       |
| CC chemokine binding protein                                                    | C23L                  | 244                     | J3R                   | 246                     | OPG001               | 246                     |
| CC and CXC chemokine binding protein                                            | A41L                  | 219                     | A41L                  | 221                     | OPG170               | 221                     |
| OMCP, inhibitor of natural killer cell-mediated NKG2D-dependent cell lysis     | –                     | –                       | N3R                   | 176                     | OPG016               | 176                     |
| Inhibitor of intracellular trafficking of MHC class I molecules                 | B9R                   | 77                      | B10R                  | 221                     | OPG195               | 221                     |
| Inhibitor of MHC class II antigen presentation                                  | A35R                  | 176                     | A37R                  | 176                     | OPG163               | 176                     |
| Viral growth factor; EGF-like protein                                           | C11R                  | 140                     | D3R                   | 142                     | OPG019               | 142                     |

Data obtained from Refs. 18,84,85,133–135. Size of each protein encoded by the displayed genes shown as number of amino acids. BCL-2, B cell lymphoma 2; EGF, epidermal growth factor; IRF3, interferon regulatory factor 3; MPXV, monkeypox virus; OMCP, orthopoxvirus MHC class I-like protein; SPI-2, serine protease inhibitor 2; VACV, vaccinia virus; –, no corresponding gene can be identified in the respective virus genome. *Name or Gene ID is provided. Locus tags are provided in the event that name or Gene ID is not available.
and caspase 8, thereby interfering with their respective pyroptotic or apoptotic pathways. In MPXV, an SPI-2 orthologue is encoded by the B12R gene.

TNFR orthologues are also commonly used by orthopoxviruses to interfere with host inflammation and apoptotic events. Decoy viral TNFRs, which lack signalling domains, are secreted and compete for the binding of TNF. Five orthopoxviral viral TNFRs — CrmB, CrmC, CrmD, CrmE and vCD30 — have been identified. The MPXV genome encodes only CrmB, which is reported to bind to both TNF and TNFβ based on investigations performed with the CPXV CrmB. Interestingly, CrmB from VARV is extremely potent and exhibits an affinity for TNF that is stronger than etanercept, a commercially available competitive inhibitor of TNF.

**Antagonism of immune mediators.** Orthopoxviruses also evade host immune responses by secreting proteins that antagonize the functions of host IFNγ, CC and CXC chemokines, IL-1β and the complement system. Interestingly, the West African MPXV clade does not express any complement-modulating proteins, whereas the Central African strains encode the monkeypox inhibitor of complement enzyme (MOPICE) from the D14L gene. Despite truncation in one of its short consensus repeat (SCR) domains, MOPICE inhibits complement activation by binding to C3 and C5 convertases. However, deletion of MOPICE did not affect MPXV virulence in rhesus macaques infected with a Central African isolate, although it did dampen the adaptive immune response.

**Reduction of cellular activation.** Orthopoxviruses also evade T cell-mediated and natural killer cell-mediated cytotoxicity. T cells identify virus-infected cells by detecting foreign peptides loaded on surface-expressed MHC class I. Meanwhile, natural killer cells continually survey cells via NKG2D for the absence of MHC class I, thereby ensuring that the MHC system is not compromised. MPXV overcomes this system first by secreting the orthopoxvirus MHC class I-like protein (OMCP) encoded by the N3R gene, which resembles the MHC class I molecule and binds to NKG2D. This suppresses the typical NKG2D-dependent natural killer cell lysis of infected cells that do not express MHC class I. Evasion of natural killer cell surveillance allows the virus to reduce MHC class I expression, thus reducing T cell recognition. In addition, CPXV also expresses proteins D10 and B8 that impair peptide loading and MHC class I trafficking within the endoplasmic reticulum. In MPXV, the B10R gene encodes for an orthologue of the CPXV B8 protein. Orthopoxviruses also directly modulate natural killer cells and T cells in a paracrine fashion. For instance, orthopoxviruses produce an IL-18-binding protein that further blocks the cytotoxic activities of natural killer cells. MPXV also suppresses T cell-mediated immunity by triggering a state of T cell unresponsiveness via an MHC-independent mechanism. Subversion of T cell responses may explain why orthopoxvirus-specific memory T cells in vaccinated NHPs failed to protect against lethal MPXV infection in the absence of neutralizing antibodies.

**Vaccines.** Vaccines against smallpox are known to have cross-protective activity against monkeypox. Two vaccines are approved by the US Food and Drug Administration (FDA) for pre-exposure vaccination against orthopoxviruses including monkeypox: a second-generation live VACV vaccine, ACAM2000, and an attenuated third-generation vaccine based on modified vaccinia Ankara (MVA), JYNNEOS. National stockpiles of second-generation vaccines such as ACAM2000 are most common. However, these vaccines are associated with several rare side effects including myocarditis and pericarditis, with higher risks for certain groups of individuals, such as those with eczema and those who are pregnant. Nevertheless, a study demonstrated the efficacy of ACAM2000 smallpox vaccination in eliciting VACV-specific T cell responses by reporting the presence of activated CD4+ and CD8+ T cell responses at 1, 3, 6 and 12 months post vaccination. Following VACV stimulation, activated CD8+ T cells expressed much higher levels of the degranulation marker CD107, as well as IFNγ, TNF, IL-2 and CCL4, indicating the presence of robust memory T cell responses. The elevated CD8+ T cell responses were also observed in another study that compared the efficacy of the MVA vaccination with the first-generation Dryvax immunization. Both VACV-specific CD8+ T cells were observed to secrete IFNγ, TNF, IL-2 and CCL4 following stimulation. Moreover, both ACAM2000 and Dryvax also triggered the production of VACV-neutralizing antibodies in the vaccinees, despite apparent differences in the viral proteins being targeted.

In unvaccinated individuals, post-exposure vaccination can be effective both for preventing disease and for reducing disease severity. Protection is strongest if vaccination is carried out immediately after exposure, and decreases with increasing time since exposure, with most studies agreeing that vaccination up to 3 days post exposure provides significant clinical benefit. In animal studies, post-exposure vaccination against monkeypox varies in efficacy depending on the animal model used, the challenge dose and the timing of post-exposure vaccination. Notably, vaccination with ACAM2000 at 1–3 days after a 50x median lethal dose MPXV challenge in prairie dogs resulted in survival for 50–62% of the animals, compared with only 25% survival in the unvaccinated group. Human clinical studies are currently in progress to determine the efficacy of post-exposure vaccination against monkeypox.

Third-generation attenuated vaccines such as JYNNEOS have a better safety profile compared with earlier vaccines. However, because JYNNEOS is given in two doses 28 days apart, the second dose would likely be too late to help protect from disease in cases of post-exposure vaccination, and it is not known whether the first dose is sufficient for therapeutic efficacy. However, at 2 weeks post vaccination, neutralizing antibody titres from one dose of JYNNEOS were equivalent to those from ACAM2000, although they subsequently became inferior to those of the ACAM2000 group. Thus, if viral neutralization correlates with
Box 3 | Ring-fencing vaccination against monkeypox

To halt the potential spread of monkeypox virus (MPXV) globally, ring vaccination has been implemented in several countries including the United Kingdom, the United States and Canada. This usually involves vaccinating people who may have been exposed to the virus through interactions with an infected person. Vaccination is performed using smallpox vaccines, which are thought to offer protection against MPXV. Some of the challenges facing a successful ring vaccination are discussed below.

- Contact tracing: to identify all individuals who are potentially exposed, extensive contact tracing and robust testing needs to be carried out. For logistic reasons this may not be possible in every country. Furthermore, given the current characterization of non-endemic monkeypox as a disease that spreads primarily within the community of men who have sex with men (MSM), patients may be reluctant to come forward for diagnosis, especially in countries where homosexuality is stigmatized or criminalized.

- Efﬁciencies of second-generation and third-generation vaccines: past data showed that smallpox vaccination was 85% protective against MPXV. However, this value was obtained from individuals vaccinated with the first-generation vaccine. Nevertheless, currently available second-generation and third-generation vaccines were also reported to be approximately 85% protective against MPXV in animals models, but there are no data on their efﬁcacy in humans exposed to MPXV.

- Willingness to comply: not everyone identified will be willing to receive the vaccine. In a recent report, only ~14% of community contacts and ~69% of potentially exposed health-care workers in the United Kingdom were willing to be vaccinated. Moreover, the stigmatization of potentially being associated with the community of MSM might further deter vaccination efforts.

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Therapeutics

Several therapeutics that were developed for smallpox are available for monkeypox, but their effectiveness for monkeypox is based on preclinical evidence with little supporting clinical data from humans. The antiviral drug tecovirimat does not affect the intracellular form of the virus (the intracellular mature virus), but targets the VP37 membrane protein of MPXV to prevent the formation of enveloped virions that are capable of cell egress, thus disrupting viral spread. Tecovirimat was effective at reducing monkeypox severity in NHP models, but its effectiveness is reduced if treatment is given more than 5 days post challenge. This suggests a caveat if tecovirimat is given based on the onset of symptoms, which may appear much later following infection. Nevertheless, a small study used tecovirimat in a single patient infected with monkeypox, who then showed a shorter duration of viral shedding and illness compared with six untreated patients.

Another smallpox therapeutic is brincidofovir, which is approved by the FDA but not the European Medicines Agency (EMA). Brincidofovir inhibits orthopoxvirus DNA polymerase-mediated DNA synthesis. It has shown efficacy against monkeypox in prairie dog and mouse models. In the same small study discussed above, three patients were treated with brincidofovir, but all three ceased therapy owing to toxicity (elevated liver enzymes). Cidofovir, the active drug form of brincidofovir, also shows anti-poxvirus activity in vitro and in animal studies, but it is nephrotoxic. VIG showed promising activity in preventing smallpox in exposed individuals and has exhibited cross-neutralizing activity against MPXV in rhesus macaques. However, neither VIG nor other mAbs or antibody cocktails have yet been studied against monkeypox in the clinic.

Concluding remarks

Immunotherapeutics and preventive strategies are important public health tools that complement stringent contact tracing in curbing the spread of monkeypox. Similarly, serology-based diagnostics are valuable surveillance tools for contact tracing and understanding exposure history. However, such serological diagnostic tools must be specific for MPXV, given the baseline of vaccine-induced poxvirus immunity. Expansion of surveillance networks and identification of surveillance gaps are also critical for a successful ring-fencing strategy (see Box 3 for details). Notably, there is a major need in public health to better inform potentially exposed persons of the benefits and risks of vaccination.

Historically, the collection of clinical data on monkeypox has been hindered by the sparseness and unpredictability of cases in endemic countries, impeding accurate risk–benefit calculations in managing monkeypox. The current outbreaks provide an opportunity to evaluate current and novel treatments and vaccine options and to establish correlates of vaccine protection. Notably, the ring vaccination trial design can provide high statistical power with relatively few subjects owing to the high potential attack rate. However, in generalizing such findings, caution must be taken to account for variance in the route of transmission.

Several key research questions remain to be answered. Studies of the human systemic and mucosal immune responses during MPXV infection are currently limited.
and will be required to better understand the mechanisms of immune defence against MPXV. Importantly, it is not currently known whether prior infection with VACV or MPXV, or smallpox vaccination, induces any form of mucosal immunity. It will be critical to characterize the mucosal immune responses given that MPXV and other poxviruses can be transmitted via respiratory aerosols. Knowledge of the roles of IgA and tissue-resident memory T cells in MPXV infection would be particularly critical, enabling a deeper understanding of MPXV-related respiratory complications. Likewise, the preputial mucosal immunity should also be characterized, as MPXV DNA has been identified in semen.

Defining the immunological correlates of protection is also an important goal for the evaluation of newer vaccines, especially those targeted towards the vulnerable populations of pregnant women and children. Therefore, other than being unvaccinated, what other factors (for example, behavioural, geographical, nutritional, medical, immunological or genetic factors) are involved? It was recently reported that the quality of innate immune responses in young children determines the severity of respiratory viral infection. Likewise, as reported for SARS-CoV-2 infections, children who are infected tend to have reduced B cell and T cell responses compared with adults. The characterization of adaptive immune responses in children infected with MPXV could clarify why children tend to exhibit a more severe disease and lower vaccine effectiveness. It is also important to understand the risks of vaccination in vulnerable populations, especially young children and pregnant women. Clinical studies are lacking in these populations for most of the available therapeutics and vaccines. Furthermore, some important vaccines and therapeutics (for example, ACAM2000 and brincidofovir) are not recommended or are even contraindicated for these populations due to increased potential risk of side effects. Nevertheless, it was recommended by the UK Health Security Agency that vaccination should be given to men who are homosexual or bisexual who are at a higher risk of exposure to help control the current outbreak.

Many other infectious diseases are endemic to Africa, and co-infection with MPXV is possible. For instance, co-infection of alphaviral infections and malaria can significantly modulate host immunity and affect infection outcomes. Given the current transmission among MSM in non-endemic countries, there is also a need to understand monkeypox disease and vaccination in the context of co-infection with other diseases that have a disproportionate burden in the community of MSM; this is especially true for HIV-1, which can severely impair adaptive immune responses.

Risk factors for severe MPXV should also be identified. Pregnant women, young children and unvaccinated individuals are known to be especially susceptible. However, other populations also need characterization, including older people, individuals on long-term medications and individuals with underlying metabolic diseases who may manifest the disease differently.

Long-term disease sequelae should be tracked in patients, including children born to mothers infected with MPXV. Experience with the Zika outbreak showed that children with in utero exposure can develop some developmental issues later in life. This research focus should be given more urgency, given that available data suggest that only 20–25% of pregnant women infected with MPXV would successfully give birth. Moreover, young children are apparently more susceptible to severe monkeypox. Currently, data on fetal development following congenital MPXV infection are lacking. In addition, longitudinal monitoring of patients with MPXV would also allow the determination of whether infection with MPXV can lead to long-term effects, as observed after infection with SARS-CoV-2 during the current pandemic.

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