Monkeypox: disease epidemiology, host immunity and clinical interventions

Fok-Moon Lum[1], Anthony Torres-Ruesta[1], Matthew Z. Tay[1], Raymond T. P. Lin[2,3,4], David C. Lye[3,5,6,7], Laurent Rénia[1,5,8] and Lisa F. P. Ng[1,9,10]✉

Abstract | Monkeypox virus (MPXV), which causes disease in humans, has for many years been restricted to the African continent, with only a handful of sporadic cases in other parts of the world. However, unprecedented outbreaks of monkeypox in non-endemic regions have recently taken the world by surprise. In less than 4 months, the number of detected MPXV infections has soared to more than 48,000 cases, recording a total of 13 deaths. In this Review, we discuss the clinical, epidemiological and immunological features of MPXV infections. We also highlight important research questions and new opportunities to tackle the ongoing monkeypox outbreak.

Monkeypox is a zoonotic viral disease caused by the monkeypox virus (MPXV), an orthopoxvirus belonging to the Poxviridae family of viruses[1]. MPXV was first identified in 1958 in research monkeys that were shipped from Singapore[2], 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[3]. 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[1,4].

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[5].

Possible reasons for this current outbreak could be attributed to waning smallpox immunity in the general population and termination of the smallpox vaccination regime[6]. Vaccination against smallpox has been shown to offer protection against monkeypox. An early study from Zaire in 1988 (REF.[7]) 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[4]. Recently, a study on 528 infections diagnosed during this current outbreak reported that only 9% of the individuals who were infected received prior smallpox vaccination[8]. Interestingly, one characteristic of the recent outbreaks is a disproportionate number of infections in men who have sex with men (MSM)[8,9].

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%[10] or >99% between the two clades[11] 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[12]. MPXV of the Central African clade has been identified in cases appearing in the Democratic Republic of the Congo (DRC) and South Sudan[12], whereas cases in Nigeria between 2010 and 2019 appeared to be due to the West African clade[12]. Cases reported outside Africa, including those currently in circulation, have all been caused by the West African clade[9,13]. Whether genetic changes in the MPXV genome could be responsible for this current outbreak is currently being investigated[14] (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.

✉*e-mail: Lisa_Ng@idlabs.a-star.edu.sg*

https://doi.org/10.1038/s41577-022-00775-4

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)[15–17]. Genes known to be important for orthopoxviruses are present within the central region of the MPXV genome (between ORFs *C10L* and *A25R*)[16,17]. However, a subset of ORFs are either missing or truncated in the MPXV genome compared with the genomes of other orthopoxviruses[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[6,11,18].

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[19]. MPXV replication is a complex process but is generally thought to be identical to other orthopoxviruses[19]. 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[20] or heparan sulfate[21,22]. In VACV, surface proteins H3, A27 and D8 have been associated with viral binding[20–22]. Following binding, VACV gains entry into the cell through 11 conserved proteins, which form a complex known as the entry fusion complex[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 swelling at the maxillary, cervical or inguinal lymph nodes (lymphadenopathy)[1,4]. In the recent outbreak, a report from Portugal suggested that inguinal lymphadenopathy was more common than cervical and axillary lymphadenopathy[24]. 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[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[24–26].

Disease severity can be classified using the lesion count, as higher lesion counts correlate with increased risk of severe complications[4]. Patients with severe complications may experience respiratory and gastrointestinal issues[27], encephalitis[4], septicaemia[27,28] and ocular infections leading to permanent vision loss[29]. Skin lesions also increase the likelihood of dermal bacterial infections, especially in patients who are not vaccinated against smallpox[4].

Lesions typically progress through four stages — macular, papular, vesicular and pustular — before falling off as scabs[1]. 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[30], which may indicate the presence of infectious viral material. Of note, viable VARV has been isolated from scabs of patients with smallpox[31].

During pregnancy, MPXV can be vertically transmitted from the mother to the fetus[32]. 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[32]. 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[30]. 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[30].

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[4]. This disparity could potentially be due to differences in their immune responses[4]. Currently, data on severity of infection in children infected with the West African clade are lacking[33]. 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[34] (FIG. 2). For several orthopoxviruses, such as the highly

Author addresses

[1]A*STAR Infectious Diseases Labs, Agency for Science, Technology and Research (A*STAR), Singapore, Singapore.
[2]National Public Health Laboratory, Singapore, Singapore.
[3]National Centre for Infectious Diseases, Singapore, Singapore.
[4]Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore.
[5]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore.
[6]Tan Tock Seng Hospital, Singapore, Singapore.
[7]Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore.
[8]School of Biological Sciences, Nanyang Technological University, Singapore, Singapore.
[9]National Institute of Health Research, Health Protection Research Unit in Emerging and Zoonotic Infections, University of Liverpool, Liverpool, UK.
[10]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, UK.

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[212]. 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[12,213–215]. 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)[12,216–222]. Between 2000 and 2020, MPXV became more widespread within the African continent, with cases reported in the Republic of the Congo[29,223,224], Liberia[12,215], South Sudan[225], Sierra Leone[226], Nigeria, Cameroon[222], the CAR[227,228] and the DRC[1,12]. During this period, Nigeria[229] reported 181 confirmed and/or suspected cases, and the DRC reported more than 20,000 suspected cases[12]. 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[27,230]. This outbreak was caused by human exposure to prairie dogs that had been infected by imported Gambian pouched rats[27]. Other countries with reported MPXV infections were Israel (2018; one case)[231], the United Kingdom (2018–2021; seven cases)[28,232–234], Singapore (2019; one case)[235] and the United States (2021; two cases)[236,237] (reviewed in REF.[12]).

As described above, MPXV was largely restricted to endemic regions prior to the current outbreak[12,238]. 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[239].

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[9,13,240] (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[240], with 13 deaths[240]. Recently, Singapore confirmed several imported and local cases of monkeypox, which are the first reported in Southeast Asia during this recent outbreak[241] and the first time that monkeypox has reappeared in Singapore since 2019 (REF.[235]). On 23 July 2022, the World Health Organization (WHO) declared the monkeypox outbreak a global health emergency[5].

contagious VARV and MPXV, the respiratory/oral cavity is a possible common entry route following the inhalation of aerosolized respiratory secretions or the ingestion of bodily fluids from individuals who are infected[35]. The virus then infects the oral and respiratory tract mucosae, with the upper, middle and lower airway epithelium as main targets for primary infection[36]. This phase of infection is asymptomatic, with no signs of oropharyngeal lesions[34]. Virus spread progresses with the infection of nearby tissue-resident immune cells, potentially including antigen-presenting cells such as monocytes, macrophages, B cells and dendritic cells[37–41]. The mechanisms whereby orthopoxviruses relocate from the initial site of infection to nearby draining lymph nodes are a matter of debate. It has been observed, for example, that VACV-infected murine dendritic cells migrate from the lung epithelium to draining lymph nodes, likely contributing to virus dissemination[42]. Conversely, VACV infection of human monocyte-derived dendritic cells has been shown to be abortive, affecting dendritic cell maturation and their migratory potential[39,43–45], and arguing against a role of dendritic cells supporting the initial lymphatic spread of VACV. Importantly, the rapid relocation of VACV to draining lymph nodes within hours of inoculation[46,47] indicates direct viral access to lymphatic vessels as a dissemination mechanism.

Following the infection of nearby draining lymph nodes, orthopoxviruses replicate extensively in lymphoid tissues. Clinical studies of human monkeypox suggest that lymphoid tissues in the neck and throat are areas of primary MPXV replication[37]. This was supported by studies in a cynomolgus macaque model of aerosolized MPXV infection, where the tonsils and the mandibular and mediastinal lymph nodes were active areas of early virus replication[36]. Poxvirus tropism in lymphoid tissue has been associated with infection of monocyte/macrophages, dendritic cells, B cells and activated T cells, which could also be targets for MPXV[48]. The processes leading to abnormal lymph node swelling upon natural MPXV infection have not been elucidated; however, during experimental infection of non-human primates (NHPs) with MPXV, massive proliferation and accumulation of natural killer cells was observed in the lymph nodes surrounding the site of inoculation[37].

After the development of low-grade primary viraemia resulting from the infection of the lymphoid tissues, orthopoxviruses can disseminate to distant organs via the lymphohaematogenous route[36,49]. In experimental mousepox models, the spleen and liver are the main targets for infection after primary lymphatic spread[50]. Virus infection in these organs releases a second major viraemia wave (believed to be through infected cells) that results in viral dissemination to the lungs, kidneys, intestines, skin and other organs[50]. Similarly, in an NHP model of aerosolized MPXV infection, viral antigen was observed in the liver, particularly in highly specialized macrophages such as Kupffer cells[36]. Antigen was also detected in hepatocytes, although to a lower extent. Enlarged spleen and liver have also been reported during MPXV infection in humans[30]. In VARV 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[51].

The presence of orthopoxviruses in the small dermal blood vessels marks the start of skin infection and development of skin lesions[51]. 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[36]. Infiltration of macrophages, dendritic cells and $CD3^+$ T cells has been observed around the infective pustule[52]. 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[53]. Importantly, epithelial lesions (enanthema) also appear during MPXV infection in the oropharyngeal mucosa, tongue, pharynx, larynx, trachea and oesophagus[51], eventually evolving into ulcers which release infectious viral particles into the saliva[51].

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[46,54,55]. Nonetheless, recent evidence from a mouse model of orthopoxvirus

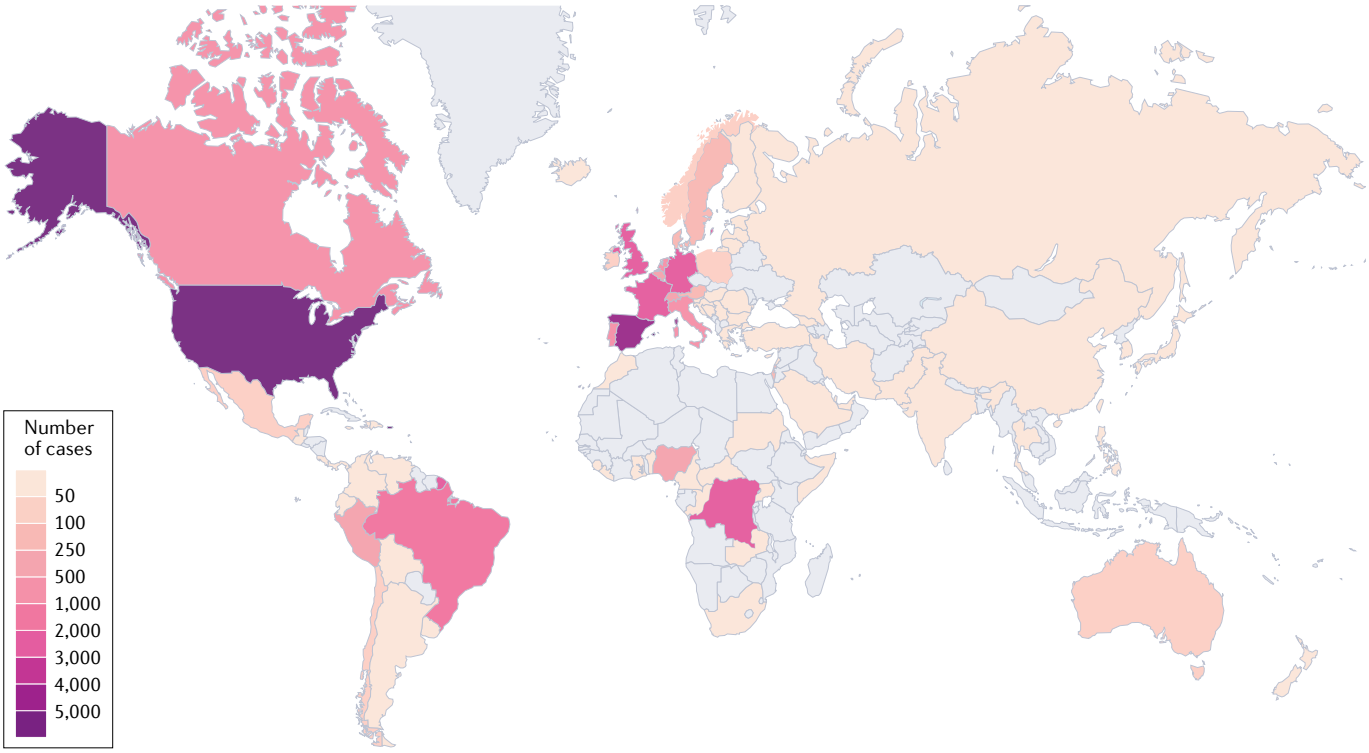


Fig. 1 | **Geographical distribution of confirmed and suspected monkeypox cases during the outbreak between May and August 2022.** Data presented as of 5 August 2022 were obtained from Global.health. Diagram generated with Datawrapper.

skin infection suggested that dendritic cell migration from the skin to draining lymph nodes is impaired on VACV[56]. 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[57].

Sexual transmission of monkeypox has been speculated[52], 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[58], which might indicate preferential MPXV tropism into the testes. Being an immune-privileged tissue[59], 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[60,61]. Viral shedding was also reported in faeces and contact with the rectal mucosa might increase the likelihood of MPXV transmission[35]. It was previously noted in patients with human immunodeficiency virus 1 (HIV-1) that these tissues could act as a reservoir for the virus[62]. A recent study[63] 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 (REF.[64]). 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[70]. Mouse $CD45^+CD11b^+GR\text{-}1^{int}$ inflammatory monocytes have also been shown to be permissive to VACV replication and may be plausible vehicles for virus dissemination[47]. It was also reported that human primary M2-like macrophages allowed VACV replication and dissemination[71]. 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.

cell linkages, lamellipodia and branching structures associated with the VACV virions, indicating that these cells might participate in virus spread[71]. However, it was also reported that depletion of phagocytic cells did not abolish spread of VACV[72] in infected mice, suggesting that monocytes and macrophages are not the only immune cells that are capable of facilitating virus dissemination. Nevertheless, Ly6G$^+$ innate immune cells (both neutrophils and Ly6G$^+$ monocytes[72]) were responsible for infiltrating and controlling virus-infected cells, thus limiting viral tissue damage[47,72]. These results were indirectly confirmed by a study that found an association between low numbers of blood neutrophils with moribundity in MPXV-infected animals[73]. It is also worth noting that immune cells recruited to the site of infection control only local pathogenesis and tissue pathology, but not virus dissemination, and a systemic immune response is required to prevent widespread infection[74].

Natural killer cells are an important component of innate immunity and, similar to monocytes, are capable of shaping the adaptive immune response[75]. In MPXV-infected rhesus macaques, numbers of natural killer cells expand significantly in both peripheral blood (a mean 23-fold increase by day 7 post infection) and lymph nodes (a mean 46.1-fold increase by days 8–9 post infection)[37]. Prior to this rapid proliferation, the migratory capacity of the various natural killer cell subsets was significantly impaired by MPXV infection, which severely affected their recruitment into the lymphoid and/or inflamed tissues[37]. A downregulation of chemokine receptors such as CXCR3, CCR5, CCR6 and CCR7 on these cells was also reported[37]. Moreover, natural killer cells isolated from both lymph nodes and blood were reported to lose their ability to degranulate and to secrete IFNγ and TNF[37]. Although no correlation between viral clearance and natural killer cell numbers and activities was reported in this NHP model[37], the importance of natural killer cells in controlling MPXV viral load was demonstrated in CAST/EiJ mice. This strain is exceptionally susceptible to orthopoxvirus infection[76] owing to low numbers of natural killer cells. IL-15 treatment protected CAST/EiJ mice from lethal MPXV infection even when both CD4$^+$ and CD8$^+$ T cells were depleted[76]. This implies that the expanded natural killer cells were responsible for the protective effect as treatment with IL-15 is known to transiently increase the numbers of IFNγ-secreting natural killer cells and CD8$^+$ T cells[77].

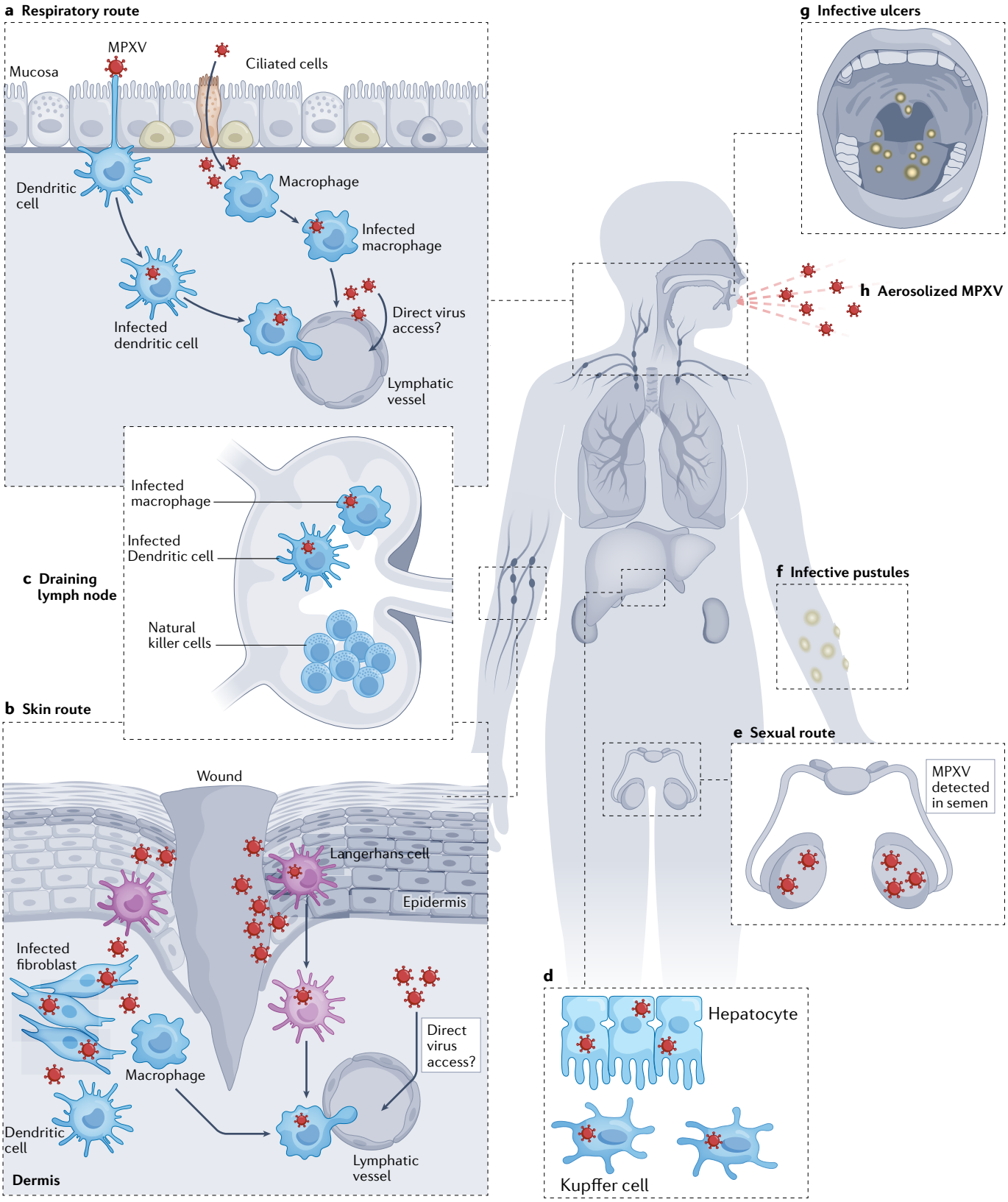
Similarly, natural killer cells are also needed to control both Ectromelia virus (ECTV) and VACV infection in C57BL/6 mice[78,79]. ECTV is a natural orthopoxviral pathogen of mice, and is often used to induce experimental mousepox as a model for other clinically important orthopoxviruses[80]. Interestingly, it was suggested that the expression of CD94 on natural killer cells in C57BL/6 mice is absolutely essential for conferring resistance to ECTV infection[81]. This is mediated by the NKG2E and CD94 receptors on natural killer cells, which bind complexes of MHC class I with the peptide Qa-1^b, which are expressed by infected cells[81]. NKG2D has also been reported to participate in natural killer cell-mediated protection against ECTV infection[79] and it was postulated that CD94-NKG2E and NKG2D may have synergistic activity in inducing optimally protective natural killer cells[81]. However, the exact mechanisms behind this apparent synergism remain to be elucidated, and further studies will be required to better understand the roles of natural killer cells in mousepox infections. Given that CD94 and NKG2 are highly conserved between humans and rodents[82], these receptors may also play a protective role against MPXV infection in humans.

In humans infected with MPXV, the roles of many innate immune cells — including monocytes/macrophages, neutrophils, natural killer cells, conventional dendritic cells, plasmacytoid dendritic cells and innate lymphoid cells — are currently unknown. The characterization and profiling of these immune cells during MPXV infection will be essential for understanding their functions and identifying important biomarkers for disease prognosis.

VARV infection in animal models triggers systemic cytokine responses that correlate with disease outcomes. In unvaccinated cynomolgus macaques, significant changes in host gene expression were detected following infection with VARV[66]. 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* (REF.[66]). The animals that succumbed to the infection (two out of seven) had minimal interferon responses, indicating that this early interferon response protects

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)[242]. At least 46 single-nucleotide polymorphisms have been found to be specific for this lineage, including 24 non-synonymous single-nucleotide polymorphisms[243]. Recent molecular epidemiology studies have shown a higher than expected rate of genomic variance among the outbreak sequences, suggesting accelerated evolution[242] and, potentially, APOBEC3 editing[244]. 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[14]. 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[243,245]. 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 do not have well-defined functions[15–17]. Identifying the potential impact of particular genetic mutations on the viral phenotype is therefore complex and time-consuming. That said, the DNA-based genome of MPXV has a much greater capacity to repair errors incurred during viral replication[246] compared with the RNA-based coronaviruses. However, the related orthopoxvirus vaccinia virus (VACV) is capable of 'phenotypic mutations' in the form of genetic accordions (multistep events involving gene application and mutation(s) that allow poxviruses to subvert host antiviral responses)[247]. Specifically, a variant of VACV was identified that has a significant amplification of the *K3L* gene, which, together with a beneficial point mutation in the same gene, allows the virus to largely overcome the host protein kinase R (PKR)-mediated antiviral responses[247]. Such genetic accordions have not been reported in MPXV and further investigations are needed to investigate whether such a phenomenon could be a plausible mechanism for MPXV evolution and spread.



against fatality[66]. Human IFNβ has been demonstrated to inhibit MPXV replication and spread[83]. However, MPXV does not robustly activate TNF-regulated and NF-κB-regulated genes, especially in animals that succumb to infection[66]. This is not surprising, given that VARV and other orthopoxviruses harbour genes that can modulate TNF[84,85] and NF-κB[86,87] pathways.

Although host immunity is required to combat infections, aberrant immune signalling can adversely affect infection outcomes. In another study of VARV-infected

◀ Fig. 2 | **Immunopathogenesis of human monkeypox. a–h** | Monkeypox virus (MPXV) might enter the body via the respiratory (panel **a**) or skin (panel **b**) route. In the respiratory tract, the virus can infect airway epithelial cells such as ciliated cells. Antigen-presenting cells such as dendritic cells and macrophages (MΦ) are also susceptible to MPXV infection. Upon inoculation in the skin, the virus infects keratinocytes and fibroblasts. Skin-resident immune cells such as Langerhans cells, dendritic cells and macrophages are also targeted. In both scenarios (panels **a** and **b**), it is hypothesized that infected antigen-presenting cells travel to nearby draining lymph nodes and facilitate its spread through the lymphatic system (panel **c**). Direct viral access to the lymphatics has been also speculated. A common feature of human monkeypox is swelling of lymph nodes (lymphadenopathy). The abnormal proliferation and retention of natural killer cells might be one of the causes. Following its spread through lymphoid tissue, MPXV may target other large organs such as the spleen and liver (panel **d**). Of note, MPXV antigens have been previously been detected in both hepatocytes and Kupffer cells in non-human primate (NHP) models. The viraemia wave could then allow the virus to further spread to distant organs such as the skin and gonads. Recently, MPXV was isolated from semen of infected individuals, highlighting the possibility of sexual transmission (panel **e**). The infection of skin and mucosae leads to the appearance of infective pustules (panel **f**) and ulcers (panel **g**). The latter release high quantities of virus into the saliva, which potentially leads to aerosolized transmission of MPXV (panel **h**).

cynomolgus macaques, the levels of IL-8, CCL2 (also known as MCP1), CCL4 (also known as MIP1β), IL-6 and IFNγ were significantly increased during the first 4 days post infection[67]. These cytokines drive monocytosis[88], which might facilitate enhanced virus dissemination through monocytic cell-associated viraemia[67]. Importantly, the macaques eventually succumbed to VARV infection, where high levels of these cytokines might have contributed to a 'cytokine storm' leading to their demise[67]. Likewise, the levels of IL-1RA, IL-2, IL-6, IL-8, IFNγ, CCL2, CCL5 (also known as RANTES), G-CSF, GM-CSF and sCD40L were found to be elevated in MPXV-infected cynomolgus macaques[70]. Furthermore, a relative expansion (0.97-fold to 16.3-fold) of $CD14^+$ monocytes was reported during acute infection, suggesting that the general immune milieu promoted the development and recruitment of monocytes following MPXV infection[70].

In reported cases of human MPXV infection, numerous cytokines are elevated following infection (regardless of disease severity) — these include IL-1β, IL-1RA, IL-2R, IL-4, IL-5, IL-6, IL-8, IL-13, IL-15, IL-17, CCL2 and CCL5[89]. However, in patients with serious disease (defined as having >250 lesions), concentrations of IL-2R, IL-10, GM-CSF and CCL5 were higher compared with those in patients with less severe disease, whereas the concentration of pro-inflammatory IL-6 was lower[89]. This cytokine profile suggests a dominant T helper 2 cell response characterized by higher levels of IL-4, IL-13 and IL-10. Likewise, the reduced levels of IL-2, TNF, IFNα and IFNγ also suggest an anti-inflammatory microenvironment involving regulatory T cells[89].

VACV can evade immune responses by downregulating inflammatory and antiviral immune responses[44,90,91], and MPXV may use a similar strategy to subvert host immunity[92]. Given that immune mediators often facilitate crosstalk between immune cells[93], future studies should identify the functional relationships between cytokine profiles and immune cells in order to clarify the mechanisms of pathogenesis and determine immune correlates of protection during MPXV infection.

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[94,95]. 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[96]. In rhesus macaques, VACV-specific B cell responses were instrumental in protecting against a lethal MPXV infection[53]. Importantly, epidemiological studies have further demonstrated that the VACV vaccine confers protection against other poxviruses, including MPXV[97]. The VACV-specific memory B cells and antibody levels induced by vaccination were exceptional, in some cases lasting longer than 50 years[98,99]. However, only ~50% of vaccinated individuals at >20 years after vaccination[65] had neutralizing antibody titres greater than 1:32, a correlate of protection suggested to confer protective immunity against smallpox[100]. 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 vacinees[101]. Three proteins in particular — MPXV (Zaire-1979_005) proteins D8, H3 and A26 — were targeted by neutralizing antibodies against MPXV in infected macaques[101]. In VACV, orthologue D8 and H3 proteins are involved in the attachment of mature virions[22], whereas A26 associates with A27 (REF.[102]) to bind surface laminin[103]. 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[101]. In another study, prophylactic treatment with a cocktail of two mAbs — c7D11 and c8A — successfully protected marmosets against lethal MPXV infection[104]. C7D11 and c8A target the VACV proteins L1 (REF.[105]) and B5 (REF.[106]), respectively, and have been recently formulated as a potential mRNA vaccine encapsulated in lipid nanoparticles[107]. Gilchuk et al.[108] 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[30]. 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[109] compared with those with mild disease, and anti-orthopoxvirus IgG responses were much reduced and less frequent in patients with moderate/severe disease[109]. 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[110]. 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[99]. These VACV-specific CD4$^+$ T cells were capable of producing IFNγ and TNF following stimulation[99]. However, no direct correlation was reported between numbers of virus-specific CD4$^+$ T cells and anti-VACV antibody titres[99]. 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[53]. 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[53]. 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[111]. This group of individuals is therefore at high risk of developing severe MPXV infection if exposed[112]. 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[113]. 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[25]. 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[36,65–68], 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[47]. 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[114]. Moreover, γδT cells also upregulate co-stimulatory molecules CD80 and CD86 and secrete IL-1 and IFNα for activation of CD8$^+$ T cells[114]. In a mouse model of VACV respiratory infection, IFNγ secretion by primary activated effector CD8$^+$ T cells was shown to protect against lethality[115]. Indeed, CD8$^+$ T cell-derived IFNγ was sufficient for protection even in the absence of CD4$^+$ T cells and B cells[115], 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[116]. 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[116]. In humans, standard smallpox vaccine administered by scarification[117] was also able to induce primary cytotoxic CD8$^+$ T cells and IFNγ-producing T cells[118]. This observation was supported by another study, in which participants received live vaccinia smallpox vaccine (Dryvax)[119]. High levels of IFNγ-producing CD8$^+$ and CD4$^+$ T cells were detected following immunization[119]. In humans infected with VACV, activated CD4$^+$ T cells were shown to upregulate genes related to cytolytic activities[120]. Interestingly, MHC class II-restricted cytolytic CD4$^+$ T cells have also been reported in individuals immunized with VACV[121]. These cells could be responsible for virus clearance in vaccinees with reduced or missing memory CD8$^+$ T cell responses[99]. In the experimental mousepox model, perforin-dependent cytolytic CD4$^+$ T cells have been reported[122]. 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[123–125]. Many are conserved among the major orthopoxviruses and bind to human MHC class I and class II molecules[126–128]. In particular, CD8$^+$ T cells specific for two identified epitopes (MHC class II-restricted GRVFDKADGKSKRDA and MHC class I-restricted NPVTVINEY) in the immediate-early E3 protein of VACV were capable of killing infected cells and halting the spread of VACV[129]. Both epitopes are conserved in the MPXV homologue, which is encoded by the MPXV *F3L* gene[130]. An earlier study showed that VACV missing the E3 protein did not protect cynomolgus macaques from subsequent MPXV infection[131]. As E3 protein is detected within 30 min of VACV infection[132], 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[109]. 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

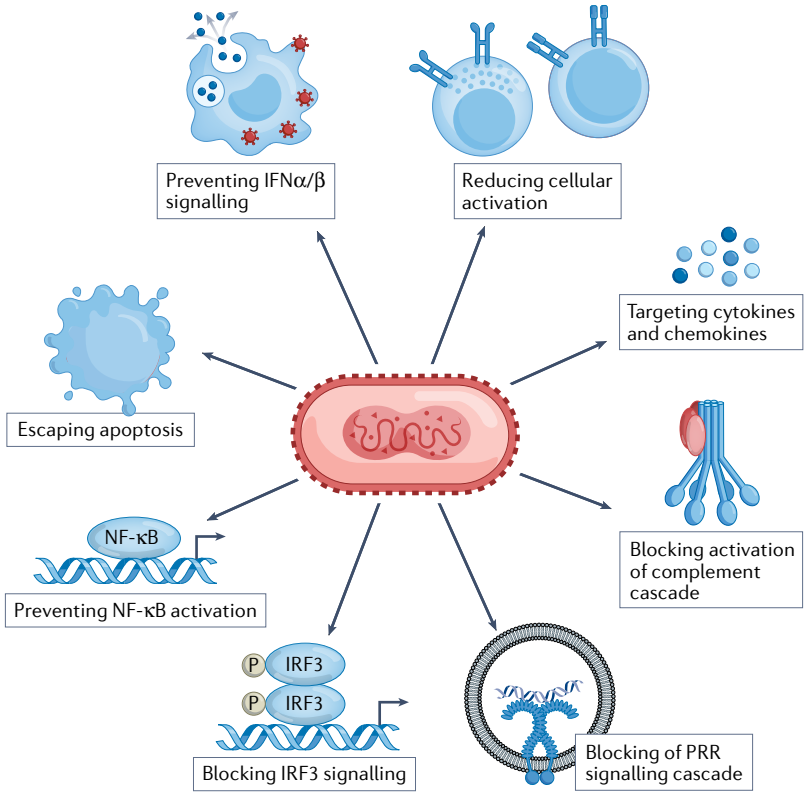


Fig. 3 | **Immune evasion by MPXV.** Monkeypox virus (MPXV) is known to encode numerous viral proteins that are involved in evading the host immunity. These can be involved in interfering with the signalling cascade of pathogen recognition receptors, disrupting key transcription factors for the expression 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). PRR, pattern recognition receptor.

fact higher in unvaccinated patients[109]. 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 virus recognition, apoptosis and immune regulation[18,84,85,133–136] (FIG. 3). Here, we discuss some of the evasion mechanisms used by MPXV during active infection (TABLE 1). For a more in-depth review of orthopoxvirus immune evasion strategies, see REFS.[85,133–136].

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)[137,138]. Numerous orthopoxviral proteins can antagonize these signalling processes[18,84,85,133]. 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[85,139–141]. BCL-2-like proteins are generally conserved across orthopoxviruses — A47, B13, P1, C6 and D11 are orthologues of BCL-2-like proteins in MPXV[17,84]. VACV also encodes the E3 protein, which binds to double-stranded RNA (dsRNA)[142] that is produced late in its replication cycle[143], and prevents the dsRNA from being detected by host intracellular PRRs[54]. This leads to complete inhibition of the protein kinase R (PKR)-mediated pathway[54], which can otherwise block protein synthesis by phosphorylating the eukaryotic translation initiation factor subunit 2α (eIF2α)[144]. The F3 protein of MPXV is a homologue of the VACV E3 protein with a 37 amino acid truncation at the amino terminus[130]. Unlike a similarly truncated recombinant VACV (VACVΔ37N), MPXV can still inhibit host immune responses[130]. Interestingly, another recombinant VACV expressing the MPVX *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[130].

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[145]. The VACV B19 protein directly interacts with soluble interferons and inhibits their binding to receptors[146]. 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[147]. The MPXV orthologue B16 can inhibit IFNβ signalling[83,148]. Interestingly, the interferon response is known to be stronger in children and has been shown to protect against severe SARS-CoV-2 (REF.[149]) and RSV[150] infections, but the reverse was observed in children infected with MPXV[1,4]. This 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[151]. NF-κB consists of two subunits (p65/p50), and in its inactive form the p65 subunit is bound by the inhibitory protein IκBα (which contains six ankyrin repeats). During NF-κB activation, IκBα is phosphorylated by IκBα kinase (IKK), followed by ubiquitination and subsequent degradation[151]. To prevent NF-κB activation, orthopoxviruses express ankyrin-like proteins that compete with IκBα for phosphorylation by IKK[84,85]. Eight ankyrin-like genes (*J3L, D1L, D7L, D9L, O1L, C1L, B5R, B17R, N4R* and *J1R*) are encoded by the MPXV genome[84] — *J3L* and *J1R* as well as *D1L* and *N4R* are duplicated ORFs in left and right inverted terminal repeats within the viral genome[84].

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

Table 1 | **Virulence and immune evasion genes of monkeypox virus (MPXV)**

Protein function	VACV (Western Reserve)		MPXV (Central African)		MPXV (Western African)	
	Accession No. NC_006998		Accession No. NC_003310		Accession No. NC_063383	
	Gene[a]	Size, amino acids	Gene	Size, amino acids	Gene	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	*VACWR017*	71	*D7L*	660	*OPG023*	660
Ankyrin-like protein	*C9L*	634	*D9L*	630	*OPG025*	630
Ankyrin-like protein	*M1L*	472	*O1L*	442	*OGP037*	442
Ankyrin-like protein	*K1L*	284	*C1L*	284	*OPG039*	284
Ankyrin-like protein	*B4R*	558	*B5R*	561	*OPG189*	561
Ankyrin-like protein	–	–	*B17R*	793	*OPG205*	787
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	*OPG021*	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.[18,84,85,133–136]. 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. [a]Name or Gene ID is provided. Locus tags are provided in the event that name or Gene ID is not available.

regulation of the intrinsic apoptotic pathway. Numerous orthopoxvirus-encoded serine protease inhibitors (SPIs; serpins) have also been reported[85,133,152,153], such as CrmA in cowpox virus (CPXV)[153]; its homologue SPI-2 (B13) in VACV is considered the virus's most potent inhibitor of apoptosis[154]. CrmA interferes with granzyme B[155], which is secreted by cytotoxic T cells to initiate cell death in the virus-infected target cells[156]. It also inhibits caspase 1

and caspase 8, thereby interfering with their respective pyroptotic or apoptotic pathways[152]. In MPXV, an SPI-2 orthologue is encoded by the *B12R* gene[84].

TNFR orthologues are also commonly used by orthopoxviruses to interfere with host inflammation and apoptotic events[85,86]. Decoy viral TNFRs, which lack signalling domains, are secreted and compete for the binding of TNF[157]. Five orthopoxviral viral TNFRs — CrmB, CrmC, CrmD, CrmE and vCD30 — have been identified[84,85,157]. The MPXV genome encodes only CrmB, which is reported to bind to both TNF and TNFβ based on investigations performed with the CPXV CrmB[158]. Interestingly, CrmB from VARV is extremely potent and exhibits an affinity for TNF[159] that is stronger than etanercept, a commercially available competitive inhibitor of TNF[160].

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[161–166]. Interestingly, the West African MPXV clade does not express any complement-modulating proteins[11,18], whereas the Central African strains encode the monkeypox inhibitor of complement enzyme (MOPICE) from the *D14L* gene[11,167]. Despite truncation in one of its short consensus repeat (SCR) domains, MOPICE inhibits complement activation by binding to C3 and C5 convertases[11,168]. 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[169].

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[170]. Evasion of natural killer cell surveillance allows the virus to reduce MHC class I expression, thus reducing T cell recognition[171]. In addition, CPXV also expresses proteins D10 and B8 that impair peptide loading and MHC class I trafficking within the endoplasmic reticulum[172]. In MPXV, the *B10R* gene encodes for an orthologue of the CPXV B8 protein[84]. 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[173]. MPXV also suppresses T cell-mediated immunity by triggering a state of T cell unresponsiveness via an MHC-independent mechanism[65]. 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[53].

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[174,175]. National stockpiles of second-generation vaccines such as ACAM2000 are most common[176]. However, these vaccines are associated with several rare side effects including myocarditis and pericarditis[176], 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[177]. 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 (REF.[177]), 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[178]. In both vaccination groups, VACV-specific $CD8^+$ T cells were observed to secrete IFNγ, TNF, IL-2 and CCL4 following stimulation[178]. Moreover, both ACAM2000 and Dryvax also triggered the production of VACV-neutralizing antibodies in the vaccinees[177,179], despite apparent differences in the viral proteins being targeted[179].

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[180]. 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[181]. Notably, vaccination with ACAM2000 at 1–3 days after a 50× 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[181]. Human clinical studies are currently in progress to determine the efficacy of post-exposure vaccination against monkeypox[182].

Third-generation attenuated vaccines such as JYNNEOS have a better safety profile compared with earlier vaccines[183]. 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[183]. 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[248].
- Efficiencies of second-generation and third-generation vaccines: past data showed that smallpox vaccination was 85% protective against MPXV[60]. 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[249,250], 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[40]. Moreover, the stigmatization of potentially being associated with the community of MSM might further deter vaccination efforts.

protection, the efficacy of a single dose of JYNNEOS may be similar to ACAM2000 within the first critical weeks of post-exposure prophylaxis. In the same prairie dog study referenced above, post-exposure vaccination with JYNNEOS resulted in 38–88% survival[181].

A critical aspect of vaccine development is understanding the mechanisms by which different vaccines elicit immune responses, as different formulations can induce either specific B cell-mediated or T cell-mediated immunity[184]. Likewise, the immunocompetency of vaccine recipients as well as the route of immunization will also affect the quality of the immune response[184]. Given the frequent mucosal route of infection, the specific induction of mucosal immunity may be crucial in the context of MPXV, as documented for other infectious pathogens that transmit via the mucosal route[185]. Currently, it is not well understood whether any of the available vaccines are able to induce mucosal immunity, and this remains a key future research need.

Another research need is to understand whether there is a risk of creating new virus strains when individuals infected with monkeypox are vaccinated. Co-infecting poxviruses can evolve by exchanging genetic information via homologous or non-homologous recombination[186]. It is currently unknown how frequently genes from live or attenuated poxvirus-based vaccines may recombine with monkeypox.

In addition to the VACV or MVA-based vaccines, newer genetically engineered VACV or other poxvirus strains such as NYVAC and ALVAC have also been experimented with as potential vaccine vectors for other diseases. However, they generally induce lower antibody titres[187], and their effectiveness against monkeypox is unclear.

Ring vaccination trial design
A recently developed approach that recruits subjects linked to an infected case and allows for the simultaneous evaluation of vaccine efficacy and effectiveness during ongoing outbreaks.

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[188]. Tecovirimat was effective at reducing monkeypox severity in NHP models[189,190], but its effectiveness is reduced if treatment is given more than 5 days post challenge[191]. This suggests a caveat if tecovirimat is given based on the onset of symptoms, which may appear much later following infection[1,4]. 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[28].

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[192,193]. In the same small study discussed above[28], three patients were treated with brincidofovir, but all three ceased therapy owing to toxicity (elevated liver enzymes). Cidofovir[28], the active drug form of brincidofovir, also shows anti-poxvirus activity in vitro and in animal studies[194], but it is nephrotoxic[195–197].

VIG showed promising activity in preventing smallpox in exposed individuals[198] and has exhibited cross-neutralizing activity against MPXV in rhesus macaques[53]. 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[199]. 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[200]. 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[1]. 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[22]. Likewise, the preputial mucosal immunity should also be characterized, as MPXV DNA has been identified in semen[26].

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[201]. Likewise, as reported for SARS-CoV-2 infections, children who are infected tend to have reduced B cell[202] and T cell[203] 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[1,4,7]. It is also important to understand the risks of vaccination in vulnerable populations, especially young children and pregnant women[1,4,30,32]. 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[204]. 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[205].

Many other infectious diseases are endemic to Africa[206], 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[207–209]. 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[210].

Risk factors for severe MPXV should also be identified. Pregnant women, young children and unvaccinated individuals are known to be especially susceptible[1,4,30,32]. 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[211]. 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[30,32]. Moreover, young children are apparently more susceptible to severe monkeypox[4]. 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.

Published online: 05 September 2022

1. McCollum, A. M. & Damon, I. K. Human monkeypox. *Clin. Infect. Dis.* **58**, 260–267 (2014).
2. Cho, C. T. & Wenner, H. A. Monkeypox virus. *Bacteriol. Rev.* **37**, 1–18 (1973).
3. Cohen, J. Monkeypox outbreak questions intensify as cases soar. *Science* **376**, 902–903 (2022).
4. Jezek, Z., Szczeniowski, M., Paluku, K. M. & Mutombo, M. Human monkeypox: clinical features of 282 patients. *J. Infect. Dis.* **156**, 293–298 (1987).
5. Kimball, S. WHO declares rapidly spreading monkeypox outbreak a global health emergency. *CNBC* https://www.cnbc.com/2022/07/23/who-declares-spreading-monkeypox-outbreak-a-global-health-emergency.html (2022).
6. Reynolds, M. G. & Damon, I. K. Outbreaks of human monkeypox after cessation of smallpox vaccination. *Trends Microbiol.* **20**, 80–87 (2012).
7. Fine, P. E. M., Jezek, Z., Grab, B. & Dixon, H. The transmission potential of monkeypox virus in human populations. *Int. J. Epidemiol.* **17**, 643–650 (1988).
8. Thornhill, J. P. et al. Monkeypox virus infection in humans across 16 countries — April–June 2022. *N. Engl. J. Med.* https://doi.org/10.1056/NEJMoa2207323 (2022).
This paper reports on the clinical findings of 528 patients infected with monkeypox from the current outbreak.
9. Otu, A., Ebenso, B., Walley, J., Barcelo, J. M. & Ochu, C. L. Global human monkeypox outbreak: atypical presentation demanding urgent public health action. *Lancet Microbe* https://doi.org/10.1016/S2666-5247(22)00153-7 (2022).
10. Likos, A. M. et al. A tale of two clades: monkeypox viruses. *J. Gen. Virol.* **86**, 2661–2672 (2005).
11. Chen, N. et al. Virulence differences between monkeypox virus isolates from West Africa and the Congo Basin. *Virology* **340**, 46–63 (2005).
12. Bunge, E. M. et al. The changing epidemiology of human monkeypox — a potential threat? A systematic review. *PLoS Negl. Trop. Dis.* **16**, e0010141 (2022).
13. Minhaj, F. S. et al. Monkeypox outbreak — nine states, May 2022. *MMWR Morb. Mortal. Wkly. Rep.* **71**, 764–769 (2022).
14. Desingu, P. A. & Nagarajan, K. Genomic regions insertion and deletion in monkeypox virus causing multi-country outbreak — 2022. Preprint at *bioRxiv* https://doi.org/10.1101/2022.06.28.497936 (2022).
15. Kugelman, J. R. et al. Genomic variability of monkeypox virus among humans, Democratic Republic of the Congo. *Emerg. Infect. Dis.* **20**, 232–239 (2014).
16. Shchelkunov, S. N. et al. Human monkeypox and smallpox viruses: genomic comparison. *FEBS Lett.* **509**, 66–70 (2001).
17. Shchelkunov, S. N. et al. Analysis of the monkeypox virus genome. *Virology* **297**, 172–194 (2002).
18. Weaver, J. R. & Isaacs, S. N. Monkeypox virus and insights into its immunomodulatory proteins. *Immunol. Rev.* **225**, 96–113 (2008).
19. McFadden, G. Poxvirus tropism. *Nat. Rev. Microbiol.* **3**, 201–213 (2005).
20. Hsiao, J. C., Chung, C. S. & Chang, W. Vaccinia virus envelope D8L protein binds to cell surface chondroitin sulfate and mediates the adsorption of intracellular mature virions to cells. *J. Virol.* **73**, 8750–8761 (1999).
21. Chung, C. S., Hsiao, J. C., Chang, Y. S. & Chang, W. A27L protein mediates vaccinia virus interaction with cell surface heparan sulfate. *J. Virol.* **72**, 1577–1585 (1998).
22. Lin, C. L., Chung, C. S., Heine, H. G. & Chang, W. Vaccinia virus envelope H3L protein binds to cell surface heparan sulfate and is important for intracellular mature virion morphogenesis and virus infection in vitro and in vivo. *J. Virol.* **74**, 3353–3365 (2000).
23. Moss, B. Membrane fusion during poxvirus entry. *Semin. Cell Dev. Biol.* **60**, 89–96 (2016).
24. Perez Duque, M. et al. Ongoing monkeypox virus outbreak, Portugal, 29 April to 23 May 2022. *Eurosurveillance* **27**, 2200424 (2022).
This study reports the epidemiological and clinical characteristics of a group of 27 confirmed cases from Portugal during this current outbreak.
25. Hammerschlag, Y. et al. Monkeypox infection presenting as genital rash, Australia, May 2022. *Eurosurveillance* **27**, 2200411 (2022).
This study describes the case report of an individual returning to Australia from Europe, who later went on to develop genital lesions arising from MPXV infection.
26. Antinori, A. et al. Epidemiological, clinical and virological characteristics of four cases of monkeypox support transmission through sexual

contact, Italy, May 2022. *Eurosurveillance* **27**, 2200421 (2022).
This paper describes the clinical findings obtained from four infected males, all of whom participate in activities with MSM.
27. Huhn, G. D. et al. Clinical characteristics of human monkeypox, and risk factors for severe disease. *Clin. Infect. Dis.* **41**, 1742–1751 (2005).
28. Adler, H. et al. Clinical features and management of human monkeypox: a retrospective observational study in the UK. *Lancet Infect. Dis.* https://doi.org/10.1016/S1473-3099(22)00228-6 (2022).
This paper describes the retrospective clinical and virological findings of seven patients infected with MPXV in the United Kingdom between 2018 and 2021.
29. Learned, L. A. et al. Extended interhuman transmission of monkeypox in a hospital community in the Republic of the Congo, 2003. *Am. J. Trop. Med. Hyg.* **73**, 428–434 (2005).
30. Pittman, P. R. et al. Clinical characterization of human monkeypox infections in the Democratic Republic of the Congo. Preprint at *medRxiv* https://doi.org/10.1101/2022.05.26.22273379 (2022).
31. Downie, A. W. & Dumbell, K. R. Survival of variola virus in dried exudate and crusts from smallpox patients. *Lancet* **1**, 550–553 (1947).
32. Mbala, P. K. et al. Maternal and fetal outcomes among pregnant women with human monkeypox infection in the Democratic Republic of Congo. *J. Infect. Dis.* **216**, 824–828 (2017).
This study describes the effect of MPXV infection in pregnant women and reports the maternal and fetal outcomes following infection.
33. Centers for Disease Control and Prevention. Clinical considerations for Monkeypox in children and adolescents. *CDC* https://www.cdc.gov/poxvirus/monkeypox/clinicians/pediatric.html (2022).
34. Stanford, M. M., McFadden, G., Karupiah, G. & Chaudhri, G. Immunopathogenesis of poxvirus infections: forecasting the impending storm. *Immunol. Cell Biol.* **85**, 93–102 (2007).
35. Rimoin, A. W. et al. Major increase in human monkeypox incidence 30 years after smallpox vaccination campaigns cease in the Democratic Republic of Congo. *Proc. Natl Acad. Sci. USA* **107**, 16262–16267 (2010).
36. Zaucha, G. M., Jahrling, P. B., Geisbert, T. W., Swearengen, J. R. & Hensley, L. The pathology of experimental aerosolized monkeypox virus infection in cynomolgus monkeys (*Macaca fascicularis*). *Lab. Invest.* **81**, 1581–1600 (2001).
37. Song, H. et al. Monkeypox virus infection of rhesus macaques induces massive expansion of natural killer cells but suppresses natural killer cell functions. *PLoS ONE* **8**, e77804 (2013).
38. Dai, P. et al. Modified vaccinia virus ankara triggers type I IFN production in murine conventional dendritic cells via a cGAS/STING-mediated cytosolic DNA-sensing pathway. *PLoS Pathog.* **10**, e1003989 (2014).
39. Engelmayer, J. et al. Vaccinia virus inhibits the maturation of human dendritic cells: a novel mechanism of immune evasion. *J. Immunol.* **163**, 6762–6768 (1999).
40. Li, P. et al. Disruption of MHC class II-restricted antigen presentation by vaccinia virus. *J. Immunol.* **175**, 6481–6488 (2005).
41. Humlová, Z., Vokurka, M., Esteban, M. & Mělková, Z. Vaccinia virus induces apoptosis of infected macrophages. *J. Gen. Virol.* **83**, 2821–2832 (2002).
42. Beauchamp, N. M., Busick, R. Y. & Alexander-Miller, M. A. Functional divergence among CD103$^+$ dendritic cell subpopulations following pulmonary poxvirus infection. *J. Virol.* **84**, 10191–10199 (2010).
43. Humrich, J. Y. et al. Vaccinia virus impairs directional migration and chemokine receptor switch of human dendritic cells. *Eur. J. Immunol.* **37**, 954–965 (2007).
44. Liu, L. et al. Vaccinia virus induces strong immunoregulatory cytokine production in healthy human epidermal keratinocytes: a novel strategy for immune evasion. *J. Virol.* **79**, 7363–7370 (2005).
45. Walzer, T., Galibert, L. & De Smedt, T. Poxvirus semaphorin A39R inhibits phagocytosis by dendritic cells and neutrophils. *Eur. J. Immunol.* **35**, 391–398 (2005).
46. Norbury, C. C., Malide, D., Gibbs, J. S., Bennink, J. R. & Yewdell, J. W. Visualizing priming of virus-specific CD8$^+$ T cells by infected dendritic cells in vivo. *Nat. Immunol.* **3**, 265–271 (2002).
47. Hickman, H. D. et al. Anatomically restricted synergistic antiviral activities of innate and adaptive immune cells in the skin. *Cell Host Microbe* **13**, 155–168 (2013).
48. Chahroudi, A. et al. Vaccinia virus tropism for primary hematolymphoid cells is determined by restricted expression of a unique virus receptor. *J. Virol.* **79**, 10397–10407 (2005).
49. Chapman, J. L., Nichols, D. K., Martinez, M. J. & Raymond, J. W. Animal models of orthopoxvirus infection. *Vet. Pathol.* **47**, 852–870 (2010).
50. Moulton, E. A., Atkinson, J. P. & Buller, R. M. L. Surviving mousepox infection requires the complement system. *PLoS Pathog.* **4**, e1000249 (2008).
51. Institute of Medicine (US) Committee on the Assessment of Future Scientific Needs for Live Variola Virus. *Assessment of Future Scientific Needs for Live Variola Virus* (National Academies Press (US), 1999).
52. Alakunle, E., Moens, U., Nchinda, G. & Okeke, M. I. Monkeypox virus in Nigeria: infection biology, epidemiology, and evolution. *Viruses* **12**, 1257 (2020).
53. Edghill-Smith, Y. et al. Smallpox vaccine-induced antibodies are necessary and sufficient for protection against monkeypox virus. *Nat. Med.* **11**, 740–747 (2005).
54. Deng, L. et al. Vaccinia virus subverts a mitochondrial antiviral signaling protein-dependent innate immune response in keratinocytes through its double-stranded RNA binding protein, E3. *J. Virol.* **82**, 10735–10746 (2008).
55. Cann, J. A., Jahrling, P. B., Hensley, L. E. & Wahl-Jensen, V. Comparative pathology of smallpox and monkeypox in man and macaques. *J. Comp. Pathol.* **148**, 6–21 (2013).
56. Aggio, J. B., Krmeskā, V., Ferguson, B. J., Wowk, P. F. & Rothfuchs, A. G. Vaccinia virus infection inhibits skin dendritic cell migration to the draining lymph node. *J. Immunol.* **206**, 776–784 (2021).
57. Reynoso, G. V. et al. Lymph node conduits transport virions for rapid T cell activation. *Nat. Immunol.* **20**, 602–612 (2019).
58. Davido, B., D'anglejan, E., Jourdan, J., Robinault, A. & Davido, G. Monkeypox 2022 outbreak: cases with exclusive genital lesions. *J. Travel. Med.* https://doi.org/10.1093/jtm/taac077 (2022).
This paper describes the presence of genital lesions in recent cases involving young men engaging in activities with MSM.
59. Forrester, J. V., Mölzer, C. & Kuffova, L. Immune privilege furnishes a niche for latent infection. *Front. Ophthalmol.* https://doi.org/10.3389/fopht.2022.869046 (2022).
60. Prow, N. A. et al. A vaccinia-based single vector construct multi-pathogen vaccine protects against both Zika and chikungunya viruses. *Nat. Commun.* **9**, 1230 (2018).
61. Zhao, Y., Adams, Y. F. & Croft, M. Preferential replication of vaccinia virus in the ovaries is independent of immune regulation through IL-10 and TGF-β. *Viral Immunol.* **24**, 387–396 (2011).
62. Khoury, G. et al. Human immunodeficiency virus persistence and T-cell activation in blood, rectal, and lymph node tissue in human immunodeficiency virus-infected individuals receiving suppressive antiretroviral therapy. *J. Infect. Dis.* **215**, 911–919 (2017).
63. Kelley, C. F. et al. The rectal mucosa and condomless receptive anal intercourse in HIV-negative MSM: implications for HIV transmission and prevention. *Mucosal Immunol.* **10**, 996–1007 (2017).
64. Mikulak, J., Di Vito, C., Zaghi, E. & Mavilio, D. Host immune responses in HIV-1 infection: the emerging pathogenic role of siglecs and their clinical correlates. *Front. Immunol.* **8**, 314 (2017).
65. Hammarlund, E. et al. Monkeypox virus evades antiviral CD4$^+$ and CD8$^+$ T cell responses by suppressing cognate T cell activation. *Proc. Natl Acad. Sci. USA* **105**, 14567–14572 (2008).
66. Rubins, K. H. et al. The host response to smallpox: analysis of the gene expression program in peripheral blood cells in a nonhuman primate model. *Proc. Natl Acad. Sci. USA* **101**, 15190–15195 (2004).
67. Jahrling, P. B. et al. Exploring the potential of variola virus infection of cynomolgus macaques as a model for human smallpox. *Proc. Natl Acad. Sci. USA* **101**, 15196–15200 (2004).
68. Rubins, K. H., Hensley, L. E., Relman, D. A. & Brown, P. O. Stunned silence: gene expression programs in human cells infected with monkeypox or vaccinia virus. *PLoS ONE* **6**, e15615 (2011).
69. Song, H. et al. Poxvirus antigen staining of immune cells as a biomarker to predict disease outcome in monkeypox and cowpox virus infection in non-human primates. *PLoS ONE* **8**, e60533 (2013).
70. Johnson, R. F. et al. Comparative analysis of monkeypox virus infection of cynomolgus macaques by the intravenous or intrabronchial inoculation route. *J. Virol.* **85**, 2112–2125 (2011).
71. Byrd, D. et al. Primary human macrophages serve as vehicles for vaccinia virus replication and dissemination. *J. Virol.* **88**, 6819–6831 (2014).
72. Fischer, M. A. et al. CD11b$^+$, Ly6G$^+$ cells produce type I interferon and exhibit tissue protective properties following peripheral virus infection. *PLoS Pathog.* **7**, e1002374 (2011).
73. Nagata, N. et al. Pathogenesis of fulminant monkeypox with bacterial sepsis after experimental infection with West African monkeypox virus in a cynomolgus monkey. *Int. J. Clin. Exp. Pathol.* **7**, 4359–4370 (2014).
74. Davies, M. L. et al. A systemic macrophage response is required to contain a peripheral poxvirus infection. *PLoS Pathog.* **13**, e1006435 (2017).
75. Paust, S., Senman, B. & von Andrian, U. H. Adaptive immune responses mediated by natural killer cells. *Immunol. Rev.* **235**, 286–296 (2010).
76. Earl, P. L., Americo, J. L. & Moss, B. Natural killer cells expanded in vivo or ex vivo with IL-15 overcomes the inherent susceptibility of CAST mice to lethal infection with orthopoxviruses. *PLoS Pathog.* **16**, e1008505 (2020).
77. Jayaraman, A. et al. IL-15 complexes induce NK- and T-cell responses independent of type I IFN signaling during rhinovirus infection. *Mucosal Immunol.* **7**, 1151–1164 (2014).
78. Bukowski, J. F., Woda, B. A., Habu, S., Okumura, K. & Welsh, R. M. Natural killer cell depletion enhances virus synthesis and virus-induced hepatitis in vivo. *J. Immunol.* **131**, 1531–1538 (1983).
79. Fang, M., Lanier, L. L. & Sigal, L. J. A role for NKG2D in NK cell-mediated resistance to poxvirus disease. *PLoS Pathog.* **4**, e30 (2008).
80. Fenner, F. Adventures with poxviruses of vertebrates. *FEMS Microbiol. Rev.* **24**, 123–133 (2000).
81. Fang, M. et al. CD94 is essential for NK cell-mediated resistance to a lethal viral disease. *Immunity* **34**, 579–589 (2011).
82. Vance, R. E., Jamieson, A. M. & Raulet, D. H. Recognition of the class Ib molecule Qa-1^b by putative activating receptors CD94/NKG2C and CD94/NKG2E on mouse natural killer cells. *J. Exp. Med.* **190**, 1801–1812 (1999).
83. Johnston, S. C. et al. In vitro inhibition of monkeypox virus production and spread by Interferon-β. *Virol. J.* **9**, 5 (2012).
84. Shchelkunov, S. N. Orthopoxvirus genes that mediate disease virulence and host tropism. *Adv. Virol.* **2012**, 524743 (2012).
85. Suraweera, C. D., Hinds, M. G. & Kvansakul, M. Poxviral strategies to overcome host cell apoptosis. *Pathogens* **10**, 6 (2020).
86. Mohamed, M. R. & McFadden, G. NF-κB inhibitors: strategies from poxviruses. *Cell Cycle* **8**, 3125–3132 (2009).
87. Shisler, J. L. & Jin, X. L. The vaccinia virus K1L gene product inhibits host NF-κB activation by preventing IκBα degradation. *J. Virol.* **78**, 3553–3560 (2004).
88. Dutta, P. & Nahrendorf, M. Regulation and consequences of monocytosis. *Immunol. Rev.* **262**, 167–178 (2014).
89. Johnston, S. C. et al. Cytokine modulation correlates with severity of monkeypox disease in humans. *J. Clin. Virol.* **63**, 42–45 (2015).
90. Howell, M. D. et al. Cytokine milieu of atopic dermatitis skin subverts the innate immune response to vaccinia virus. *Immunity* **24**, 341–348 (2006).
91. van Den Broek, M. et al. IL-4 and IL-10 antagonize IL-12-mediated protection against acute vaccinia virus infection with a limited role of IFN-γ and nitric oxide synthetase 2. *J. Immunol.* **164**, 371–378 (2000).
92. Thakur, A., Mikkelsen, H. & Jungersen, G. Intracellular pathogens: host immunity and microbial persistence strategies. *J. Immunol. Res.* **2019**, 1356540 (2019).
93. Srivastava, R. M., Marincola, F. M. & Shanker, A. Editorial: lymphocyte functional crosstalk and regulation. *Front. Immunol.* **10**, 2916 (2019).
94. Strassburg, M. A. The global eradication of smallpox. *Am. J. Infect. Control.* **10**, 53–59 (1982).
95. Cherry, J. D. et al. Clinical and serologic study of four smallpox vaccines comparing variations of dose and route of administration. Primary percutaneous vaccination. *J. Infect. Dis.* **135**, 145–154 (1977).
96. Kempe, C. H. et al. The use of vaccinia hyperimmune γ-globulin in the prophylaxis of smallpox. *Bull. World Health Organ.* **25**, 41–48 (1961).
97. Jacobs, B. L. et al. Vaccinia virus vaccines: past, present and future. *Antivir. Res.* **84**, 1–13 (2009).

98. Crotty, S. et al. Cutting edge: long-term B cell memory in humans after smallpox vaccination. *J. Immunol.* **171**, 4969–4973 (2003).
99. Hammarlund, E. et al. Duration of antiviral immunity after smallpox vaccination. *Nat. Med.* **9**, 1131–1137 (2003).
100. Mack, T. M., Noble, J. Jr. & Thomas, D. B. A prospective study of serum antibody and protection against smallpox. *Am. J. Trop. Med. Hyg.* **21**, 214–218 (1972).
101. Keasey, S. et al. Proteomic basis of the antibody response to monkeypox virus infection examined in cynomolgus macaques and a comparison to human smallpox vaccination. *PLoS ONE* **5**, e15547 (2011).
102. Howard, A. R., Senkevich, T. G. & Moss, B. Vaccinia virus A26 and A27 proteins form a stable complex tethered to mature virions by association with the A17 transmembrane protein. *J. Virol.* **82**, 12384–12391 (2008).
103. Chiu, W. L., Lin, C. L., Yang, M. H., Tzou, D. L. & Chang, W. Vaccinia virus 4c (A26L) protein on intracellular mature virus binds to the extracellular cellular matrix laminin. *J. Virol.* **81**, 2149–2157 (2007).
104. Mucker, E. M. et al. Intranasal monkeypox marmoset model: prophylactic antibody treatment provides benefit against severe monkeypox virus disease. *PLoS Negl. Trop. Dis.* **12**, e0006581 (2018). **This study highlights the feasibility of using nanoparticle technology as a vehicle for delivering antibody-encoding mRNA targeting poxviruses**.
105. Wolffe, E. J., Vijaya, S. & Moss, B. A myristylated membrane protein encoded by the vaccinia virus L1R open reading frame is the target of potent neutralizing monoclonal antibodies. *Virology* **211**, 53–63 (1995).
106. Chen, Z. et al. Chimpanzee/human mAbs to vaccinia virus B5 protein neutralize vaccinia and smallpox viruses and protect mice against vaccinia virus. *Proc. Natl Acad. Sci. USA* **103**, 1882–1887 (2006).
107. Mucker, E. M., Thiele-Suess, C., Baumhof, P. & Hooper, J. W. Lipid nanoparticle delivery of unmodified mRNAs encoding multiple monoclonal antibodies targeting poxviruses in rabbits. *Mol. Ther. Nucleic Acids* **28**, 847–858 (2022).
108. Gilchuk, I. et al. Cross-neutralizing and protective human antibody specificities to poxvirus infections. *Cell* **167**, 684–694.e9 (2016). **In this study, a panel of mAbs were evaluated for their potential to cross-neutralize orthopoxviruses**.
109. Karem, K. L. et al. Monkeypox-induced immunity and failure of childhood smallpox vaccination to provide complete protection. *Clin. Vaccin. Immunol.* **14**, 1318–1327 (2007).
110. MacLeod, M. K., Clambey, E. T., Kappler, J. W. & Marrack, P. CD4 memory T cells: what are they and what can they do? *Semin. Immunol.* **21**, 53–61 (2009).
111. Jayani, I., Susmiati, E. & Sulistyawati, W. The correlation between CD4 count cell and opportunistic infection among HIV/AIDS patients. *J. Phys. Conf. Ser.* **1569**, 032066 (2020).
112. Smith, Y. E. et al. Smallpox vaccine does not protect macaques with AIDS from a lethal monkeypox virus challenge. *J. Infect. Dis.* **191**, 372–381 (2005).
113. Sklenovská, N. & Van Ranst, M. Emergence of monkeypox as the most important orthopoxvirus infection in humans. *Front. Public Health* **6**, 241 (2018).
114. Dai, R., Huang, X. & Yang, Y. γδT cells are required for $CD8^+$ T cell response to vaccinia viral infection. *Front. Immunol.* **12**, 727046 (2021).
115. Goulding, J. et al. CD8 T cells use IFN-γ to protect against the lethal effects of a respiratory poxvirus infection. *J. Immunol.* **192**, 5415 (2014).
116. Remakus, S. et al. Memory $CD8^+$ T cells can outsource IFN-γ production but not cytolytic killing for antiviral protection. *Cell Host Microbe* **13**, 546–557 (2013).
117. McClain, D. J. et al. Immunologic responses to vaccinia vaccines administered by different parenteral routes. *J. Infect. Dis.* **175**, 756–763 (1997).
118. Ennis, F. A., Cruz, J., Demkowicz, W. E. Jr, Rothman, A. L. & McClain, D. J. Primary induction of human $CD8^+$ cytotoxic T lymphocytes and interferon-γ-producing T cells after smallpox vaccination. *J. Infect. Dis.* **185**, 1657–1659 (2002).
119. Miller, J. D. et al. Human effector and memory $CD8^+$ T cell responses to smallpox and yellow fever vaccines. *Immunity* **28**, 710–722 (2008).
120. Munier, C. M. L. et al. The primary immune response to vaccinia virus vaccination includes cells with a distinct cytotoxic effector CD4 T-cell phenotype. *Vaccine* **34**, 5251–5261 (2016).
121. Littaua, R. A., Takeda, A., Cruz, J. & Ennis, F. A. Vaccinia virus-specific human $CD4^+$ cytotoxic T-lymphocyte clones. *J. Virol.* **66**, 2274–2280 (1992).
122. Fang, M. et al. Perforin-dependent $CD4^+$ T-cell cytotoxicity contributes to control a murine poxvirus infection. *Proc. Natl Acad. Sci. USA* **109**, 9983–9988 (2012).
123. Kennedy, R. & Poland, G. A. T-cell epitope discovery for variola and vaccinia viruses. *Rev. Med. Virol.* **17**, 93–113 (2007).
124. Sette, A. et al. Definition of epitopes and antigens recognized by vaccinia specific immune responses: their conservation in variola virus sequences, and use as a model system to study complex pathogens. *Vaccine* **27**, G21–G26 (2009).
125. Walsh, S. R. et al. Diverse recognition of conserved orthopoxvirus $CD8^+$ T cell epitopes in vaccinated rhesus macaques. *Vaccine* **27**, 4990–5000 (2009).
126. Calvo-Calle, J. M., Strug, I., Nastke, M. D., Baker, S. P. & Stern, L. J. Human $CD4^+$ T cell epitopes from vaccinia virus induced by vaccination or infection. *PLoS Pathog.* **3**, 1511–1529 (2007).
127. Kennedy, R. B. & Poland, G. A. The identification of HLA class II-restricted T cell epitopes to vaccinia virus membrane proteins. *Virology* **408**, 232–240 (2010).
128. Molero-Abraham, M., Glutting, J. P., Flower, D. R., Lafuente, E. M. & Reche, P. A. EPIPOX: immunoinformatic characterization of the shared T-cell epitome between variola virus and related pathogenic orthopoxviruses. *J. Immunol. Res.* **2015**, 738020 (2015).
129. Ando, J., Ngo, M. C., Ando, M., Leen, A. & Rooney, C. M. Identification of protective T-cell antigens for smallpox vaccines. *Cytotherapy* **22**, 642–652 (2020). **In this study, the *E3L* gene, which is conserved across orthopoxviruses, is identified as a $CD8^+$ T cell specific epitope, which could be used to monitor the protective immunity following infection**.
130. Arndt, W. D. et al. Evasion of the innate immune type I interferon system by monkeypox virus. *J. Virol.* **89**, 10489–10499 (2015).
131. Denzler, K. L. et al. Attenuated NYCBH vaccinia virus deleted for the *E3L* gene confers partial protection against lethal monkeypox virus disease in cynomolgus macaques. *Vaccine* **29**, 9684–9690 (2011).
132. Resch, W., Hixson, K. K., Moore, R. J., Lipton, M. S. & Moss, B. Protein composition of the vaccinia virus mature virion. *Virology* **358**, 233–247 (2007).
133. Nichols, D. B., De Martini, W. & Cottrell, J. Poxviruses utilize multiple strategies to inhibit apoptosis. *Viruses* **9**, 215 (2017).
134. Shchelkunov, S. N. & Shchelkunova, G. A. Genes that control vaccinia virus immunogenicity. *Acta Nat.* **12**, 33–41 (2020).
135. Smith, G. L. et al. Vaccinia virus immune evasion: mechanisms, virulence and immunogenicity. *J. Gen. Virol.* **94**, 2367–2392 (2013).
136. Yu, H., Bruneau, R. C., Brennan, G. & Rothenburg, S. Battle royale: innate recognition of poxviruses and viral immune evasion. *Biomedicines* **9**, 765 (2021).
137. Franz, K. M. & Kagan, J. C. Innate immune receptors as competitive determinants of cell fate. *Mol. Cell* **66**, 750–760 (2017).
138. Mogensen, T. H. Pathogen recognition and inflammatory signaling in innate immune defenses. *Clin. Microbiol. Rev.* **22**, 240–273 (2009).
139. Di Pilato, M., Mejias-Perez, E., Sorzano, C. O. S. & Esteban, M. Distinct roles of vaccinia virus NF-κB inhibitor proteins A52, B15, and K7 in the immune response. *J. Virol.* **91**, e00575-17 (2017).
140. Graham, S. C. et al. Vaccinia virus proteins A52 and B14 share a Bcl-2-like fold but have evolved to inhibit NF-κB rather than apoptosis. *PLoS Pathog.* **4**, e1000128 (2008).
141. Maluquer de Motes, C. et al. Inhibition of apoptosis and NF-κB activation by vaccinia protein N1 occur via distinct binding surfaces and make different contributions to virulence. *PLoS Pathog.* **7**, e1002430 (2011).
142. Chang, H. W., Watson, J. C. & Jacobs, B. L. The *E3L* gene of vaccinia virus encodes an inhibitor of the interferon-induced, double-stranded RNA-dependent protein kinase. *Proc. Natl Acad. Sci. USA* **89**, 4825–4829 (1992).
143. Silverman, R. H. Caps off to poxviruses. *Cell Host Microbe* **17**, 287–289 (2015).
144. Zhang, P., Jacobs, B. L. & Samuel, C. E. Loss of protein kinase PKR expression in human HeLa cells complements the vaccinia virus E3L deletion mutant phenotype by restoration of viral protein synthesis. *J. Virol.* **82**, 840–848 (2008).
145. Jefferies, C. A. Regulating IRFs in IFN driven disease. *Front. Immunol.* **10**, 325 (2019).
146. Colamonici, O. R., Domanski, P., Sweitzer, S. M., Larner, A. & Buller, R. M. Vaccinia virus *B18R* gene encodes a type I interferon-binding protein that blocks interferon α transmembrane signaling. *J. Biol. Chem.* **270**, 15974–15978 (1995).
147. Xu, R. H. et al. The orthopoxvirus type I IFN binding protein is essential for virulence and an effective target for vaccination. *J. Exp. Med.* **205**, 981–992 (2008).
148. Fernandez de Marco Mdel, M., Alejo, A., Hudson, P., Damon, I. K. & Alcami, A. The highly virulent variola and monkeypox viruses express secreted inhibitors of type I interferon. *FASEB J.* **24**, 1479–1488 (2010).
149. Yoshida, M. et al. Local and systemic responses to SARS-CoV-2 infection in children and adults. *Nature* **602**, 321–327 (2022).
150. Hijano, D. R. et al. Role of type I interferon (IFN) in the respiratory syncytial virus (RSV) immune response and disease severity. *Front. Immunol.* **10**, 566 (2019).
151. Oeckinghaus, A. & Ghosh, S. The NF-κB family of transcription factors and its regulation. *Cold Spring Harb. Perspect. Biol.* **1**, a000034 (2009).
152. Bloomer, D. T. et al. CrmA orthologs from diverse poxviruses potently inhibit caspases-1 and -8, yet cleavage site mutagenesis frequently produces caspase-1-specific variants. *Biochem. J.* **476**, 1335–1357 (2019).
153. Komiyama, T. et al. Inhibition of interleukin-1β converting enzyme by the cowpox virus serpin CrmA. An example of cross-class inhibition. *J. Biol. Chem.* **269**, 19331–19337 (1994).
154. Veyer, D. L. et al. Analysis of the anti-apoptotic activity of four vaccinia virus proteins demonstrates that B13 is the most potent inhibitor in isolation and during viral infection. *J. Gen. Virol.* **95**, 2757–2768 (2014).
155. Quan, L. T., Caputo, A., Bleackley, R. C., Pickup, D. J. & Salvesen, G. S. Granzyme B is inhibited by the cowpox virus serpin cytokine response modifier A. *J. Biol. Chem.* **270**, 10377–10379 (1995).
156. Devadas, S. et al. Granzyme B is critical for T cell receptor-induced cell death of type 2 helper T cells. *Immunity* **25**, 237–247 (2006).
157. Alvarez-de Miranda, F. J., Alonso-Sanchez, I., Alcami, A. & Hernaez, B. TNF decoy receptors encoded by poxviruses. *Pathogens* **10**, 1065 (2021).
158. Hu, F. Q., Smith, C. A. & Pickup, D. J. Cowpox virus contains two copies of an early gene encoding a soluble secreted form of the type II TNF receptor. *Virology* **204**, 343–356 (1994).
159. Pontejo, S. M., Alejo, A. & Alcami, A. Comparative biochemical and functional analysis of viral and human secreted tumor necrosis factor (TNF) decoy receptors. *J. Biol. Chem.* **290**, 15973–15984 (2015).
160. Marotte, H. & Cimaz, R. Etanercept—TNF receptor and IgG1 Fc fusion protein: is it different from other TNF blockers? *Expert. Opin. Biol. Ther.* **14**, 569–572 (2014).
161. Bahar, M. W. et al. Structure and function of A41, a vaccinia virus chemokine binding protein. *PLoS Pathog.* **4**, e5 (2008).
162. Kotwal, G. J. & Moss, B. Vaccinia virus encodes a secretory polypeptide structurally related to complement control proteins. *Nature* **335**, 176–178 (1988).
163. Miller, C. G., Shchelkunov, S. N. & Kotwal, G. J. The cowpox virus-encoded homolog of the vaccinia virus complement control protein is an inflammation modulatory protein. *Virology* **229**, 126–133 (1997).
164. Ruiz-Arguello, M. B. et al. An ectromelia virus protein that interacts with chemokines through their glycosaminoglycan binding domain. *J. Virol.* **82**, 917–926 (2008).
165. Seregin, S. V., Babkina, I. N., Nesterov, A. E., Sinyakov, A. N. & Shchelkunov, S. N. Comparative studies of γ-interferon receptor-like proteins of variola major and variola minor viruses. *FEBS Lett.* **382**, 79–83 (1996).
166. Upton, C., Mossman, K. & McFadden, G. Encoding of a homolog of the IFN-γ receptor by myxoma virus. *Science* **258**, 1369–1372 (1992).
167. Uvarova, E. A. & Shchelkunov, S. N. Species-specific differences in the structure of orthopoxvirus complement-binding protein. *Virus Res.* **81**, 39–45 (2001).
168. Liszewski, M. K. et al. Structure and regulatory profile of the monkeypox inhibitor of complement: comparison to homologs in vaccinia and variola and evidence for dimer formation. *J. Immunol.* **176**, 3725–3734 (2006).
169. Estep, R. D. et al. Deletion of the monkeypox virus inhibitor of complement enzymes locus impacts the adaptive immune response to monkeypox virus in a

nonhuman primate model of infection. *J. Virol.* **85**, 9527–9542 (2011).
170. Campbell, J. A., Trossman, D. S., Yokoyama, W. M. & Carayannopoulos, L. N. Zoonotic orthopoxviruses encode a high-affinity antagonist of NKG2D. *J. Exp. Med.* **204**, 1311–1317 (2007).
171. Dasgupta, A., Hammarlund, E., Slifka, M. K. & Fruh, K. Cowpox virus evades CTL recognition and inhibits the intracellular transport of MHC class I molecules. *J. Immunol.* **178**, 1654–1661 (2007).
172. Byun, M. et al. Two mechanistically distinct immune evasion proteins of cowpox virus combine to avoid antiviral CD8 T cells. *Cell Host Microbe* **6**, 422–432 (2009).
173. Born, T. L. et al. A poxvirus protein that binds to and inactivates IL-18, and inhibits NK cell response. *J. Immunol.* **164**, 3246–3254 (2000).
174. Petersen, B. W., Harms, T. J., Reynolds, M. G. & Harrison, L. H. Use of vaccinia virus smallpox vaccine in laboratory and health care personnel at risk for occupational exposure to orthopoxviruses — recommendations of the Advisory Committee on Immunization Practices (ACIP), 2015. *MMWR Morb. Mortal. Wkly. Rep.* **65**, 257–262 (2016).
175. Rao, A. K. et al. Use of JYNNEOS (smallpox and monkeypox vaccine, live, nonreplicating) for preexposure vaccination of persons at risk for occupational exposure to orthopoxviruses: recommendations of the Advisory Committee on Immunization Practices — United States, 2022. *MMWR Morb. Mortal. Wkly. Rep.* **71**, 734–742 (2022).
176. Nalca, A. & Zumbrun, E. E. ACAM2000: the new smallpox vaccine for United States strategic national stockpile. *Drug Des. Devel. Ther.* **4**, 71–79 (2010).
177. Sukhumvittaya, S., Ampol, S., Pattanapanyasat, K. & Kantakamalakul, W. Polyfunctional T cell and neutralizing antibody responses to ACAM2000TM smallpox vaccine immunization in primary-vaccinated individuals. *Adv. Microbiol.* **6**, 169–177 (2016).
178. Precopio, M. L. et al. Immunization with vaccinia virus induces polyfunctional and phenotypically distinctive $CD8^+$ T cell responses. *J. Exp. Med.* **204**, 1405–1416 (2007).
179. Pugh, C. et al. Human antibody responses to the polyclonal Dryvax vaccine for smallpox prevention can be distinguished from responses to the monoclonal replacement vaccine ACAM2000. *Clin. Vaccin. Immunol.* **21**, 877–885 (2014).
180. Keckler, M. S., Reynolds, M. G., Damon, I. K. & Karem, K. L. The effects of post-exposure smallpox vaccination on clinical disease presentation: addressing the data gaps between historical epidemiology and modern surrogate model data. *Vaccine* **31**, 5192–5201 (2013).
181. Keckler, M. S. et al. IMVAMUNE® and ACAM2000® provide different protection against disease when administered postexposure in an intranasal monkeypox challenge prairie dog model. *Vaccines* **8**, 396 (2020).
This study evaluates and characterizes the differences in protection provided by IMVAMUNE and ACAM2000 as post-exposure prophylactic vaccines in protection against MPXV infection.
182. Luong Nguyen, L. B. et al. A prospective national cohort evaluating ring MVA vaccination as post-exposure prophylaxis for monkeypox. *Nat. Med.* https://doi.org/10.1038/d41591-022-00077-1 (2022).
This study highlights the potential usefulness of a post-exposure prophylaxis ring vaccination as an effort to control spread of MPXV infection in a recruited cohort from France.
183. Yang, S. & Yonts, A. BLA clinical review memorandum. *FDA* https://www.fda.gov/media/131870/download (2019).
184. Pollard, A. J. & Bijker, E. M. A guide to vaccinology: from basic principles to new developments. *Nat. Rev. Immunol.* **21**, 83–100 (2021).
185. Lavelle, E. C. & Ward, R. W. Mucosal vaccines — fortifying the frontiers. *Nat. Rev. Immunol.* **22**, 236–250 (2022).
186. Qin, L. & Evans, D. H. Genome scale patterns of recombination between coinfecting vaccinia viruses. *J. Virol.* **88**, 5277–5286 (2014).
187. Midgley, C. M., Putz, M. M., Weber, J. N. & Smith, G. L. Vaccinia virus strain NYVAC induces substantially lower and qualitatively different human antibody responses compared with strains Lister and Dryvax. *J. Gen. Virol.* **89**, 2992–2997 (2008).
188. Russo, A. T. et al. An overview of tecovirimat for smallpox treatment and expanded anti-orthopoxvirus applications. *Expert. Rev. Anti Infect. Ther.* **19**, 331–344 (2021).
189. Berhanu, A. et al. Treatment with the smallpox antiviral tecovirimat (ST-246) alone or in combination with ACAM2000 vaccination is effective as a postsymptomatic therapy for monkeypox virus infection. *Antimicrob. Agents Chemother.* **59**, 4296–4300 (2015).
190. Huggins, J. et al. Nonhuman primates are protected from smallpox virus or monkeypox virus challenges by the antiviral drug ST-246. *Antimicrob. Agents Chemother.* **53**, 2620–2625 (2009).
191. Russo, A. T. et al. Effects of treatment delay on efficacy of tecovirimat following lethal aerosol monkeypox virus challenge in cynomolgus macaques. *J. Infect. Dis.* **218**, 1490–1499 (2018).
192. Hutson, C. L. et al. Pharmacokinetics and efficacy of a potential smallpox therapeutic, brincidofovir, in a lethal monkeypox virus animal model. *mSphere* **6**, e00927-20 (2021).
193. Stabenow, J. et al. A mouse model of lethal infection for evaluating prophylactics and therapeutics against monkeypox virus. *J. Virol.* **84**, 3909–3920 (2010).
194. Andrei, G. & Snoeck, R. Cidofovir activity against poxvirus infections. *Viruses* **2**, 2803–2830 (2010).
195. Lalezari, J. P. et al. (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (cidofovir): results of a phase I/II study of a novel antiviral nucleotide analogue. *J. Infect. Dis.* **171**, 788–796 (1995).
196. Ortiz, A. et al. Tubular cell apoptosis and cidofovir-induced acute renal failure. *Antivir. Ther.* **10**, 185–190 (2005).
197. Wachsman, M. et al. Pharmacokinetics, safety and bioavailability of HPMPC (cidofovir) in human immunodeficiency virus-infected subjects. *Antivir. Res.* **29**, 153–161 (1996).
198. Hopkins, R. J. & Lane, J. M. Clinical efficacy of intramuscular vaccinia immune globulin: a literature review. *Clin. Infect. Dis.* **39**, 819–826 (2004).
199. Dubois, M. E., Hammarlund, E. & Slifka, M. K. Optimization of peptide-based ELISA for serological diagnostics: a retrospective study of human monkeypox infection. *Vector Borne Zoonotic Dis.* **12**, 400–409 (2012).
200. Ebola ça Suffit Ring Vaccination Trial Consortium. The ring vaccination trial: a novel cluster randomised controlled trial design to evaluate vaccine efficacy and effectiveness during outbreaks, with special reference to Ebola. *BMJ* **351**, h3740 (2015).
201. Heinonen, S. et al. Immune profiles provide insights into respiratory syncytial virus disease severity in young children. *Sci. Transl. Med.* **12**, eaaw0268 (2020).
202. Toh, Z. Q. et al. Comparison of seroconversion in children and adults with mild COVID-19. *JAMA Netw. Open* **5**, e221313 (2022).
203. Cohen, C. A. et al. SARS-CoV-2 specific T cell responses are lower in children and increase with age and time after infection. *Nat. Commun.* **12**, 4678 (2021).
204. Ryan, M. A. & Seward, J. F., Smallpox Vaccine in Pregnancy Registry Team. Pregnancy, birth, and infant health outcomes from the national smallpox vaccine in pregnancy registry, 2003–2006. *Clin. Infect. Dis.* **46**, S221–S226 (2008).
205. UK Health Security Agency. Monkeypox outbreak: vaccination strategy. *gov.uk* https://www.gov.uk/guidance/monkeypox-outbreak-vaccination-strategy (2022).
206. Nkengasong, J. N. & Tessema, S. K. Africa needs a new public health order to tackle infectious disease threats. *Cell* **183**, 296–300 (2020).
207. Teo, T. H. et al. Co-infection with chikungunya virus alters trafficking of pathogenic $CD8^+$ T cells into the brain and prevents *Plasmodium*-induced neuropathology. *EMBO Mol. Med.* **10**, 121–138 (2018).
208. Teo, T. H. et al. *Plasmodium* co-infection protects against chikungunya virus-induced pathologies. *Nat. Commun.* **9**, 3905 (2018).
209. Torres-Ruesta, A. et al. Malaria abrogates O'nyong-nyong virus pathologies by restricting virus infection in nonimmune cells. *Life Sci. Alliance* **5**, e202101272 (2022).
210. Yohannes, E. & Chance, M. R. in *Encyclopedia of Systems Biology* (eds Dubitzky, W., Wolkenhauer, O., Cho, K. H. & Yokota, H.) 2078–2084 (Springer, 2013).
211. Mulkey, S. B. et al. Neurodevelopmental abnormalities in children with in utero Zika virus exposure without congenital Zika syndrome. *JAMA Pediatr.* **174**, 269–276 (2020).
212. Heymann, D. L., Szczeniowski, M. & Esteves, K. Re-emergence of monkeypox in Africa: a review of the past six years. *Br. Med. Bull.* **54**, 693–702 (1998).
213. Breman, J. G. et al. Human monkeypox, 1970–79. *Bull. World Health Organ.* **58**, 165–182 (1980).
214. Breman, J. G., Nakano, J. H., Coffi, E., Godfrey, H. & Gautun, J. C. Human poxvirus disease after smallpox eradication. *Am. J. Trop. Med. Hyg.* **26**, 273–281 (1977).
215. Foster, S. O. et al. Human monkeypox. *Bull. World Health Organ.* **46**, 569–576 (1972).
216. Centers for Disease Control and Prevention. Human monkeypox — Kasai Oriental, Democratic Republic of Congo, February 1996–October 1997. *MMWR Morb. Mortal. Wkly. Rep.* **46**, 1168–1171 (1997).
217. Jezek, Z. et al. Four generations of probable person-to-person transmission of human monkeypox. *Am. J. Epidemiol.* **123**, 1004–1012 (1986).
218. Jezek, Z., Grab, B., Szczeniowski, M., Paluku, K. M. & Mutombo, M. Clinico-epidemiological features of monkeypox patients with an animal or human source of infection. *Bull. World Health Organ.* **66**, 459–464 (1988).
219. Khodakevich, L. et al. Monkey pox virus infection in humans in the Central African Republic [French]. *Bull. Soc. Pathol. Exot. Filiales* **78**, 311–320 (1985).
220. Merouze, F. & Lesoin, J. J. Monkeypox: second human case observed in Ivory Coast (rural health sector of Daloa) [French]. *Med. Trop.* **43**, 145–147 (1983).
221. Meyer, A. et al. First appearance of monkey pox in human beings in Gabon [French]. *Med. Trop.* **51**, 53–57 (1991).
222. Tchokoteu, P. F. et al. Variola or a severe case of varicella? A case of human variola due to monkeypox virus in a child from the Cameroon [French]. *Ann. Soc. Belg. Med. Trop.* **71**, 123–128 (1991).
223. Doshi, R. H. et al. Epidemiologic and ecologic investigations of monkeypox, Likouala department, Republic of the Congo, 2017. *Emerg. Infect. Dis.* **25**, 281–289 (2019).
224. Reynolds, M. G. et al. Detection of human monkeypox in the Republic of the Congo following intensive community education. *Am. J. Trop. Med. Hyg.* **88**, 982–985 (2013).
225. Formenty, P. et al. Human monkeypox outbreak caused by novel virus belonging to Congo Basin clade, Sudan, 2005. *Emerg. Infect. Dis.* **16**, 1539–1545 (2010).
226. Reynolds, M. G. et al. Human monkeypox in Sierra Leone after 44-year absence of reported cases. *Emerg. Infect. Dis.* **25**, 1023–1025 (2019).
227. Berthet, N. et al. Maculopapular lesions in the Central African Republic. *Lancet* **378**, 1354 (2011).
228. Nakoune, E. et al. A nosocomial outbreak of human monkeypox in the Central African Republic. *Open Forum Infect. Dis.* **4**, ofx168 (2017).
229. Yinka-Ogunleye, A. et al. Outbreak of human monkeypox in Nigeria in 2017–18: a clinical and epidemiological report. *Lancet Infect. Dis.* **19**, 872–879 (2019).
230. Reynolds, M. G. et al. Spectrum of infection and risk factors for human monkeypox, United States, 2003. *Emerg. Infect. Dis.* **13**, 1332–1339 (2007).
231. Erez, N. et al. Diagnosis of imported monkeypox, Israel, 2018. *Emerg. Infect. Dis.* **25**, 980–983 (2019).
232. Vaughan, A. et al. Two cases of monkeypox imported to the United Kingdom, September 2018. *Eurosurveillance* **23**, 1800509 (2018).
233. Vaughan, A. et al. Human-to-human transmission of monkeypox virus, United Kingdom, October 2018. *Emerg. Infect. Dis.* **26**, 782–785 (2020).
234. Hobson, G. et al. Family cluster of three cases of monkeypox imported from Nigeria to the United Kingdom, May 2021. *Eurosurveillance* **26**, 2100745 (2021).
235. Yong, S. E. F. et al. Imported monkeypox, Singapore. *Emerg. Infect. Dis.* **26**, 1826–1830 (2020).
236. Costello, V. et al. Imported monkeypox from international traveler, Maryland, USA, 2021. *Emerg. Infect. Dis.* **28**, 1002–1005 (2022).
237. Rao, A. K. et al. Monkeypox in a traveler returning from Nigeria — Dallas, Texas, July 2021. *MMWR Morb. Mortal. Wkly. Rep.* **71**, 509–516 (2022).
238. Zumla, A. et al. Monkeypox outbreaks outside endemic regions: scientific and social priorities. *Lancet Infect. Dis.* https://doi.org/10.1016/S1473-3099(22)00354-1 (2022).
239. Nigeria Centre for Disease Control. Update on monkeypox (MPX) in Nigeria. *NCDC* https://ncdc.gov.ng/themes/common/files/sitreps/4f9bc59b967d1f4b19d61358595d3546.pdf (2022).

240. Kraemer, M. U. G. et al. Tracking the 2022 monkeypox outbreak with epidemiological data in real-time. *Lancet Infect. Dis.* https://doi.org/10.1016/S1473-3099(22)00359-0 (2022).
This is the first paper that creates an open-access database and visualization tool for tracking the occurrences of MPXV infection globally.
241. Channel News Asia. Singapore confirms imported case of monkeypox after flight attendant develops fever and rashes. *CNA* https://www.channelnewsasia.com/singapore/monkeypox-singapore-imported-case-flight-attendant-fever-rashes-moh-2760996 (2022).
242. Isidro, J. et al. Phylogenomic characterization and signs of microevolution in the 2022 multi-country outbreak of monkeypox virus. *Nat. Med.* https://doi.org/10.1038/s41591-022-01907-y (2022).
This recent article characterizes the MPXV outbreak genome sequence and highlights the involvement of host APOBEC3 protein in the virus evolution.
243. Luna, N. et al. Phylogenomic analysis of the monkeypox virus (MPXV) 2022 outbreak: emergence of a novel viral lineage. *Travel. Med. Infect. Dis.* https://doi.org/10.1016/j.tmaid.2022.102402 (2022).
244. Gigante, C. M. et al. Multiple lineages of monkeypox virus detected in the United States, 2021–2022. Preprint at *bioRxiv* https://doi.org/10.1101/2022.06.10.495526 (2022).
245. Hammarlund, E. et al. Multiple diagnostic techniques identify previously vaccinated individuals with protective immunity against monkeypox. *Nat. Med.* **11**, 1005–1011 (2005).
246. Sanjuan, R. & Domingo-Calap, P. Mechanisms of viral mutation. *Cell Mol. Life Sci.* **73**, 4433–4448 (2016).
247. Elde, N. C. et al. Poxviruses deploy genomic accordions to adapt rapidly against host antiviral defenses. *Cell* **150**, 831–841 (2012).
This paper describes the possibility of poxviruses using genomic accordions as a mechanism of evading host antiviral responses.
248. Daskalakis, D., McClung, R. P., Mena, L. & Mermin, J. Monkeypox: avoiding the mistakes of past infectious disease epidemics. *Ann. Intern. Med.* https://doi.org/10.7326/M22-1748 (2022).
249. Centers for Disease Control and Prevention. Monkeypox and smallpox vaccine guidance. *CDC* https://www.cdc.gov/poxvirus/monkeypox/clinicians/smallpox-vaccine.html (2022).
250. Kimball, S. U.S. has more than 36,000 Jynneos monkeypox vaccine doses immediately available in national stockpile. *CNBC* https://www.cnbc.com/2022/06/06/us-has-36000-doses-of-jynneos-monkeypox-vaccine-immediately-available-in-national-stockpile.html (2022).

Acknowledgements
The authors thank D. Ackerman of Insight Editing London for his valuable inputs on an early draft. This work was supported by core funds at the A*STAR Infectious Diseases Labs (ID Labs) through the Biomedical Medical Research Council (BMRC), A*STAR and the PREPARE (Programme for Research in Epidemic Preparedness and REsponse) Outbreak Research Strategic Funds (PREPARE-SF-2022-001).

Author contributions
All authors contributed equally to all aspects of the article.

Competing interest
The authors declare no competing interests.

Peer review information
Nature Reviews Immunology thanks Jacqueline Williams who co-reviewed with Bertram Jacobs, Sathesh Panayampalli and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.