Elsevier has created a Monkeypox Information Center in response to the declared public health emergency of international concern, with free information in English on the monkeypox virus. The Monkeypox Information Center is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its monkeypox related research that is available on the Monkeypox Information Center - including this research content - immediately available in publicly funded repositories, with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the Monkeypox Information Center remains active.

Trends in
Pharmacological Sciences

CellPress

Forum

Monkeypox: potential vaccine development strategies

José Manuel Lozano [1,*] and Sylviane Muller [2,3,4,*]

A multicountry outbreak of monkeypox has gained global attention. Basic research including structural and immunological investigation on monkeypox virus (MPXV) is central to design effective solutions of treatment with antivirals and appropriate vaccines. We summarize some information about this virus and its re-emergence and the current vaccines that are proposed to limit its spread and present some possible avenues for developing new vaccines.

Introduction

MPXV is a member of the Orthopoxvirus genus in the family Poxviridae, a large group of double-stranded DNA (dsDNA) viruses [1]. Since the officially announced global eradication of smallpox in 1980 and subsequent cessation of routine smallpox vaccination, monkeypox has been considered to be the most important orthopoxvirus infection in humans. The first outbreak of monkeypox, in 1958, was described in a colony of cynomolgus monkeys held at the State Serum Institute in Copenhagen; other outbreaks followed in monkey colonies. Its first description in humans dates back to 1970 with cases in the Democratic Republic of the Congo (DRC) and later in Sudan in 2006. Monkeypox can be acquired through direct contact with an infected person or animal (lesions, body fluids, respiratory droplets) or with material contaminated with the virus (e.g., bedding). Monkeypox is a viral **zoonosis** (see Glossary) that occurs primarily in tropical rainforest areas of Central and West Africa and was occasionally exported to other regions. Since early May 2022, however, cases of monkeypox have been reported from countries in disparate geographic areas where the disease was not endemic [2–4]. Today, monkeypox is thus considered as a re-emerging infectious disease. In a way that cannot yet be predicted, the monkeypox re-emergence can be time limited as found in the case of viral hemorrhagic fevers that caused severe, life-threatening illness (Marburg and Ebola virus infections); on the contrary, it can spread all over the globe and for a longer period, as in the case of the coronavirus disease that emerged in 2019 (COVID-19), which still requires large-scale management and priority intervention worldwide. Currently, in the case of monkeypox, although it is not a newly emerging infection, a number of gaps in knowledge still remain to enable development of effective disease control strategies [1,4,5]. This includes understanding how different clades of the virus are precisely transmitted and spread (especially in high-risk patient groups), how infection develops in patients at the cellular and tissue level, how innate and adaptive cellular and humoral immune responses coordinate to fight MPXV, and obviously, based on answers to the foregoing questions, how effective prevention and treatment approaches such as appropriate vaccines and antivirals could be designed. Here, we briefly discuss cellular and humoral immunity to MPXV and highlight potential approaches that could help in the development of new vaccines against human monkeypox.

Cellular and humoral immunity to MPXV

The initial attachment of MPXV to the host cell surface occurs through interactions between multiple viral ligands (e.g., proteins A34 and B5) and, depending on the type of cells that are targeted, several possible cell surface receptors such as chondroitin sulfate, heparan sulfate, macrophage receptor with collagenous structure (MARCO, a class A scavenger receptor), and laminin, a major component of the basal lamina. Thereafter, the virus may enter the target cell by direct fusion or via the endosomal pathway and release its content (especially a linear, dsDNA genome and enzymes required for virus uncoating and replication) into the host cell cytosol. Earlier data obtained after the US monkeypox outbreak of 2003 showed that classical immune responses are generated against this virus [4]. Orthopoxvirus-specific immunoglobulin G and M, $CD4^+$ and $CD8^+$ T cells, and B cell responses were measured at ~7–14

Glossary

γδ T cells: a small subset of CD3-positive T cells in the peripheral blood that occurs at increased frequency in mucosal tissues. Human γδ T cells do not recognize peptides presented by human leukocyte antigen (HLA) molecules like the conventional $CD4^+$ or $CD8^+$ T cells that carry the αβ T cell receptor, but rather recognize nonpeptidic phosphorylated molecules secreted by pathogens.
Neutralizing antibody: in contrast to blocking antibody, which binds its target but does not interfere with the infectivity of a virus, for example, a neutralizing antibody binds its target and negates its downstream cellular effects, such as cell proliferation or chemotaxis, or infectivity. Only a small subset of the many antibodies that bind a virus are capable of neutralization.
mRNA vaccine: a vaccine that delivers antigen-encoding mRNA molecules into immune cells, which use the designed mRNA to build foreign protein that would normally be produced by a pathogen (e.g., a virus). The mRNA is generally delivered by a co-formulation of the RNA encapsulated in lipid nanoparticles that protect the RNA strands and aid in their absorption into the cells.
Synthetic vaccine: a vaccine consisting mainly of synthetic peptides, carbohydrates, or lipid antigens or combinations thereof. They are usually considered to be safe compared with vaccines prepared from bacterial cultures, less costly, and relatively rapid to develop.
Ubiquitin system: a cellular machinery used by viruses to facilitate many aspects of the infectious cycle in the acquired immune response. This system is also involved in major histocompatibility complex (MHC) class II expression and turnover with impact on MHC I molecule.
Zoonotic infection or zoonosis: infection that can spread between animals and humans.

weeks and 1 year after exposure [6]. Recent data have also highlighted the importance of the host **ubiquitin system** [7]. The importance of memory **γδ T cells**, which play an important role in protective immunity but also have the potential to worsen the progression of autoimmunity, has been highlighted in the acquired immune memory to MPXV (shown in macaques). More broadly, however, in humans infected with MPXV, the effective roles of innate immune cells (e.g., monocytes/macrophages, neutrophils, natural killer cells, conventional dendritic cells, plasmacytoid dendritic cells, and innate lymphoid cells) remain largely unknown [4]. The fundamental basis for immune evasion of MPXV also deserves much more study to be fully understood [4]. Specific anti-MPXV antibodies, especially induced by site-directed vaccination, could hamper MPXV binding and infection of host cells by either targeting virus receptors at the host cell surface or by blocking viral molecules that interact with those receptors.

Vaccine strategies to prevent monkeypox infection

To date, there are no specific treatments or vaccines for the prevention of MPXV infection. Bearing in mind that monkeypox and smallpox viruses are genetically similar and expose low polymorphic antigens on their surface, allowing conferment of indirect protection against MPXV infection via cross-reactive antibodies, several countries are currently using antiviral drugs and vaccines that were developed earlier against smallpox as treatment and prevention approaches for MPXV infection [4,5] (Box 1). International organizations for public health monitoring and infection prevention, such as the World Health Organization (WHO), recommend the administration of these vaccines before or after a recent exposure to MPXV to help protect people against the disease and reduce the spread of infection. To date, however, information regarding the efficacy of these vaccines against MPXV remains insufficiently documented [5]. We summarize current vaccination resources for MPXV, propose new avenues for vaccine development, and discuss future resources in development.

Box 1. Antivirals and vaccinia immune globulin intravenous (VIGIV) in monkeypox infection

In general, MPXV-infected patients with intact immune systems recover without strong medication, by only receiving supportive care and pain control. However, depending on the profile of the patient (health status, comorbidities, previous vaccination program), a complementary treatment may be necessary. Several drugs developed to treat smallpox (tecovirimat, brincidofovir, and cidofovir), and VIGIV may be recommended. Although the efficacy of smallpox antivirals has been shown to lower virus levels and reduce clinical symptoms in animal models with monkeypox, data are much more limited in humans, and the results of ongoing clinical trials will not be known for several months. Tecovirimat (ST-246), the only FDA- and European Medicines Agency (EMA)-approved drug for orthopoxvirus infection in humans, is an inhibitor of extracellular virus effects. It interferes with the cellular localization of the major p37 envelope protein, which is necessary for the replication of viral particles; p37 has no homologs among proteins of humans or other mammals. However, much more information is needed with regard to the mechanism of action of the proposed anti-MPXV antivirals. Regarding VIGIV, data are not yet available on their effectiveness for treating MPXV infection. To date, it is unknown whether a person with severe MPXV infection would benefit from treatment with VIGIV.

Current vaccination resources

Today, three vaccines are in use against MPXV [4,5] (Table 1). They have been previously used for protection against smallpox infection. The first one, an attenuated, nonreplicating, smallpox vaccine developed in collaboration with the US government to ensure the supply of a smallpox vaccine for the entire population, including immunocompromised individuals who are not recommended vaccination with traditional replicating smallpox vaccines, is produced by the Bavarian Nordic company and marketed as either Imvanex (Europe), Jynneos (USA), or Imvamune (Canada) [8,9] (see Table 1 for further information). Bearing in mind that this product is a repurposed vaccine, it would require extensive research and even Phase 3 clinical trials for monkeypox efficacy. This vaccine contains a modified smallpox virus hence has been named as modified vaccinia Ankara (MVA), a biological product that has completed Phase 3 clinical trials, and has been approved in different countries. An open-label prospective cohort study including healthcare personnel was performed in DRC. Adult personnel at risk for monkeypox received two doses of attenuated live virus smallpox vaccine. They received a liquid (1000 subjects) or lyophilized formulation (600 subjects) on days 0 and 28 via subcutaneous injection [1×10^8 median tissue culture infectious dose (TCID50) per 0.5 ml]. Blood samples were regularly collected over 24 months. The data regarding efficacy in this study are announced but not yet available in the case of MPXV [10] [for updated information, see clinicaltrials.gov and Centers for Disease Control (CDC)[i]].

Another vaccine called ACAM2000 (Acambis, Inc./Sanofi Pasteur Biologics Co., Cambridge, MA) can be used for the prevention of monkeypox (Table 1). It is a recombinant second-generation vaccine (aimed to reduce the risks of live or attenuated vaccines, consisting of specific protein antigens or recombinant protein components including nucleic acids species), also initially developed for smallpox. As such, it has been recommended for use in the USA for preventing monkeypox infection. However, the US CDC do not recommend it for people with defective health conditions, such as congenital or acquired immune deficiency disorders (especially people with AIDS), diseases affecting skin conditions like atopic dermatitis/eczema, and cardiac diseases; for infants under 12 months of age; or during pregnancy. ACAM2000 is delivered in a single percutaneous dose with a booster dose every 3 years or at least every 10 years. ACAM2000 is a vaccinia virus vaccine derived from a plaque-purified clone of the same New York City Board of Health strain, a smallpox strain that was used to manufacture Dryvax, a related monkeypox

Table 1. Vaccines under study for the prevention of monkeypox virus infection[a]

Name	Type	Other names	Status	Study results for monkeypox	Conditions	Location	URL/Refs
IMVANEX smallpox vaccine in adult healthcare personnel at risk for monkeypox in the DRC; Bavarian Nordic (MVA-BN), Denmark	Live modified vaccinia virus Ankara	Imvanex (Europe) Jynneos (USA) Imvamune (Canada)	Active, not recruiting	No results available	Monkeypox virus infection	Tshuapa, Boende; Tshuapa, DRC	https://ClinicalTrials.gov/show/NCT02977715
ACAM2000; Acambis, Inc./Sanofi Pasteur Biologics Co., Cambridge, MA, USA	Recombinant second-generation vaccine	None	Active, not recruiting	No results available	Monkeypox virus infection	Not known	https://clinicaltrials.gov/ct2/results/details?term=ACAM2000&cond=Monkeypox
LC16m8; KM Biologics, Kumamoto, Japan	Recombinant second-generation vaccine, derived from the Lister strain of the vaccinia virus	None	Active, not recruiting	No results available	Monkeypox virus infection	Discontinued – Phase 1/2 for smallpox in USA	[19]

[a]Information source: https://www.who.int/health-topics/monkeypox/#tab=tab_1 and https://clinicaltrials.gov/ct2/results?cond=Monkeypox&term=vaccine&cntry=&state=&city=&dist=.

vaccine candidate. Data from ACAM2000 clinical trials indicate a safety profile similar to Dryvax, including risk for rare but serious cardiac adverse events [10,11].

A third approved vaccinia vaccine made by KM Biologics (Kumamoto, Japan) is also proposed for monkeypox (Table 1). This vaccine, called LC16m8, is licensed for use in healthy people in Japan, against biological terrorism [8,9]. Approximately 100 000 people have undergone vaccination with this vaccine without experiencing any severe complications. It is an attenuated smallpox vaccine derived from the Lister vaccinia strain. It was developed to lack the B5R envelope protein gene of the vaccinia virus to attenuate its neurotoxicity. It generated **neutralizing antibody** titers to multiple poxviruses, including vaccinia, monkeypox, and variola major, and generated broad T cell responses.

It has been shown that immune response takes 14 days after the second dose of Jynneos and 4 weeks after the ACAM2000 dose for maximal immunity development. No data are yet available on the efficacy of these two vaccines in the current outbreak of monkeypox. Due to uncertainty regarding the efficacy of proposed vaccines toward monkeypox, and problems created by adverse events that have been reported [12], a next generation of safer and specific vaccines is eagerly awaited. Although in the vaccine community some experts claim that developing vaccines with a large spectrum of specificity to multiple diseases can be advantageous (as found with LC16m8, for instance, but also others), past experience has also shown that epitope-focused immunogens are often more effective in boosting subdominant neutralizing antibody responses *in vivo*, resulting in enhanced neutralization.

Monkeypox vaccine candidate development: introducing synthetic peptide-based prototype vaccine formulations

Finding and developing novel vaccines against emergent and transmissible diseases represents huge conceptual and technological challenges. The COVID-19 pandemic demonstrated the complexity of producing safe, specific, and high-efficacy vaccine formulations based on the most used biological platforms, including whole attenuated or killed severe acute respiratory syndrome (SARS)-CoV-2 virus; others made by using parts of the virus such as nonreplicative human or simian adenovirus DNA vectors; and others are based on specific mRNA templates for parts of the viral spike (S) glycoprotein. Current strategies for producing and transporting biological platform-based virus vaccines require highly controlled biosafety laboratories and strict cold chains and packing lines to prevent vaccine alteration and for allowing researchers to work safely. In addition, second-generation vaccines have not been proven to be a reliable pathway toward subunit vaccines due to their low stability and specificity. On the contrary, low-cost, stable, specific, nontoxic, and safe molecules produced by controlled synthesis strategies have become a reality for obtaining vaccine formulations for

multiple lethal diseases, such as malaria and COVID-19 [13,14].

New generation vaccines, especially **synthetic vaccines** and **mRNA vaccines**, are emerging as novel tools for controlling infectious diseases. Hence, some efforts toward site-directed designed synthetic vaccines, especially those based on a rational site-directed selection of relevant epitopes exposed by the virus and their subsequent modification, have proven to be reliable pathways toward more efficient vaccines. Perhaps the first and most representative example of this strategy is a synthetic malaria vaccine proposed in the mid-80s named SPf66 that reached clinical trials [14]. Although this early trial has shown limited effectiveness, it has opened the way to the development of peptide-based synthetic vaccines that have proven to be safe and potentially useful to prevent transmissible diseases.

Our own experience developing synthetic peptide-based prototype vaccine formulations for human life-threatening infectious diseases, such as malaria and COVID-19, has led us to also assess different routes of administration such as intranasal, sub- and transcutaneous, and intramuscular routes for synthetic vaccine candidate formulation for these diseases in animal models. These routes have all proven to be able to stimulate strong humoral and cellular immunity in preclinical studies. An important finding in experiments conducted by us and others is that site-directed designed synthetic epitopes when administered in animal models as vaccine formulations stimulate not only the production of expected antihomologous peptide antibodies but also that of antibodies that cross-react with viral proteins, including antibodies displaying neutralizing properties [13,15]. We have shown that intravenous administration of site-directed antibodies stimulated by synthetic peptides encompassing specific epitopes cleared different circulating malaria pathogen strains in rodents [13], a strategy that could be applied to diseases caused by viruses such as MPXV. Synthetic platforms do not require cold chains since this sort of vaccine product has proven to be highly stable even at room temperature for long time periods.

Using the synthetic approach, we thus developed synthetic formulations of antigenic site-directed polymers on human-compatible adjuvant systems such as Alhydrogel, which stimulated neutralizing antibodies against SARS-CoV-2 and some genetic variants in *in vitro* experiments [16]. In this pursuit, clinical trials have yet to be conducted. This type of vaccine would be applied especially for booster purposes to those persons who have previously received an mRNA vaccine for COVID-19. When properly formulated, synthetic vaccines can be strong stimulators of both cell and humoral immune responses [17]. One could thus consider identifying key dominant epitopes, most preferably neutralizing, of MPXV and design potent site-directed synthetic vaccine formulations containing these immunogenic epitopes and appropriate immune stimulators (adjuvants, excipients, carriers). These formulations can be developed at moderate cost compared with biological vaccines, especially due to low biosafety requirements for production, and have remarkable immune versatility and stability. Identification of several epitopes that may be assembled to design (multi-)epitope vaccines (peptides and mRNA vaccines) could be highly advantageous. Developing such constructs requires identifying and artificially reproducing a variety of surface protein motifs endowed with immunogenic activity. This strategy of associating sequences encompassed in several SARS-CoV-2 proteins (S, E, M, and N) could be exploited in vaccines against MPXV. We suggest here that elements used by the virus to attach to key sites on the cell surface could be prime targets [18]. Induced antibodies generated by vaccination could then block the virus entry and consequently prevent it from spreading.

DNA vaccines to MPXV are in development but not yet validated in humans. For example, Tonix Pharmaceuticals associated with the University of Alberta is heading toward clinical trials with TNX-801, a live vaccine that uses a horsepox virus TNX-801 assembled from synthetic DNA fragments. In non-human primates, TNX-801 blocks the formation of monkeypox lesions, and in mice it is less virulent than old vaccinia strains. In addition to studies based on synthetic peptides, proteins, and DNA, novel vaccines based on mRNA are also being investigated with the experience gained from the new vaccines developed against COVID-19. In this context, Moderna has announced on August 5, 2022 that it has begun a preclinical program investigating possible mRNA vaccines for monkeypox [10].

Concluding remarks

As monkeypox cases rise globally, researchers are learning more about how the disease is transmitted and spreads in the general population. According to the European Centre for Disease Prevention and Control (September 27, 2022), since the start of the monkeypox outbreak in May 2022, 20 083 confirmed cases of monkeypox have been reported from 29 EU/European Economic Area countries. The five countries reporting most cases since the start of the outbreak are Spain (7149), France (3969), Germany (3607), The Netherlands (1223), and Portugal (851). According to the recently published scores from the US CDC (September 29, 2022), monkeypox has spread to 106 countries and led to more than 67 000 confirmed infections worldwide. Because MPXV is closely related to the smallpox virus, vaccines approved to prevent smallpox virus infection are thought to protect 85% of individuals against monkeypox infection [4,10]. This level of protection against monkeypox remains to be confirmed in the general population. Based on recent experience of new vaccines, an arsenal of

possible tools (including DNA and mRNA vaccines and protein or peptide vaccines) should shortly appear for monkeypox, especially synthetic vaccines, as highlighted above, that are endowed with extremely valuable properties in terms of specificity, safety, and effectiveness against transmissible diseases.

Acknowledgments

This study was supported by the ITI 2021-2028 program, University of Strasbourg-CNRS-Inserm, IdEx Unistra (ANR-10-IDEX-0002), and ANR (STRAT'US project, ANR-20-SFRI-0012) to S.M.; S.M. also acknowledges the support of the French Centre National de la Recherche Scientifique (CNRS); the University of Strasbourg Institute for Advanced Study (USIAS); the European Regional Development Fund of the European Union in the context of the INTERREG V Upper Rhine program; the FHU ARRIMAGE and the OMAGE project granted by Region Grand-Est of France and FEDER. Research on SARS-CoV-2 immunoprevention conducted at the Universidad Nacional de Colombia was sponsored by Vicerrectoría de Investigación, information system for research, extension, and laboratory facilities (HERMES; grant numbers 49586, 50090, and 54836) to J.M.L.

Declaration of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could constitute a potential conflict of interest.

Resources

[i]www.cdc.gov/poxvirus/monkeypox/interim-considerations/jynneos-vaccine.html

[1]Universidad Nacional de Colombia-Sede Bogotá, Departamento de Farmacia, Mimetismo molecular de los Agentes infecciosos, Bogotá, DC, Colombia
[2]Centre National de la Recherche scientifique–Université de Strasbourg, Biotechnology and Cell Signalling Unit, Neuroimmunology and Peptide Therapeutics Team, Strasbourg Drug Discovery and Development Institute, Strasbourg, France
[3]University of Strasbourg Institute for Advanced Study, Strasbourg, France
[4]Fédération Hospitalo-Universitaire OMICARE, Fédération de Médecine Translationnelle de Strasbourg, University of Strasbourg, Strasbourg, France

*Correspondence:
jmlozanom@unal.edu.co (J.M. Lozano) and
sylviane.muller@unistra.fr (S. Muller).

https://doi.org/10.1016/j.tips.2022.10.005

References

1. Kaler, J. *et al.* (2022) Monkeypox: a comprehensive review of transmission, pathogenesis, and manifestation. *Cureus* 14, e26531
2. Bunge, E.M. *et al.* (2022) The changing epidemiology of human monkeypox—a potential threat? A systematic review. *PLoS Negl. Trop. Dis.* 16, e0010141
3. Thornhill, J.P. *et al.* (2022) Monkeypox virus infection in humans across 16 countries — April–June 2022. *N. Engl. J. Med.* 387, 679–691
4. Lum, F.M. *et al.* (2022) Monkeypox: disease epidemiology, host immunity and clinical interventions. *Nat. Rev. Immunol.* 22, 597–613
5. Kmiec, D. and Kirchhoff, F. (2022) Monkeypox: a new threat? *Int. J. Mol. Sci.* 23, 7866
6. Karem, K.L. *et al.* (2007) Monkeypox-induced immunity and failure of childhood smallpox vaccination to provide complete protection. *Clin. Vaccine Immunol.* 14, 1318–1327
7. Lant, S. and Maluquer de Motes, C. (2021) Poxvirus interactions with the host ubiquitin system. *Pathogens* 10, 1034
8. Petersen, B.W. *et al.* (2016) 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
9. Yoshikawa, T. *et al.* (2021) A highly attenuated vaccinia virus strain LC16m8-based vaccine for severe fever with thrombocytopenia syndrome. *PLoS Pathog.* 17, e1008859
10. Harrison, C. (2022) Monkeypox response relies on three vaccine suppliers. *Nat. Biotechnol.* 40, 1306–1307
11. Rizk, J.G. *et al.* (2022) Prevention and treatment of monkeypox. *Drugs* 82, 957–963
12. Petersen, B.W. *et al.* (2019) Vaccinating against monkeypox in the Democratic Republic of the Congo. *Antivir. Res.* 162, 171–177
13. Lozano, J.M. *et al.* (2021) The search of a malaria vaccine: the time for modified immuno-potentiating probes. *Vaccines (Basel)* 9, 115
14. Patarroyo, M.E. *et al.* (1988) A synthetic vaccine protects humans against challenge with asexual blood stages of *Plasmodium falciparum* malaria. *Nature* 332, 158–161
15. Beignon, A.S. *et al.* (2005) A peptide vaccine administered transcutaneously together with cholera toxin elicits potent neutralising anti-FMDV antibody responses. *Vet. Immunol. Immunopathol.* 104, 273–280
16. Lozano, J.M. *et al.* (2020) COVID-19 infection detection and prevention by SARS-CoV-2 active antigens: a synthetic vaccine approach. *Vaccines* 8, 692
17. Bonam, S.R. *et al.* (2017) An overview of novel adjuvants designed for improving vaccine efficacy. *Trends Pharmacol. Sci.* 38, 771–793
18. Louten, J. (2016) Poxviruses. In *Essential Human Virology* (Louten, J., ed.), pp. 273–290, Academic Press
19. Kidokoro, M. and Shida, H. (2014) Vaccinia virus LC16m8 as a vaccine vector for clinical applications. *Vaccines* 2, 755–771