BIOLOGICAL SCIENCES

STUDY OF HERPESVIRUSES IN CELL CULTURE AND MECHANISM OF INFECTION IN MODERN RESEARCH

Mammadova V.
Scientific Research Institute of Medical Prophylaxis named after V.Y.Akhundov, Baku,Azerbaijan
Head of the virology laboratory
Alazova K.
Scientific Research Institute of Medical Prophylaxis named after V.Y.Akhundov, Baku,Azerbaijan
Scientist
Ismayilova S.
Scientific Research Institute of Medical Prophylaxis named after V.Y.Akhundov, Baku,Azerbaijan
Senior researcher
Mirzayeva A.
Scientific Research Institute of Medical Prophylaxis named after V.Y.Akhundov, Baku,Azerbaijan
Scientist
Mammadov P.
Scientific Research Institute of Medical Prophylaxis named after V.Y.Akhundov, Baku,Azerbaijan
Senior laboratory assistant
https://doi.org/10.5281/zenodo.18631218

Abstract

Herpesviruses (family *Herpesviridae*) are widespread double-stranded DNA viruses capable of establishing lifelong latent infections and causing diverse clinical manifestations, particularly in immunocompromised individuals. In addition to acute and recurrent infections, certain herpesviruses such as Epstein–Barr virus (EBV) and Kaposi's sarcoma–associated herpesvirus (KSHV) possess oncogenic potential, making them a major focus of modern virological and oncological research. This study provides a comprehensive overview of herpesvirus biology, replication mechanisms, latency, and pathogenesis, with a particular emphasis on in vitro cell culture models and emerging molecular technologies. Various cell lines, including Vero, HEp-2, HeLa, HFF, and L929 fibroblast cells, are discussed as experimental platforms for studying herpesvirus infection, cytopathic effects, and antiviral susceptibility. Experimental findings from L929 fibroblast cells inoculated with HSV-2 demonstrate characteristic stages of cytopathic effects, highlighting the suitability of cell culture systems for visualizing viral replication dynamics. The article also reviews current diagnostic approaches, including PCR-based molecular methods, serological assays, and immunohistochemical techniques, which are critical for detecting both active and latent infections. Furthermore, the potential of CRISPR-Cas9 technology as a novel antiviral strategy targeting latent herpesvirus genomes is critically evaluated. Although conventional antiviral therapies effectively suppress viral replication, they fail to eliminate latent infection. CRISPR-based genome editing offers a promising avenue for achieving functional cure, despite existing technical and bioethical challenges. Overall, this study emphasizes the importance of integrating cell culture models, molecular diagnostics, and advanced genetic technologies to improve the understanding, diagnosis, and treatment of herpesvirus infections.

Keywords: Herpesviruses, latent infection, CRISPR-Cas9, cell culture, viral replication, molecular diagnostics, antiviral treatment.

Introduction:

Herpesviruses (family Herpesviridae) are large-genome, double-stranded DNA viruses widely distributed in humans and animals. They are of special importance in virology because they are distinguished by their ability to reactivate latency and produce different clinical manifestations. Herpesviruses can remain latent in the cells of the body, creating lifelong infections. These viruses are acute infections that damage various organ systems. When the immune system is weakened, they have the ability to cause severe complications [1]. Herpesviruses not only cause infection, some types (eg, EBV and KSHV) can cause oncogenic transformation in humans [2]. In this regard, herpesviruses are not only infectious, but also the subject of oncological research. The spread of herpesviruses at the global level and the social and economic impact of the diseases caused by them increase the relevance of the research conducted in the direction of the pathogenesis and treatment of these viruses [3]. Herpesviridae family represents a large and complex group in virological taxonomy. Viruses included in this family are divided into three main families:

1) Alphaherpesvirinae 2) Betaherpesvirinae 3) Gammaherpesvirinae [4].

Alphaherpesvirinae: This group includes Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2), and Varicella-zoster virus (VZV). These viruses are characterized by fast replication and mainly create latency in nervous tissues. HSV-1 mainly causes oral and labial lesions, while HSV-2 causes genital herpes. VZV is the causative agent of chicken pox and shingles [5]. Betaherpesvirinae: This group includes Cytomegalovirus (CMV), Human herpesvirus 6 (HHV-6), and Human herpesvirus 7 (HHV-7). These viruses replicate slowly in cell culture and remain mostly latent

in immune cells. CMV can cause severe infections in those with weakened immune systems (eg, post-transplant or HIV patients) [6]. Gammaherpesvirinae: Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV, HHV-8) are included in this group. Gammaherpesviruses show a tropism mainly to lymphoid cells and create long-term latency. EBV is the main etiological factor of infectious mononucleosis and some lymphomas, and KSHV is the main etiological factor of Kaposi's sarcoma [7]. Genomes of herpesviruses consist of double-stranded DNA and range in size from 120–240 kbp. The genome contains a variety of structural and regulatory genes and encodes more than 70 proteins. These proteins play an important role in virus replication, synthesis of structural components, evasion of the body's immune response, and latency mechanisms [8]. Viruses pass through the cell wall membrane and replicate in the cell nucleus. The mechanism of infection and pathogenesis of herpesviruses is based on the type of virus, the site of infection and the immune state of the body. Infection can occur in different ways: skin and mucous membrane contact (HSV-1, HSV-2, VZV), oral, airborne or sexual (EBV, CMV); Transplantation or transplacental infection (CMV);

The virus initially enters epithelial cells, where rapid replication begins. Alphaherpesviruses enter the transmission nerves and reach the nerve ganglia by retrograde flow, where they take a latent state. For example, HSV may remain latent in the trigeminal or sacral ganglia, and VZV may remain latent in the dorsal root ganglia. During the latency period, the viral genome remains in the cell nucleus in an episomal form and does not show any clinical symptoms. Due to the weakening of the immune system or the influence of factors such as stress, infection, UV radiation, the virus is activated and may manifest itself with symptoms at the site of the previous infection or in new areas [9]. Betaherpesviruses maintain latency mainly in monocytes and lymphocytes, while gammaherpesviruses can remain latent in B-lymphocytes. The pathogenesis of herpesviruses is not limited to cell lysis and inflammatory reactions. Some herpesviruses can also cause the formation of tumor cells by manipulating genes that play an important role in regulating the cell cycle. For example, EBV can lead to Hodgkin and non-Hodgkin lymphomas by causing B-cell immortalization. KSHV is the etiological factor of Kaposi's sarcoma and some rare lymphoproliferative diseases [10].

Clinical signs of herpesviruses are diverse and vary depending on the type of virus, the body's immune system, and the location of the infection.

HSV-1 and HSV-2: HSV-1 causes infections involving the mouth, lips, eyes, and brain. The most common form is labial herpes (cold sore). HSV-2 is the main cause of genital herpes and is sexually transmitted. It can also cause neonatal herpes in newborns [11].

VZV (Varicella-Zoster Virus): Primary infection presents as chicken pox (varicella). During reactivation, shingles (herpes zoster) appear - this manifests as a painful, blistering rash along the nerve [12, 13].

CMV (Cytomegalovirus): Is asymptomatic in those with a healthy immune system, but can sometimes present with mononucleosis-like symptoms. In immunosuppressive patients, it can cause severe pneumonia, retinitis, hepatitis and brain pathologies. If transferred to the fetus during pregnancy, it can cause severe abnormalities before or after birth [14].

EBV (Epstein-Barr Virus): It is the main causative agent of infectious mononucleosis ("kissing disease"). It has oncogenic potential during long-term latency: Burkitt's lymphoma, nasopharyngeal carcinoma, etc. [15].

KSHV (Kaposi Sarcoma Herpesvirus): Can cause Kaposi's sarcoma, primary effusion lymphoma, and Multicentric Castleman's disease in immunocompromised individuals (especially AIDS patients) [16].

Diagnosis of herpesviruses is determined by several methods. These include -Virus isolation and cell culture, PCR and real-time PCR - provide high accuracy in the detection of viral DNA, Serological tests (ELISA, Western blot) - determine the phase of infection by detecting antibodies, Immunohistochemistry and in situ hybridization - used to detect viral antigens and genome in tissues [17].

The main goal in the treatment of herpesvirus infections is to stop the replication of the virus, relieve symptoms and prevent reactivation. The main antiviral drugs currently used against herpesviruses target the DNA polymerase enzyme of the virus [18].

In recent years, it has become possible to target specific regions of the herpesvirus genome with CRISPR-Cas9 technology. This is a promising approach for complete eradication of latent infection [19]. At the same time, work is being done in the direction of delivery of antiviral drugs, immunotherapy and antiviral vaccines with nanoparticles. Currently, only an effective vaccine against VZV is available. Extensive research is being done on the development of vaccines against other herpesviruses.

Varicella vaccine (Varivax): -Used to prevent chicken pox infection in children. Live attenuated virus based. Zoster vaccine (Shingrix, Zostavax): Used to prevent shingles in the elderly. Shingrix is a recombinant non-enveloped protein-based vaccine that is more effective and less risky [20]. Vaccination against HSV-2: Vaccines in Phase III clinical trials, especially vaccines based on the gD2 antigen, show promising results in generating an immune response [21].

CMV vaccine: Research is being done on vaccines containing different antigens that have a synergistic effect. It is critical for pregnant women and transplant patients. The development of effective vaccines is complicated by the latent capacity of herpesviruses and their immune evasion strategies. In the future, the introduction of vaccines based on mRNA technology may be a turning point in this field. In vitro cell culture is the main experimental platform for the study of herpesvirus biology, pathogenesis, and antiviral therapies. This method is widely used to analyze viral replication mechanisms, evaluate the effectiveness of antiviral drugs, and test new vaccine designs. Advantages of cell culture: It is possible to visualize the stages of the viral replication cycle. The degree of resistance of viruses to antiviral drugs can be measured. Mutant forms of viruses can be studied by genetic modification. Latent infection models are difficult to establish. The complexity of the immune system cannot be fully replicated in

cell culture. Some types of herpesviruses are activated only in certain cell types (eg B-lymphocytes for EBV). For this reason, 3D organelle models and mouse models have recently been used as complementary tools in laboratory research [22].

Cell culture is a method of growing cells in the laboratory, used in various research and experiments. In this process, cells are grown and propagated in a special nutrient medium [23].

For the study of herpesviruses in laboratory conditions, their reproduction in appropriate cell culture is one of the main research methods. Because herpesviruses have a broad cell tropism, they can be grown successfully in a number of cell lines. The cell lines used are: Vero cells (African green monkey kidney cells): Widely used to study HSV-1 and HSV-2. HEp-2 and HeLa cells: Epithelial cells of human origin, suitable for observation of cytopathic effects.

HFF (Human Foreskin Fibroblasts): Optimal for herpesviruses such as CMV and HHV-6. ARPE-19 and RPE-1: Retinal pigment epithelial cells are used to investigate ocular types of CMV and HSV [24].

For this purpose, cell lines such as Vero, HEp-2, HeLa, MRC-5 are widely used [25].

Materials and Methods

Materials obtained in our laboratory:

The L929 fibroblast cell line was obtained from a cryo bank. Cells were maintained in pre-prepared nutrient media: DMEM-10% FBS and supplemented with RPIM-10% FBS. Passage process was performed after rapid proliferation of cells was observed. During passaging, cells were detached with trypsin and transferred to new flasks. A part of the passaged cells was filled in cryotubes and frozen for future studies. During the freezing process, 10% DMSO was added to the nutrient medium. Cells were first stored at -80°C, then placed in a liquid nitrogen tank for long-term preservation. Virus inoculation: HSV-2 IgM positive serum obtained from a clinical laboratory was subjected to high-speed centrifugation (16000 rpm) and inoculated into L929 fibroblast cells. Morphological changes in the cells were observed under a microscope.

Changes observed as a result of HSV-2 inoculation:

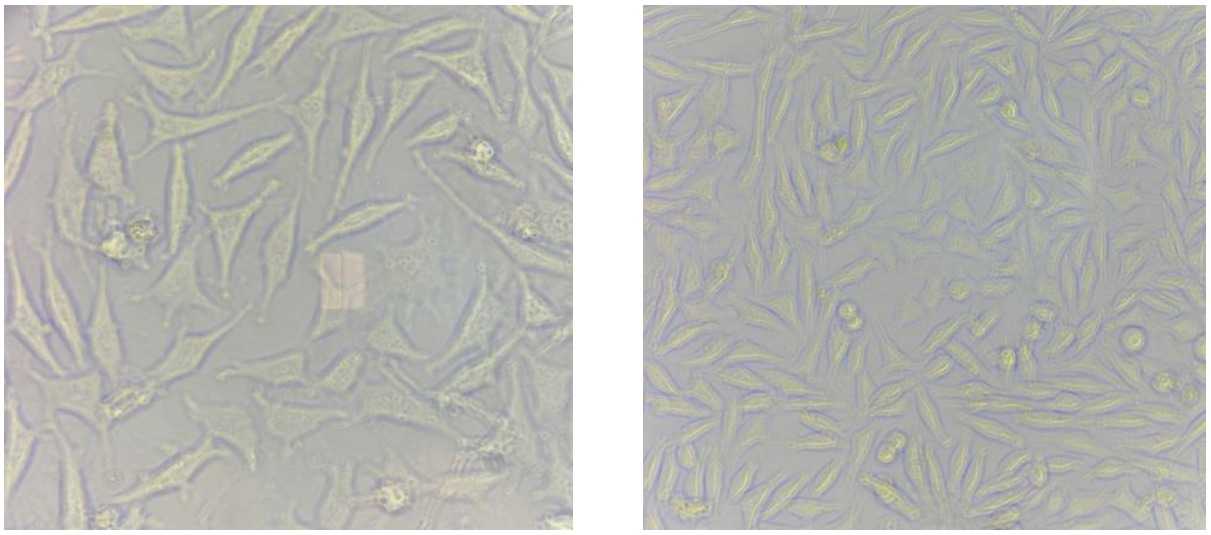
L929 fibroblast cells were observed daily after inoculation with HSV-2. Morphological changes were recorded in the following sequence:

Early stage: Cells were in normal fibroblast morphology and maintained their needle shape. No pathological changes were recorded at this stage.

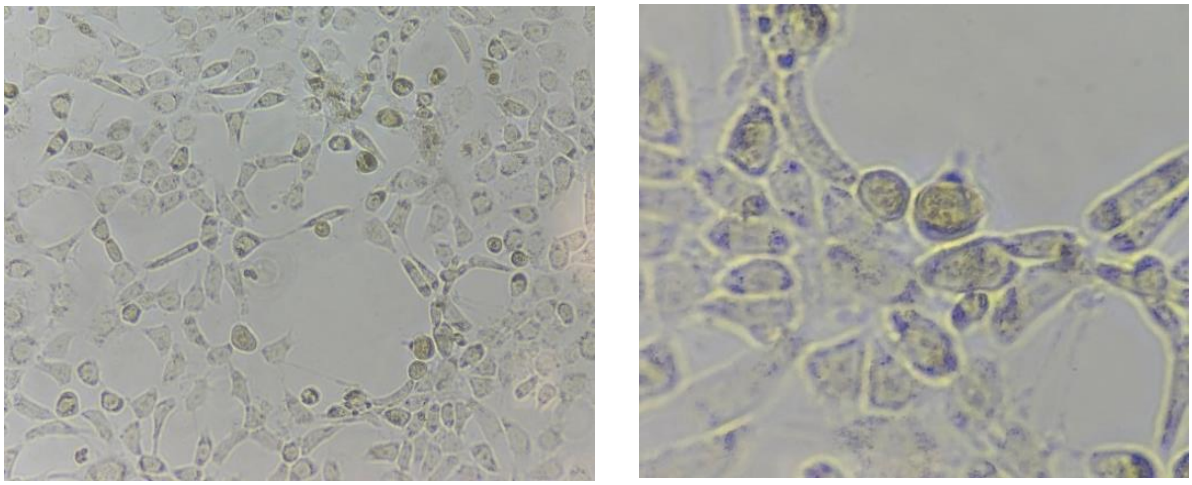
Initial cytopathic effect: Aggregation and round formation of cells were observed. The cells came close to each other and formed small clusters. This stage marked the beginning of cell-to-cell transmission of HSV-2.

Middle stage: The morphology of the cells has changed dramatically. They became round and swollen, the cytoplasm became transparent, and the cells stuck together to form large clusters. Cells that lost contact with the substrate were observed as floating cells. These changes reflected intensive reproduction of the virus and disruption of cell membrane structures.

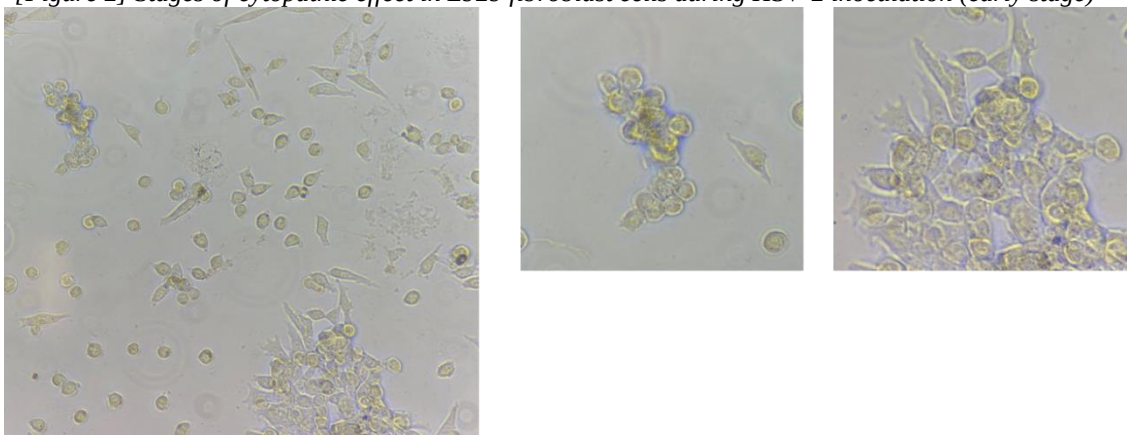
The last stage: The membrane of the cells completely collapsed and the process of fragmentation was observed. This phase was considered the terminal phase of the cytopathic effect. As a result, the majority of L929 fibroblast cells consisted of dead cells.



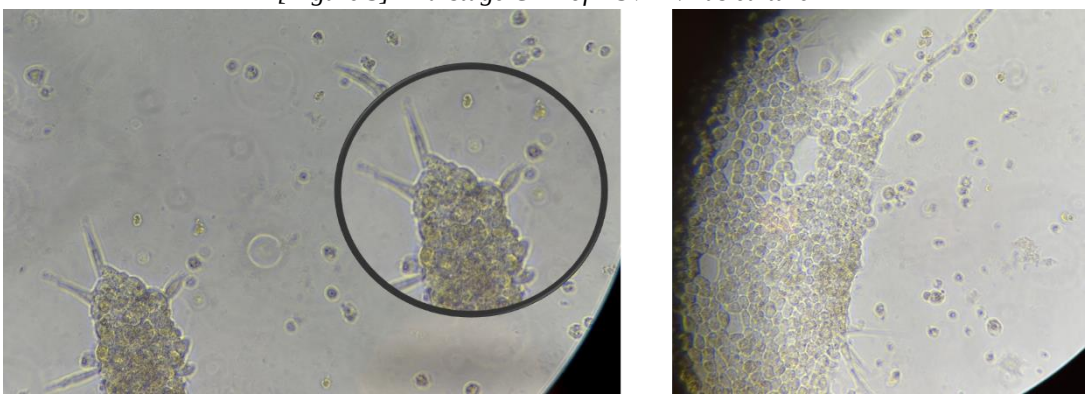
[Figure 1] L929 fibroblast cells microscopic view



[Figure 2] Stages of cytopathic effect in L929 fibroblast cells during HSV-2 inoculation (early stage)



[Figure 3] Mid-stage CPE of HSV-2 virus culture



[Figure 4] Final stage of CPE of HSV-2 virus culture

Cytopathic effects (CPE) caused by herpesviruses in cells are observed under a microscope. These include changes in cell morphology, the formation of syncytiums (the fusion of several cell cytoplasms to form a multinucleated structure), and cell lysis. These symptoms are one of the main indicators of the presence and intensity of infection. After the incubation period, the presence and quantity of the virus is determined by various methods. Plaque assay, $TCID_{50}$ (50% tissue culture infection dose), Immunofluorescence, PCR analysis are included [26, 27]. Application of CRISPR-Cas Technology Against Herpesviruses - The fact that herpesviruses create a latent infection and do not respond to conventional antiviral treatment in this case makes CRISPR-Cas9 technology promising in this field. With this technology, the replication or latent form of the virus can be disabled by targeting specific regions of the viral genome. The main directions of application are as follows:

Cleavage of the latent viral genome: The latent form of HSV-1 and CMV can be eliminated by cutting from the genome using CRISPR-Cas9 [28]. Inactivation of viral genes: Viral activity is reduced by targeting key replication genes such as UL29, UL30 (HSV) and IE genes (CMV). Enhancement of the immune response: Genes that enhance the immune response can be activated in cells through the CRISPR-system. Changes can occur in unwanted regions of the genome. Mechanisms of viral resistance to the Cas system: Eva-

sion of CRISPR targets is possible as a result of mutations. Effective delivery system: AAV vectors and nanoparticle-based systems are currently under testing [29]. Despite all these reasons, the CRISPR-Cas system is considered one of the most promising technologies for the complete elimination of herpesviruses. Research conducted in recent years provides a deeper understanding of viral replication mechanisms, molecular basis of latent infection, and interactions with the immune system. Cell culture, molecular analysis and new genetic technologies, especially the CRISPR-Cas9 system, have opened up new opportunities in the fight against herpesviruses. However, it is still not possible to completely eliminate the latent form of herpesviruses. Currently used antiviral drugs mainly target the replication phase and are ineffective against latent infection. In the future, the use of technologies such as CRISPR-Cas to target viral genomes may revolutionize this field. Also, it is important to conduct more research on immune evasion mechanisms, genetic variations and drug resistance of viruses [30]. The results of these studies will play a key role in the development of both diagnostic and new antiviral strategies. In recent years, the CRISPR-Cas system, considered a revolutionary technology in the field of genetic engineering, has shown promising results in the study and treatment of many viral infections, including herpesviruses. This system enables genetic modification by recognizing and cutting specific DNA sequences in a targeted manner [31].

CRISPR-Cas technology makes it possible to completely destroy or prevent the activation of herpesviruses by targeting the genome of latently hidden herpesviruses in the cell. This is especially relevant for Herpes Simplex Virus 1 and 2 (HSV-1 and HSV-2), Cytomegalovirus (CMV) and Epstein-Barr Virus (EBV) [32].

Because infections caused by herpesviruses have a wide clinical spectrum, accurate and rapid diagnosis is important. Molecular diagnostic methods are considered the most reliable approach to detect the presence of the virus, especially in latent or asymptomatic cases. Among these methods, the polymerase chain reaction (PCR) takes the main place [33].

PCR (Polymerase Chain Reaction)-PCR amplifies the genomic material of herpesviruses, allowing detection of even very low amounts of the virus. This method is used for the identification of viruses such as: HSV-1, HSV-2, CMV, EBV, HHV-6/7, VZV. Applied in both qualitative and quantitative (real-time PCR) formats, PCR-based technologies also allow determining virus genotypes and detecting drug resistance [34].

Conclusion and discussion

Herpesviruses are viruses that are widespread, manifest themselves with different clinical signs and cause lifelong latent infections. Viruses such as HSV-1, HSV-2, VZV, CMV, EBV, HHV-6, HHV-7, and HHV-8 pose serious risks to human health, especially in immunocompromised individuals and can cause complications and sometimes oncogenic consequences [35]. As can be seen from the data presented in the article, the study of herpesviruses requires a multidisciplinary approach. The genetic structure of viruses, replication mechanisms, latency strategies, and interactions between the body and the immune system should be thoroughly investigated. In recent years, advances in molecular biology, cell culture, CRISPR-Cas technology, molecular diagnostics, and vaccine research have led to a better understanding of herpesviruses and the development of targeted approaches [36–37].

Although the CRISPR-Cas system offers a promising approach for targeting latent forms of herpesviruses and removing them from the genome, the clinical application of this technology is still at an early stage, and bioethical, technical, and safety issues still require serious investigation [38].

New technologies—particularly mRNA, CRISPR, and NGS—may enable the development of more specific, effective, and personalized approaches to herpesviruses. Future studies should focus on viral latency mechanisms, mutational profiles and immune evasion strategies.

References:

1. Whitley RJ, Roizman B. Herpes simplex virus infections. *Lancet*. 2001;357(9267):1513–1518.

2. Cesarman E. Kaposi sarcoma–associated herpesvirus: pathogenesis and treatment. *J Clin Invest*. 2016;126(9):3165–3175.

3. Looker KJ, Magaret AS, Turner KM, et al. Global estimates of prevalent and incident HSV-2 infection. *PLoS One*. 2015;10(1): e0114989.

4. Pellet PE, Roizman B. The family *Herpesviridae*: a brief introduction. In: *Fields Virology*. 6th ed. 2013. p. 1802–1822.

5. Arvin AM, Gilden D. Varicella-zoster virus. *Clin Microbiol Rev*. 2013;26(4):728–743.

6. Cheeran MCJ, Lokensgard JR, Schleiss MR. Neuropathogenesis of congenital CMV infection. *Clin Microbiol Rev*. 2009;22(1):99–126.

7. Kenney SC, Mertz JE. Regulation of the latent–lytic switch in Epstein–Barr virus. *Semin Cancer Biol*. 2014; 26:60–68.

8. Roizman B, Knipe DM. Herpes simplex viruses and their replication. In: *Fields Virology*. 6th ed. 2013. p. 1823–1897.

9. Komatsu T, Nagata K, Wodarz D, et al. Herpesvirus latency and host immune response. *Curr Opin Virol*. 2017; 23:56–61.

10. Gantt S, Casper C. HHV-8–associated neoplasms. *Curr Opin Infect Dis*. 2011;24(4):295–301.

11. James SH, Kimberlin DW. Neonatal herpes simplex virus infection. *Infect Dis Clin North Am*. 2015;29(3):391–400.

12. Gershon AA, Breuer J, Cohen JI, et al. Varicella zoster virus infection. *Nat Rev Dis Primers*. 2015; 1:15016.

13. Gilden D, Nagel MA, Cohrs RJ, et al. Varicella zoster virus in the nervous system. *F1000Research*. 2015; 4:1356.

14. Pass RF. Cytomegalovirus. In: *Remington and Klein's Infectious Diseases of the Fetus and Newborn Infant*. 7th ed. 2011.

15. Kang M, Li Y, Zhao Y, et al. EBV-associated gastric carcinoma. *Cancer Lett*. 2019; 461:237–246.

16. Ablashi DV, Salahuddin SZ, Josephs SF, et al. HHV-6 associated lymphoproliferative disorders. *JAMA*. 1988;260(19):3144–3148.

17. Li Q, Ding X, Lin A, Liu Y. Diagnosis and therapy of CMV infection. *Virol Sin*. 2020;35(2):172–179.

18. Whitley RJ. Antiviral therapy of herpesvirus infections. *Antiviral Res*. 2006;71(2–3):134–145.

19. van Diemen FR, Kruse EM, Hooykaas MJG, et al. CRISPR-Cas9 antiviral immunity. *Nat Microbiol*. 2016; 1:16004.

20. Cunningham AL, Lal H, Kovac M, et al. Herpes zoster subunit vaccine efficacy. *N Engl J Med*. 2016; 375:1019–1032.

21. Belshe RB, Leone PA, Bernstein DI, et al. HSV vaccine trial. *N Engl J Med*. 2012;366(1):34–43.

22. Watanabe T, Bareno G, Kawai Y, et al. 3D organoids for herpesvirus infection. *Nat Biomed Eng*. 2021;5(9):876–889.

23. Freshney RI. *Culture of Animal Cells*. 7th ed. Wiley; 2016.

24. Wang Y, Su C, Yin X, et al. HSV-1 infection of Vero cells. *Virol J*. 2020; 17:22.

25. Smith JS, Robinson NJ. Global HSV prevalence. *J Infect Dis*. 2002;186(Suppl 1): S3–S28.

26. Freshney RI. Subculture and cryopreservation of cells. *Methods Mol Biol*. 2010; 731:1–18.

27. Roehm PC, Shekarabi M, Wollebo HS, et al. CRISPR inhibition of HSV-1. *Gene Ther*. 2016;23(7):540–546.

28. Corey L, Wald A. Genital herpes. In: *Sexually Transmitted Diseases*. 4th ed. 2008.

29. Smith KO, Kennell WL. CPE in viral infections. *J Virol Methods*. 1981;2(4):219–226.

30. Flint SJ, Enquist LW, Racaniello VR, Skalka AM. *Principles of Virology*. 4th ed. ASM Press; 2015.

31. Hierholzer JC, Killington RA. Virus isolation and quantitation. In: *Virology Methods Manual*. 1996.

32. Xu X, Wan Y, Hu P, et al. CRISPR/Cas9 against latent herpesviruses. *Trends Microbiol*. 2022;30(4):284–296.

33. Joung J, Konermann S, Gootenberg JS, et al. Genome-scale CRISPR screening. *Nat Protoc*. 2017;12(4):828–863.

34. Collins-McMillen D, Buehler J, Peppenelli M, Goodrum F. CMV latency regulation. *Viruses*. 2018;10(8):444.

35. Doudna JA, Charpentier E. CRISPR-Cas9 genome editing. *Science*. 2014;346(6213):1258096.

36. Ehrlich ES, Yu X, Ragheb JA. HSV latency regulation. *PLoS Pathog*. 2020;16(3): e1008366.

37. Mackay IM. Real-time PCR in virology. *Nucleic Acids Res*. 2004;32(4):1292–1305.

38. Johnston C, Gottlieb SL, Wald A. HSV vaccine development status. *Vaccine*. 2016;34(26):2948–2952.