Medical Virology for 2nd Year M.D. Students

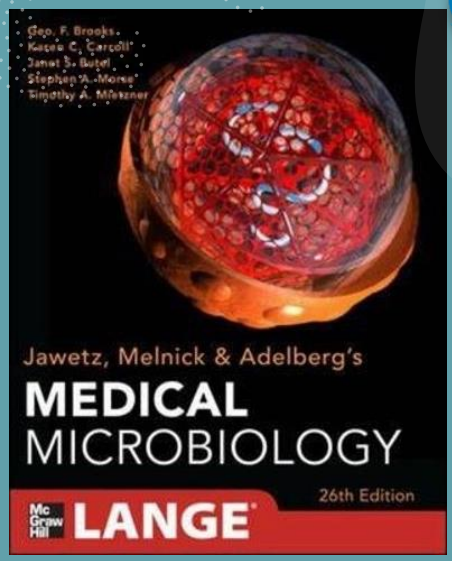
Herpesviruses
Part I

University of Jordan
School of Medicine
Section of Microbiology & Immunology
Malik Sallam, M.D., Ph.D.

1

1

Important Note:
The Required Material for the Exams:
Section IV (Virology) in the provided textbook: Jawetz Medical Microbiology



References

- Jawetz, Melnick & Adelberg's Medical Microbiology. 26th edition. New York : London: McGraw-Hill Medical ; McGraw-Hill, 2013.
- Recent original and review articles in high impact and well renowned scientific journals

2

2

Herpesviruses - Important properties

Virion: Spherical, 150–200 nm in diameter (icosahedral)

Genome: Double-stranded DNA, linear, 125–240 kbp, reiterated sequences

Proteins: More than 35 proteins in virion

Envelope: Contains viral glycoproteins, Fc receptors

Replication: Nucleus, bud from nuclear membrane

Outstanding characteristics:

- Encode many enzymes
- Establish latent infections
- Persist indefinitely in infected hosts
- Frequently reactivated in immunosuppressed hosts
- Some cause cancer

3

3

Herpesviruses – Structure and Composition

- Different members of the group share architectural details and are indistinguishable by electron microscopy
- All herpesviruses have a core of double-stranded DNA, in ring form, surrounded by a protein coat that exhibits icosahedral symmetry and has 162 capsomeres.
- The nucleocapsid is surrounded by an envelope that is derived from the nuclear membrane of the infected cell and contains viral glycoprotein spikes about 8 nm long.
- An amorphous, sometimes asymmetric structure between the capsid and envelope is designated the tegument.

4

Herpesviruses – Structure and Composition

- The enveloped form measures 150–200 nm; the "naked" virion, 125 nm.
- The double-stranded DNA genome (125–240 kbp) is linear.
- There is little DNA homology among different herpesviruses except for HSV-1 and HSV-2, which show 50% sequence homology, and human herpesviruses 6 and 7 (HHV-6 and HHV-7), which display limited (30–50%) sequence homology.
- Treatment with restriction endonucleases yields characteristically different cleavage patterns for herpesviruses and even for different strains of each type. This "fingerprinting" of strains allows epidemiologic tracing of a given strain.

5

Herpesviruses – Structure and Composition

- The herpesvirus genome is large and encodes at least 100 different proteins. Of these, more than 35 polypeptides are involved in the structure of the virus particle; at least 10 are part of the viral envelope.
- The herpesvirus genome is large and encodes at least 100 different proteins. Of these, more than 35 polypeptides are involved in the structure of the virus particle; at least 10 are part of the viral envelope.
- There is little antigenic relatedness among members of the herpesvirus group. Only HSV-1 and HSV-2 share a significant number of common antigens. HHV-6 and HHV-7 exhibit a few cross-reacting epitopes.

6

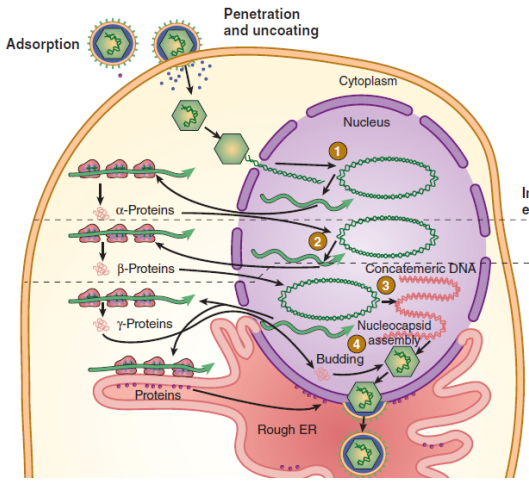
Classification of Human Herpesviruses

Subfamily ("-herpesvirinae")	Biologic Properties: Growth Cycle and Cytopathology	Biologic Properties: Latent Infections	Genus ("-*virus*")	Examples: Official Name ("Human Herpesvirus")	Examples: Common Name
Alpha	Short, cytolytic	Neurons	*Simplex*	1	Herpes simplex virus type 1
				2	Herpes simplex virus type 2
			Varicello	3	Varicella-zoster virus
Beta	Long, cytomegalic	Glands, kidneys	*Cytomegalo*	5	Cytomegalovirus
	Long, lymphoproliferative	Lymphoid tissue	*Roseolo*	6	Human herpesvirus 6
				7	Human herpesvirus 7
Gamma	Variable, lymphoproliferative	Lymphoid tissue	*Lymphocrypto*	4	Epstein-Barr virus
			Rhadino	8	Kaposi sarcoma-associated herpesvirus

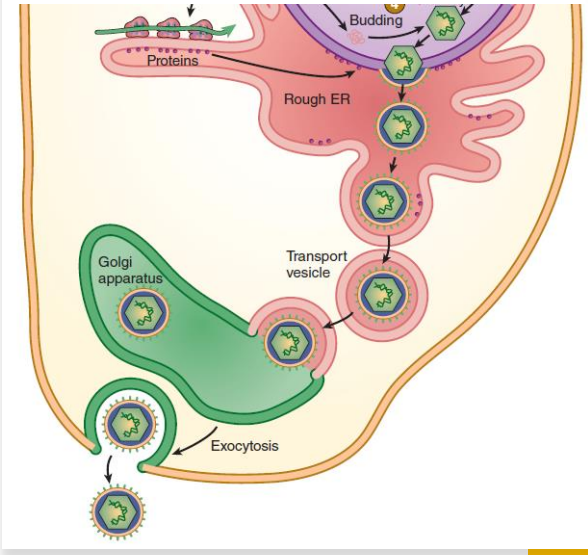
7

7

Herpesvirus Replication



Herpesvirus Replication



9

Herpesvirus Replication

- The virus enters the cell by fusion with the cell membrane after binding to specific cellular receptors via envelope glycoproteins.
- **Several herpesviruses bind to cell surface glycosaminoglycans, principally heparan sulfate**. Virus attachment also involves binding to one of several coreceptors (e.g., members of IgSF).
- After fusion, the capsid is transported through the cytoplasm to a nuclear pore, uncoating occurs, and the DNA becomes associated with the nucleus.
- The viral DNA forms a circle immediately upon release from the capsid. Expression of the viral genome is tightly regulated and sequentially ordered in a cascade fashion.

Herpesvirus Replication

- The tegument protein VP16, complexes with several cellular proteins and activates initial viral gene expression.
- Immediate-early genes are expressed, yielding "α" proteins.
- These proteins permit expression of the early set of genes, which are translated into "β" proteins.
- Viral DNA replication begins, and late transcripts are produced that give rise to "γ" proteins.
- More than 50 different proteins are synthesized in herpesvirus-infected cells.
- Many α and β proteins are enzymes or DNA-binding proteins; most of the γ proteins are structural components.

11

Herpesvirus Replication

- Viral DNA is transcribed throughout the replicative cycle by cellular RNA polymerase II but with the participation of viral factors.
- Viral DNA is synthesized by a rolling-circle mechanism.
- Herpesviruses differ from other nuclear DNA viruses in that they encode a large number of enzymes involved in DNA synthesis.
- Newly synthesized viral DNA is packaged into preformed empty nucleocapsids in the cell nucleus.
- Maturation occurs by budding of nucleocapsids through the altered inner nuclear membrane. Enveloped virus particles are then transported by vesicular movement to the surface of the cell.

12

12

Herpesvirus Replication

- The length of the replication cycle varies from about 18 hours for HSV to more than 70 hours for CMV.
- Cells productively infected with herpesviruses are invariably killed.
- Host macromolecular synthesis is shut off early in infection; normal cellular DNA and protein synthesis virtually stop as viral replication begins.
- Cytopathic effects induced by human herpesviruses are quite distinct (swelling rounding, multinucleated giant cell containing acidophilic intranuclear inclusions.
- The number of potential protein-coding open-reading frames in herpesvirus genomes ranges from about 70 to more than 200.

13

13

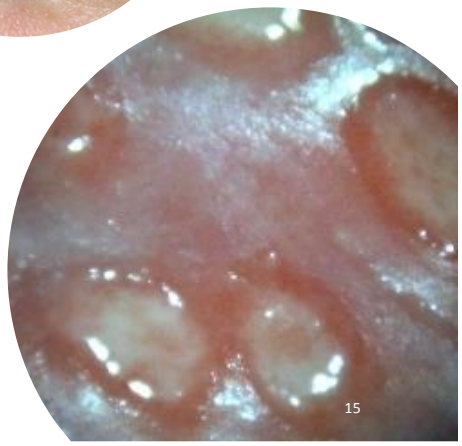
Herpesvirus Replication

- Herpesviruses have recently been found to express multiple microRNAs, small (~22 nucleotides) single-stranded RNAs that function post-transcriptionally to regulate gene expression.
- It is predicted that these viral microRNAs are important in regulating entry into or exit from (or both) the latent phase of the virus life cycle and may be attractive targets for antiviral therapy.

14

Herpesvirus Simplex Viruses - Overview

- HSV are extremely widespread humans.
- They exhibit a broad host range, being able to replicate in many types of cells and to infect many different animals.
- They grow rapidly and are highly cytolytic.
- The HSVs are responsible for a spectrum of diseases, ranging from gingivostomatitis to keratoconjunctivitis, encephalitis, genital disease, and infections of newborns.
- The HSVs establish latent infections in nerve cells; recurrences are common.



15

Comparison of Herpes Simplex Virus Types 1 and 2

Characteristics	HSV-1	HSV-2
Biochemical		
Viral DNA base composition (G + C) (%)	67	69
Buoyant density of DNA (g/cm³)	1.726	1.728
Buoyant density of virions (g/cm³)	1.271	1.267
Homology between viral DNAs (%)	~50	~50
Biologic		
Animal vectors or reservoirs	None	None
Site of latency	Trigeminal ganglia	Sacral ganglia
Epidemiologic		
Age of primary infection	Young children	Young adults
Transmission	Contact (often saliva)	Sexual

16

Comparison of Herpes Simplex Virus Types 1 and 2

Characteristics	HSV-1	HSV-2
Clinical		
Primary infection:		
Gingivostomatitis	+	–
Pharyngotonsillitis	+	–
Keratoconjunctivitis	+	–
Neonatal infections	±	+
Recurrent infection:		
Cold sores, fever blisters	+	–
Keratitis	+	–
Primary or recurrent infection:		
Cutaneous herpes		
Skin above the waist	+	±
Skin below the waist	±	+
Hands or arms	+	+
Herpetic whitlow	+	+
Eczema herpeticum	+	–
Genital herpes	±	+
Herpes encephalitis	+	–
Herpes meningitis	±	+

17

Properties of the Herpes Simplex Viruses

- There are two distinct HSVs: types 1 and 2 (HSV-1 and HSV-2)
- Their genomes are similar in organization and exhibit substantial sequence homology. However, they can be distinguished by sequence analysis or by restriction enzyme analysis of viral DNA.
- The two viruses cross-react serologically, but some unique proteins exist for each type.
- They differ in their mode of transmission. Whereas HSV-1 is spread by contact, usually involving infected saliva, HSV-2 is transmitted sexually or from a maternal genital infection to a newborn. This results in different clinical features of human infections.

18

18

Properties of the Herpes Simplex Viruses

- The HSV growth cycle proceeds rapidly, requiring 8–16 hours for completion.
- The HSV genome is large (~150 kbp) and can encode at least 70 polypeptides; the functions of many of the proteins in replication or latency are not known.
- At least eight viral glycoproteins are among the viral late gene products.
- One (gD) is the most potent inducer of neutralizing antibodies.
- Glycoprotein C is a complement (C3b)-binding protein, and gE is an Fc receptor, binding to the Fc portion of immunoglobulin G (IgG).
- Glycoprotein G is type specific and allows for antigenic discrimination between HSV-1 (gG-1) and HSV-2 (gG-2).

19

Pathogenesis and Pathology the Herpes Simplex Viruses

- Because HSV causes cytolytic infections, pathologic changes are due to necrosis of infected cells together with the inflammatory response.
- Lesions induced in the skin and mucous membranes by HSV-1 and HSV-2 are the same and resemble those of VZV.
- Changes induced by HSV are similar for primary and recurrent infections but vary in degree, reflecting the extent of viral cytopathology.
- Characteristic histopathologic changes include ballooning of infected cells, production of Cowdry type A intranuclear inclusion bodies, margination of chromatin, and formation of multinucleated giant cells.
- Cell fusion provides an efficient method for cell-to-cell spread of HSV, even in the presence of neutralizing antibody.

20

Pathogenesis and Pathology the Herpes Simplex Viruses

- In primary infection, the virus must encounter mucosal surfaces or broken skin for an infection to be initiated (unbroken skin is resistant)
- Viral replication occurs first at the site of infection. Virus then invades local nerve endings and is transported by retrograde axonal flow to dorsal root ganglia, where, after further replication, latency is established
- Whereas oropharyngeal HSV-1 infections result in latent infections in the trigeminal ganglia, genital HSV-2 infections lead to latently infected sacral ganglia.
- Viremia is more common during primary HSV-2 infections than during HSV-1 infections.
- Primary HSV infections are usually mild; (mostly asymptomatic). Only rarely does systemic disease develop.

21

21

Pathogenesis and Pathology the Herpes Simplex Viruses

- In latent infection, the virus resides in latently infected ganglia in a non-replicating state; only a very few viral genes are expressed.
- **Viral persistence in latently infected ganglia lasts for the lifetime of the host**.
- No virus can be recovered between recurrences at or near the usual site of recurrent lesions.
- Provocative stimuli can reactivate virus from the latent state, including axonal injury, fever, physical or emotional stress, and exposure to ultraviolet light.
- The virus follows axons back to the peripheral site, and replication proceeds at the skin or mucous membranes.

22

22

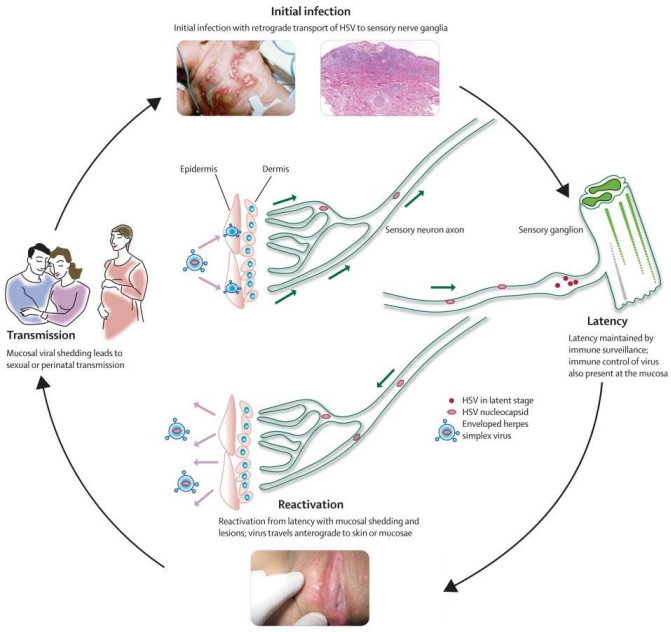
Pathogenesis and Pathology the Herpes Simplex Viruses

- Spontaneous reactivations occur despite HSV-specific humoral and cellular immunity in the host. However, this immunity limits local viral replication, so that **recurrent infections are less extensive and less severe**.
- Many recurrences are asymptomatic, reflected only by viral shedding in secretions.
- When symptomatic, episodes of recurrent HSV-1 infection are usually manifested as cold sores (fever blisters) near the lip.
- More than 80% of the human population harbor HSV-1 in a latent form, but only a small portion experience recurrences.
- **It is not known why some individuals have reactivations and others do not**.

23

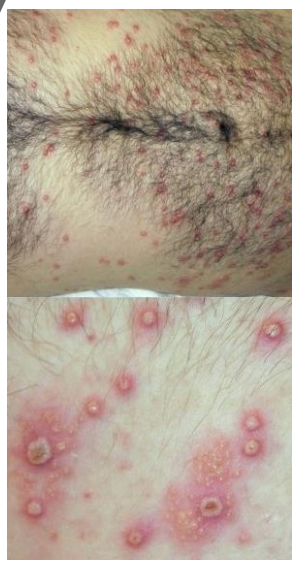
23

Pathogenesis and Pathology the Herpes Simplex Viruses



24

Disseminated HSV infection in AIDS patient

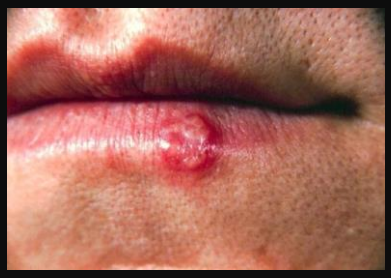


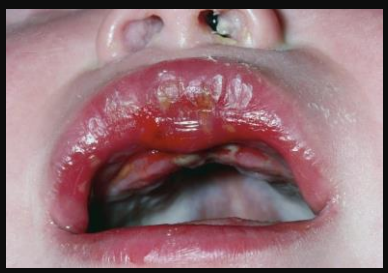
25

25

Herpes Simplex – Oropharyngeal Disease

- Primary HSV-1 infections are mostly asymptomatic.
- The incubation period is short (~3–5 days, with a range of 2–12 days), and clinical illness lasts 2–3 weeks.
- Symptoms include fever, sore throat, vesicular and ulcerative lesions, gingivostomatitis´.
- Primary infections in adults commonly cause pharyngitis and tonsillitis. Localized lymphadenopathy may occur.
- Recurrent disease is characterized by a cluster of vesicles most commonly localized at the border of the lip.

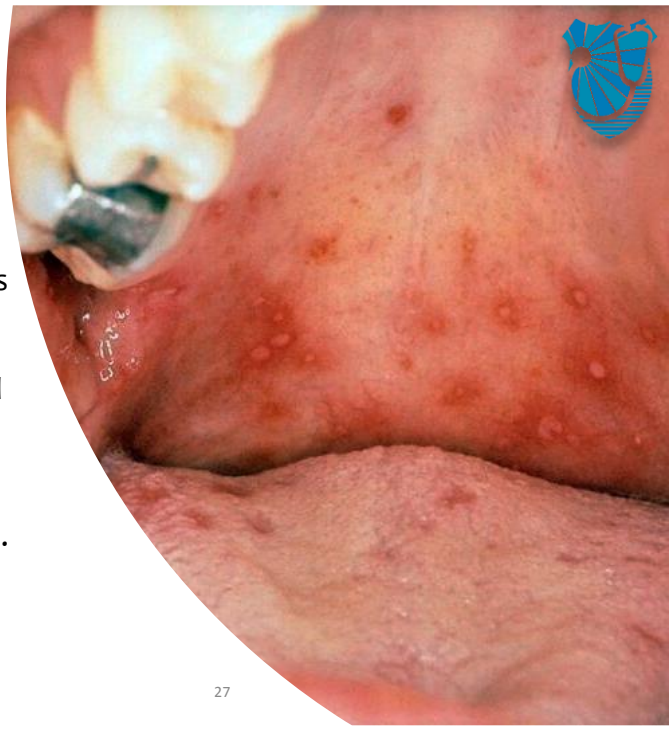




26

Herpes Simplex – oral infection

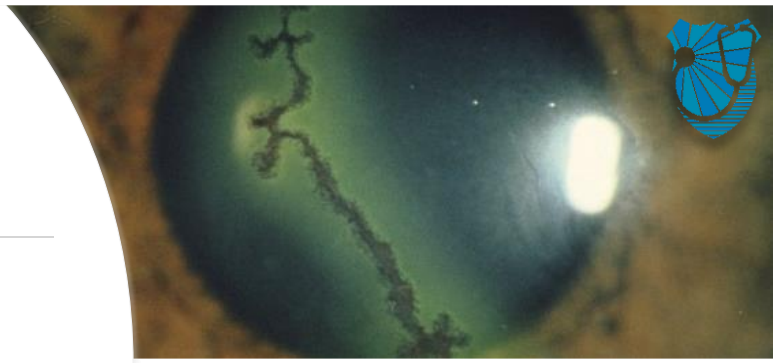
- Intense pain occurs at the outset but fades over 4–5 days.
- Lesions progress through the pustular and crusting stages, and healing without scarring is usually complete in 8–10 days.
- The lesions may recur in the same location.
- Many recurrences of oral shedding are asymptomatic and of short duration (12 – 24 hours).

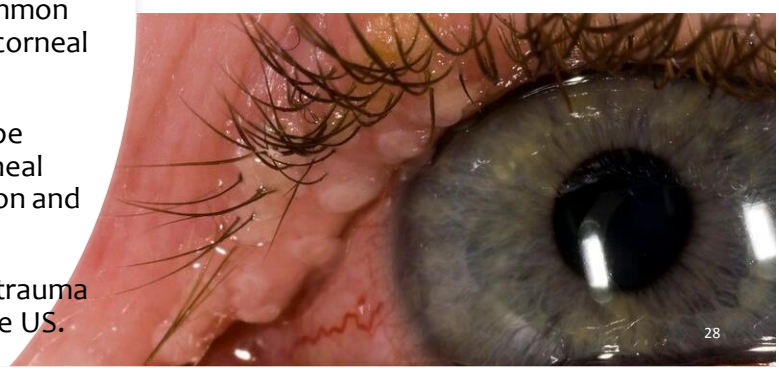


27

Herpes Simplex – Keratoconjunctivitis

- HSV-1 can cause severe keratoconjunctivitis.
- Recurrent lesions of the eye are common and appear as dendritic keratitis or corneal ulcers or as vesicles on the eyelids.
- With recurrent keratitis, there may be progressive involvement of the corneal stroma, with permanent opacification and blindness.
- HSV-1 infections are second only to trauma as a cause of corneal blindness in the US.





28

Herpes Simplex – Genital herpes

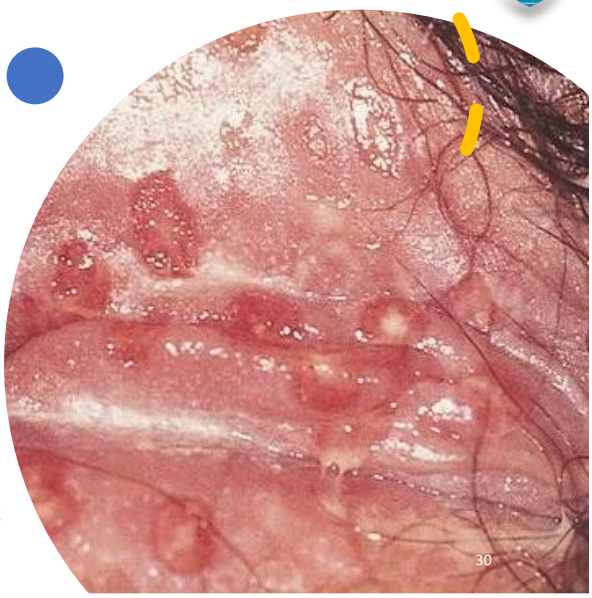
- Genital disease is usually caused by HSV-2, although HSV-1 can also cause clinical episodes of genital herpes.
- Primary genital herpes infections can be severe, with illness lasting about 3 weeks.
- Genital herpes is characterized by vesiculo-ulcerative lesions of the penis of the male or of the cervix, vulva, vagina, and perineum of the female.
- The lesions are very painful and may be associated with fever, malaise, dysuria, and inguinal lymphadenopathy.
- Complications include extragenital lesions (~20% of cases) and **aseptic meningitis** (~10% of cases).

29

29

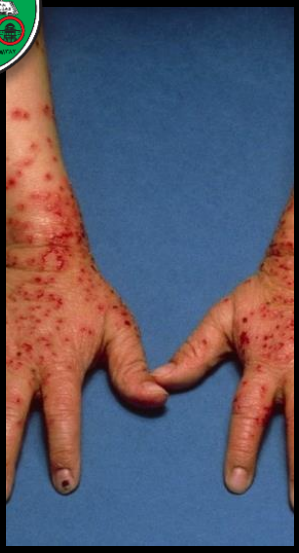
Herpes Simplex – Genital disease

- Because of the antigenic cross-reactivity between HSV-1 and HSV-2, pre-existing immunity provides some protection against heterotypic infection.
- An initial HSV-2 infection in a person already immune to HSV-1 tends to be less severe.
- Recurrences of genital herpetic infections are common and tend to be mild. A limited number of vesicles appear and heal in about 10 days.
- Some recurrences are asymptomatic with anogenital shedding lasting less than 24 hours.
- Whether a recurrence is symptomatic or asymptomatic, **a person shedding virus can transmit the infection to sexual partners**.



30

Herpes Simplex – Skin Infections

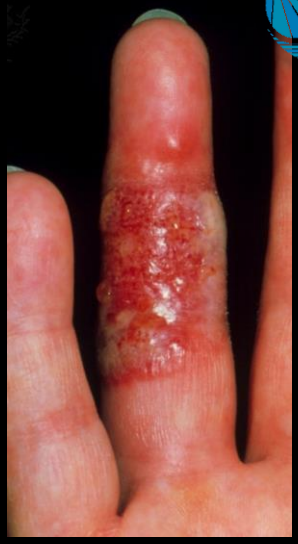


Intact skin is resistant to HSV.

Localized lesions caused by HSV-1 or HSV-2 may occur in abrasions that become contaminated with the virus (traumatic herpes); on the fingers of dentists and hospital personnel (herpetic whitlow) and on the bodies of wrestlers (herpes gladiatorum or mat herpes).

Cutaneous infections are often severe and life threatening when they occur in individuals with disorders of the skin, such as eczema or burns.

Eczema herpeticum is a primary infection, usually with HSV-1, in a person with chronic eczema. In rare instances, the illness may be fatal.



31

31

Herpes Simplex – Encephalitis

- A severe form of encephalitis may be produced by herpesvirus.
- HSV-1 infections are considered the most common cause of sporadic, fatal encephalitis in the US.
- The disease carries a high mortality rate, and those who survive often have residual neurologic defects.
- About half of patients with HSV encephalitis appear to have primary infections, and the rest appear to have recurrent infection.

32

Herpes Simplex – Neonatal Herpes

- HSV infection of the newborn may be acquired in utero, during birth, or after birth. The mother is the most common source of infection in all cases.
- Neonatal herpes is estimated to occur in about 1 in 5000 deliveries per year. The newborn infant seems to be unable to limit the replication and spread of HSV and has a propensity to develop severe disease.
- The most common route of infection (~75% of cases) is for HSV to be transmitted to a newborn during birth by contact with herpetic lesions in the birth canal. To avoid infection, delivery by C/S has been used in pregnant women with genital herpes lesions.
- However, many fewer cases of neonatal HSV infection occur than cases of recurrent genital herpes, even when the virus is present at term.

33

Herpes Simplex – Neonatal Herpes

- Neonatal herpes can be acquired postnatally by exposure to either HSV-1 or HSV-2.
- Sources of infection include family members and hospital personnel who are shedding virus.
- About 75% of neonatal herpes infections are caused by HSV-2.
- There do not appear to be any differences between the nature and severity of neonatal herpes in premature or full-term infants, in infections caused by HSV-1 or HSV-2, or in disease when virus is acquired during delivery or postpartum.

34

Herpes Simplex – Neonatal Herpes

- Neonatal herpes infections are almost always symptomatic.
- The overall mortality rate of untreated disease is 50%. Babies with neonatal herpes exhibit three categories of disease:

  (1) lesions localized to the skin, eye, and mouth;

  (2) encephalitis with or without localized skin involvement;

  (3) disseminated disease involving multiple organs, including the central nervous system.

- The worst prognosis (~80% mortality rate) applies to infants with disseminated infection, many of whom develop encephalitis. The cause of death of babies with disseminated disease is usually viral pneumonitis or intravascular coagulopathy. Many survivors of severe infections are left with permanent neurologic impairment.

35

Herpes Simplex – Immunocompromised patients

- Immunocompromised patients are at increased risk of developing severe HSV infections. These include patients immunosuppressed by disease or therapy (especially those with deficient cellular immunity) and individuals with malnutrition.
- Renal, cardiac, and bone marrow transplant recipients are at particular risk for severe herpes infections. Patients with hematologic malignancies and patients with AIDS have more frequent and more severe HSV infections.
- Herpes lesions may spread and involve the respiratory tract, esophagus, and intestinal mucosa.
- Malnourished children are prone to fatal disseminated HSV infections. In most cases, the disease reflects reactivation of latent HSV infection.

36

Herpes Simplex – Immunity

- Many newborns acquire passively transferred maternal IgG.
- The period of greatest susceptibility to primary herpes infection occurs between ages 6 months and 2 years.
- Trans-placentally acquired IgG from the mother are not totally protective against infection of newborns, but they seem to ameliorate infection if not prevent it.
- HSV-1 antibodies begin to appear in the population in early childhood; by adolescence, **they are present in most persons (for type 1)**
- Antibodies to HSV-2 rise during the age of adolescence and sexual activity.

37

37

Herpes Simplex – Immunity

- During primary infections, IgM antibodies appear transiently and are followed by IgG and IgA antibodies that persist for long periods.
- Cell-mediated immunity and non-specific host factors (NK cells, IFN) are important in controlling both primary and recurrent HSV infections.
- After recovery from a primary infection (inapparent, mild, or severe), the virus is carried in a latent state in the presence of antibodies.
- **These antibodies do not prevent reinfection or reactivation of latent virus but may modify subsequent disease.**

38

Herpes Simplex – Laboratory Diagnosis

- **PCR** on CSF has replaced viral isolation as the standard assay for specific diagnosis of HSV infections of the CNS.
- Virus isolation remains the definitive diagnostic approach. Virus may be isolated from herpetic lesions and may also be found in throat washings, CSF, both during primary infection and during asymptomatic periods.
- **Therefore, the isolation of HSV is not in itself sufficient evidence to indicate that the virus is the causative agent of a disease under investigation**.
- A rapid cytologic method is to stain scrapings obtained from the base of a vesicle (e.g., with Giemsa's stain); the presence of multinucleated giant cells indicates that herpesvirus (HSV-1, HSV-2, or varicella-zoster) is present, distinguishing lesions from those caused by coxsackieviruses (Tzanck smear).

39

39

Herpes Simplex – Laboratory Diagnosis

- Antibodies appear in 4–7 days after infection and reach a peak in 2–4 weeks.
- They persist with minor fluctuations for the life of the host.
- Detection methods available include CLA, IF, and ELISA.
- The diagnostic value of serologic assays is limited by the multiple antigens shared by HSV-1 and HSV-2.
- There may also be some heterotypic anamnestic responses to VZV in persons infected with HSV and vice versa.
- The use of HSV type-specific Abs, available in some research laboratories, allows more meaningful serologic tests.

40

Herpes Simplex Virus Infection – Epidemiology

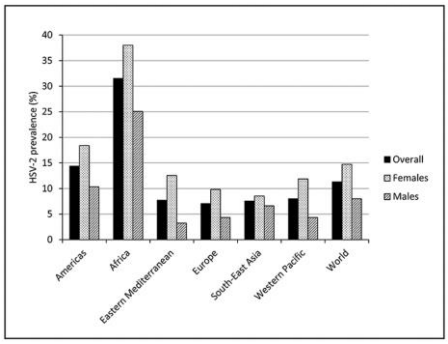
- The highest incidence of HSV-1 occurs among children 6 months to 3 years of age.
- By adulthood, 70–90% of persons HSV-1 IgG.
- Middle-class individuals in developed countries acquire antibodies later in life than those in lower socioeconomic populations (crowding, poor hygiene).
- The virus is spread by direct contact with infected saliva or through utensils contaminated with the saliva of a virus shedder.
- The source of infection for children is usually an adult with a symptomatic herpetic lesion or with asymptomatic viral shedding in saliva. The frequency of recurrent HSV-1 infections varies widely among individuals.
- At any given time, 1–5% of normal adults are excreting virus.

41

41

Herpes Simplex – Epidemiology

- HSV-2 is usually considered an STI, so antibodies to this virus are seldom found before puberty.
- Antibody prevalence studies have been complicated by the cross-reactivity between HSV types 1 and 2. Surveys using type-specific gp Ags determined that 17% of adults in the US possess HSV-2 IgG, with seroprevalence higher among women, blacks, and older individuals, reaching 56% in blacks 30–49 years.
- Frequent subclinical reactivations for both types occur in immunocompetent hosts lasting less than 12 hours.
- Both symptomatic and asymptomatic infections provide a reservoir of virus for transmission to susceptible persons.
- Studies have estimated that transmission of **genital herpes in more than 50% of cases resulted from sexual contact in the absence of lesions or symptoms**.



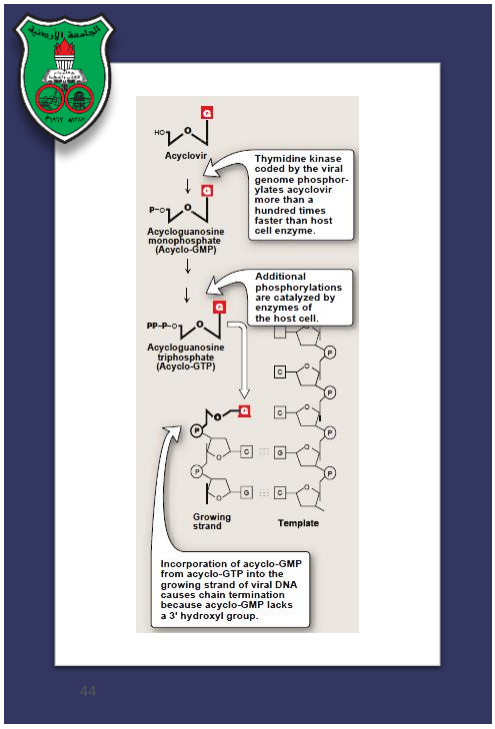
42

42

Herpes Simplex – Epidemiology

- Maternal genital HSV infections pose risks to both the mother and the fetus.
- Rarely, pregnant women may develop disseminated disease after primary infection, with a high mortality rate.
- Primary infection before 20 weeks of gestation has been associated with spontaneous abortion.
- The fetus may acquire infection as a result of viral shedding from recurrent lesions in the mother's birth canal at the time of delivery.
- Estimates of the frequency of cervical shedding of virus among pregnant women vary widely.
- Genital HSV infections increase acquisition of HIV infections.

43



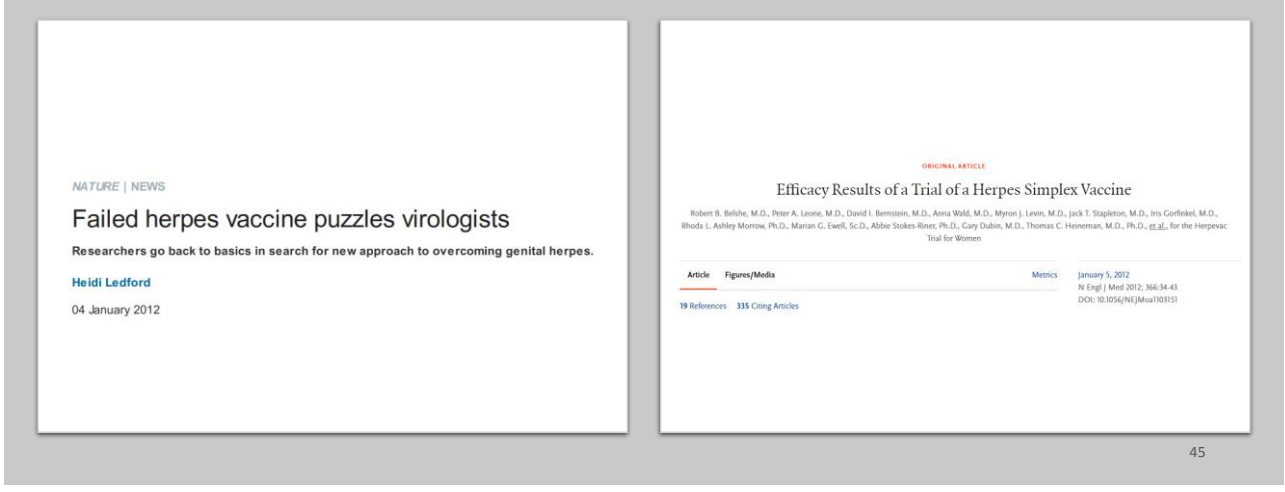
44

Herpes Simplex – Treatment

- Effective antivirals include acyclovir, valacyclovir, and vidarabine, all of which are inhibitors of viral DNA synthesis.
- Acyclovir which is a nucleoside analog, is monophosphorylated by the HSV thymidine kinase and is then converted to the triphosphate form by cellular kinases. The acyclovir triphosphate is efficiently incorporated into viral DNA by the HSV polymerase, where it then **prevents chain elongation**.
- The drugs may suppress clinical manifestations, shorten time to healing, and reduce recurrences of genital herpes. However, **HSV remains latent in sensory ganglia**.
- Drug-resistant virus strains may emerge.

Herpes Simplex – Prevention and Control

- Counselling, antiviral therapy and condom usage to prevent genital herpes.
- Experimental vaccines of various types are still under development.



NATURE | NEWS

Failed herpes vaccine puzzles virologists

Researchers go back to basics in search for new approach to overcoming genital herpes.

Heidi Ledford

04 January 2012

ORIGINAL ARTICLE

Efficacy Results of a Trial of a Herpes Simplex Vaccine

Robert B. Belshe, M.D., Peter A. Leone, M.D., David I. Bernstein, M.D., Anna Wald, M.D., Myron J. Levin, M.D., Jack T. Stapleton, M.D., Iris Gorfinkel, M.D., Rhoda L. Ashley Morrow, Ph.D., Marian G. Ewell, Sc.D., Abbie Stokes-Riner, Ph.D., Gary Dubin, M.D., Thomas C. Heineman, M.D., Ph.D., et al., for the Herpevac Trial for Women

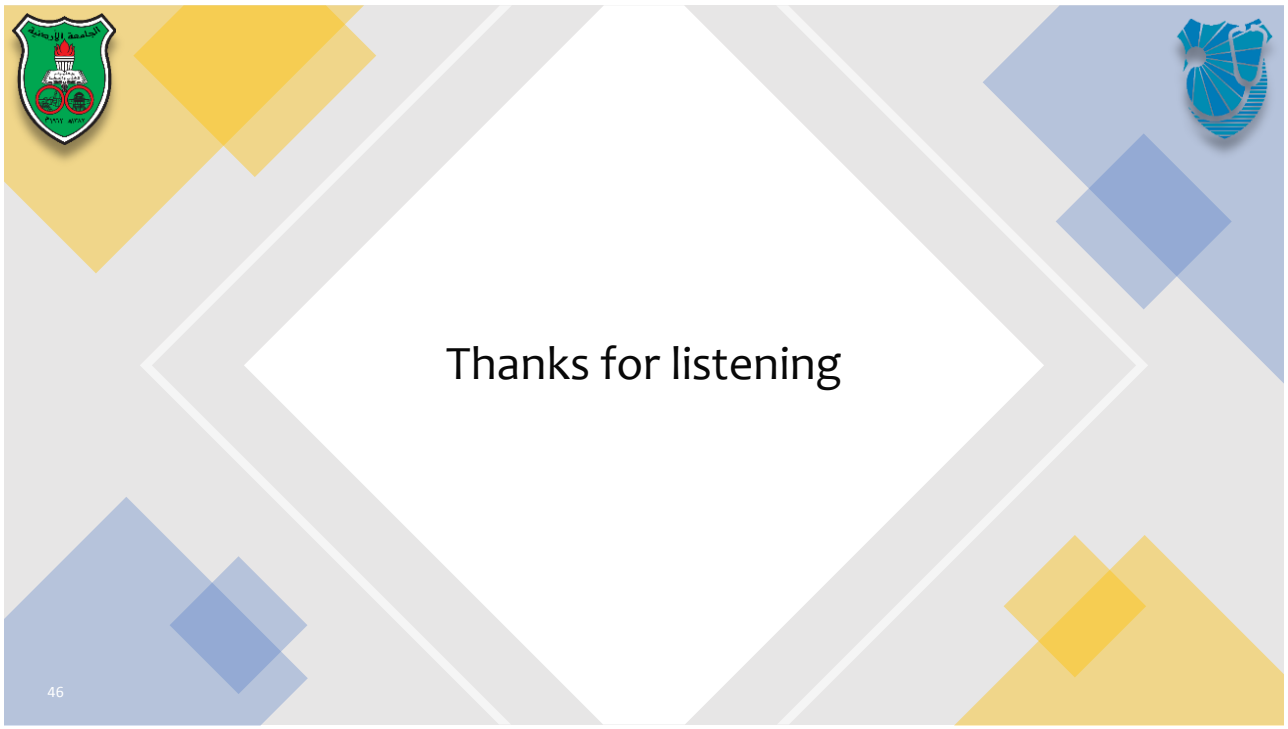
Article Figures/Media Metrics

January 5, 2012
N Engl J Med 2012; 366:34-43
DOI: 10.1056/NEJMoa1103151

19 References 335 Citing Articles

45

45


Thanks for listening
46