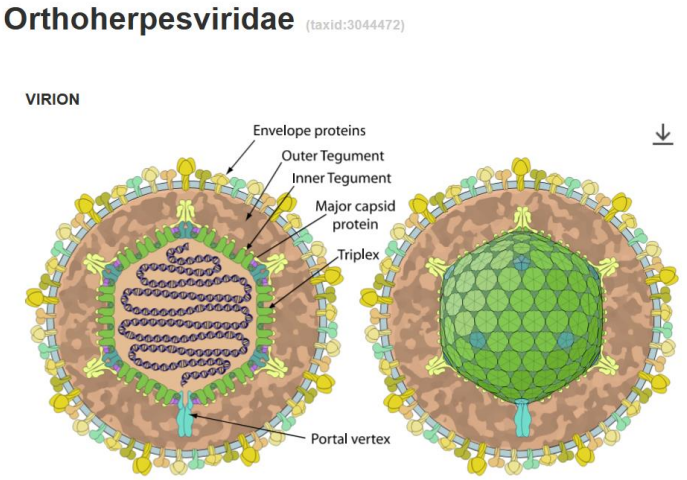
université PARIS-SACLAY

FACULTÉ DE PHARMACIE



TU 02 : Herpesviruses

Pr Audrey Esclatine

audrey.esclatine@universite-paris-saclay.fr

Herpesviruses

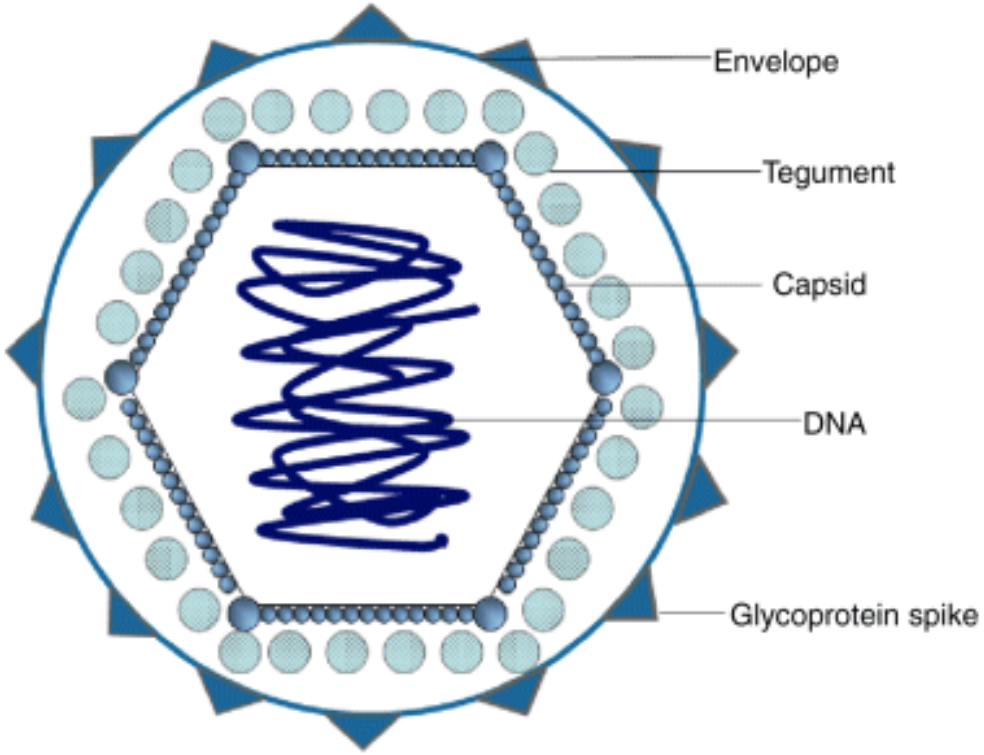
- Over 200 herpesvirus species, infect humans and animals
- 10 affect humans
  - Each of them has a unique clinical syndrome
- *Orthoherpesviridae* family
- Three sub-families
  - *Alphaherpesvirinae*
  - *Betaherpesvirinae*
  - *Gammaherpesvirinae*

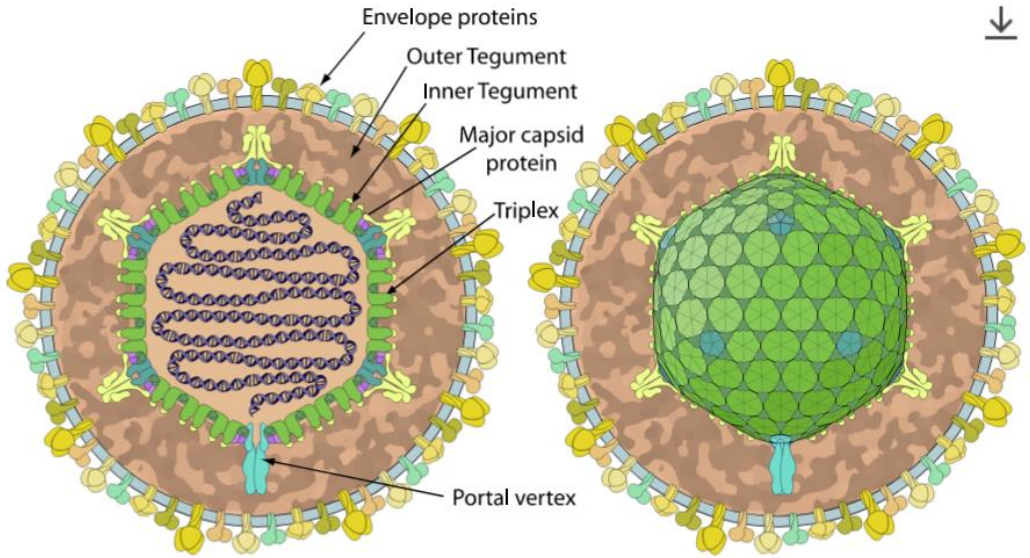
Herpesviruses

- Over 200 herpesvirus species, infect humans and animals
- 10 affect humans
  - Each of them has a unique clinical syndrome
- *Orthoherpesviridae* family
- Three sub-families
  - *Alphaherpesvirinae* — HSV-1, HSV-2, VZV
  - *Betaherpesvirinae* — CMV, HHV6A, HHV6B (HHV7)
  - *Gammaherpesvirinae* — EBV, HHV8

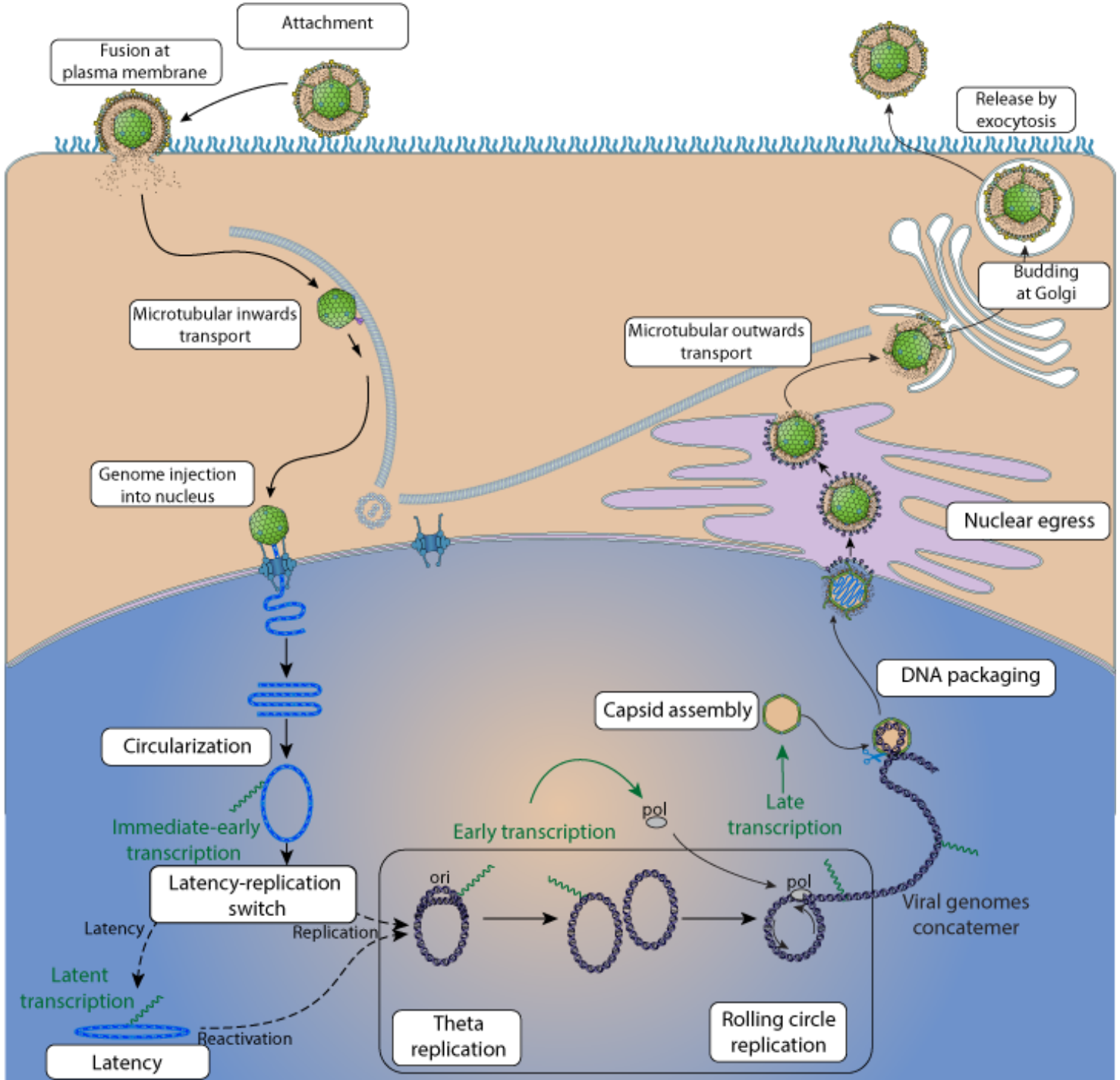
Structure

- A common structure
- Linear double stranded DNA
  - Important size, code 70 to 200 proteins
- Icosahedral capsid
  - 162 capsomeres
- Tegument (phosphoproteins)
- Envelope
  - Different glycoprotein spikes





Herpesvirus multiplication

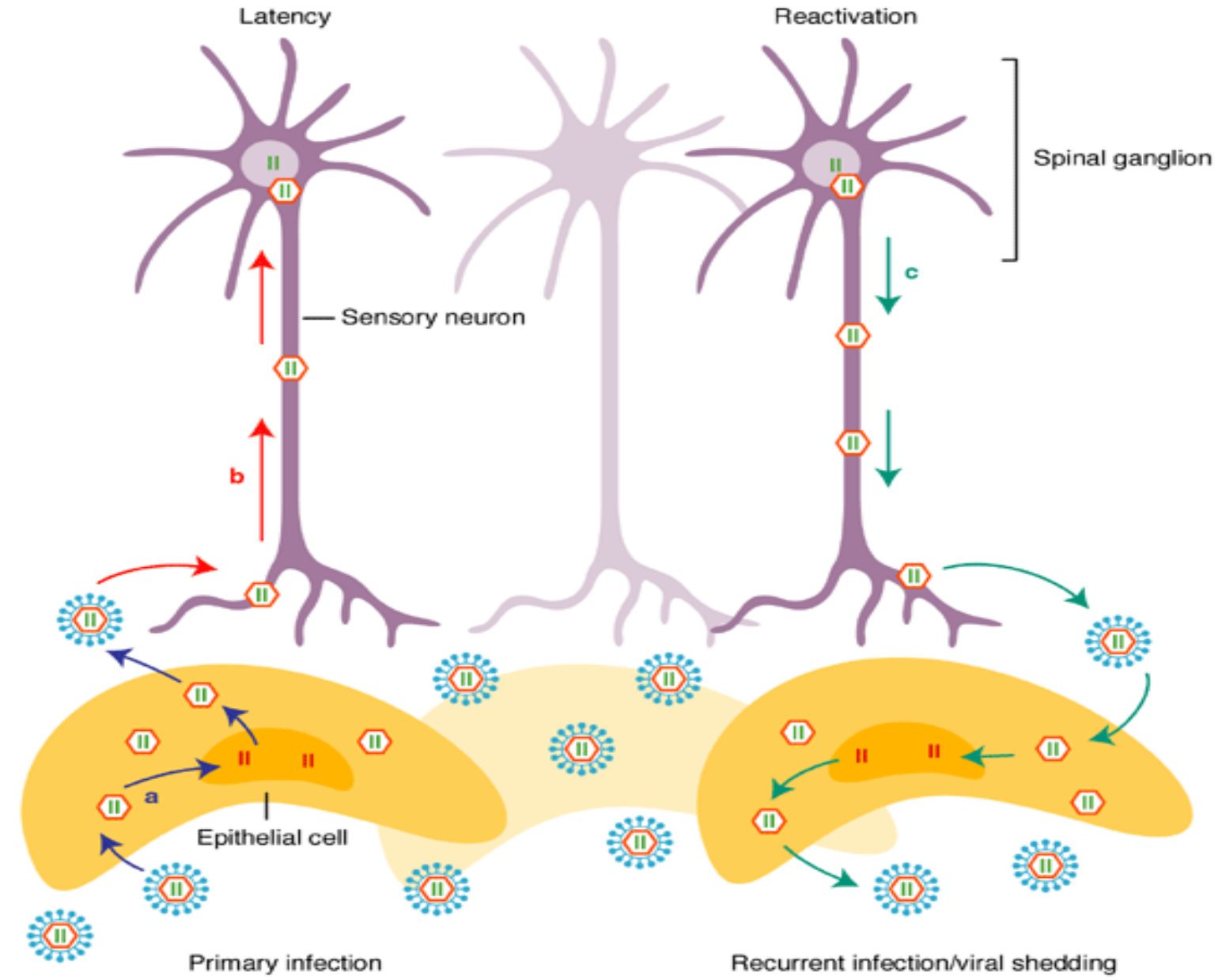


https://viralzone.expasy.org/5836

Latency

- Infections during the lifetime of the host
- retention of the viral genome in the nucleus in a silent state
  - Persistence as episome (no integration)
- No replication, no infectious particle production
  - No transmission
- Limited expression of viral genes
  - Latency associated transcripts and proteins
- Allows the virus to evade immune recognition
- Primary sites of latency vary depending of the virus
- Reactivation of latent virus leads to recurrence

Latency establishment and reactivation



Pathogenicity

- Primary infection
  - Symptomatic or not
  - The immune system effectively limits extensive virus replication
- Latency can be associated with cancers for EBV and HHV8
- Reactivation
  - Symptomatic or not
- Serious cause of morbidity and mortality in immunosuppressed persons (transplant patients) – opportunistic infections
- Serious cause of morbidity and mortality in newborns. Maternal fetal transmission CMV HSV1/2, VZV
- No vaccine except for VZV
- Virostatic treatments (drugs only exert their activities on viral replication)

Herpes simplex virus HSV-1, HSV-2

- Frequent and ubiquitous infections
- Various tropisms

  HSV-1 : most often oral infection herpes labialis

  - gingivostomatitis (primary infection)
  - cold sore (recurrence)
  - meningo-encephalitis

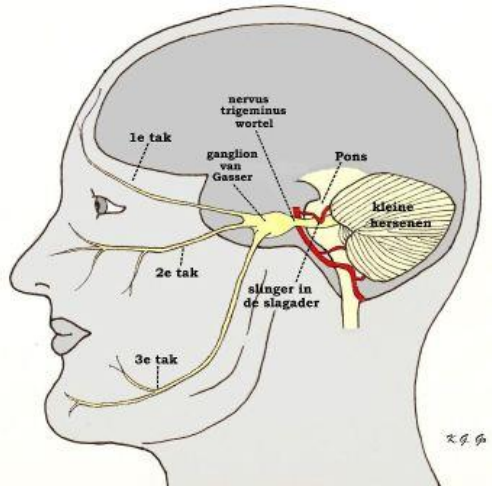
  HSV-2 : genital lesions

  - genital herpes , neonatal herpes
- Usually, primary infection is asymptomatic
- Common severe infections include encephalitis, meningitis, neonatal herpes, and, in immunocompromised patients, disseminated infection
- Prevalence depending on the socio-economic standards
- Genital HSV1 primary infection in developed countries

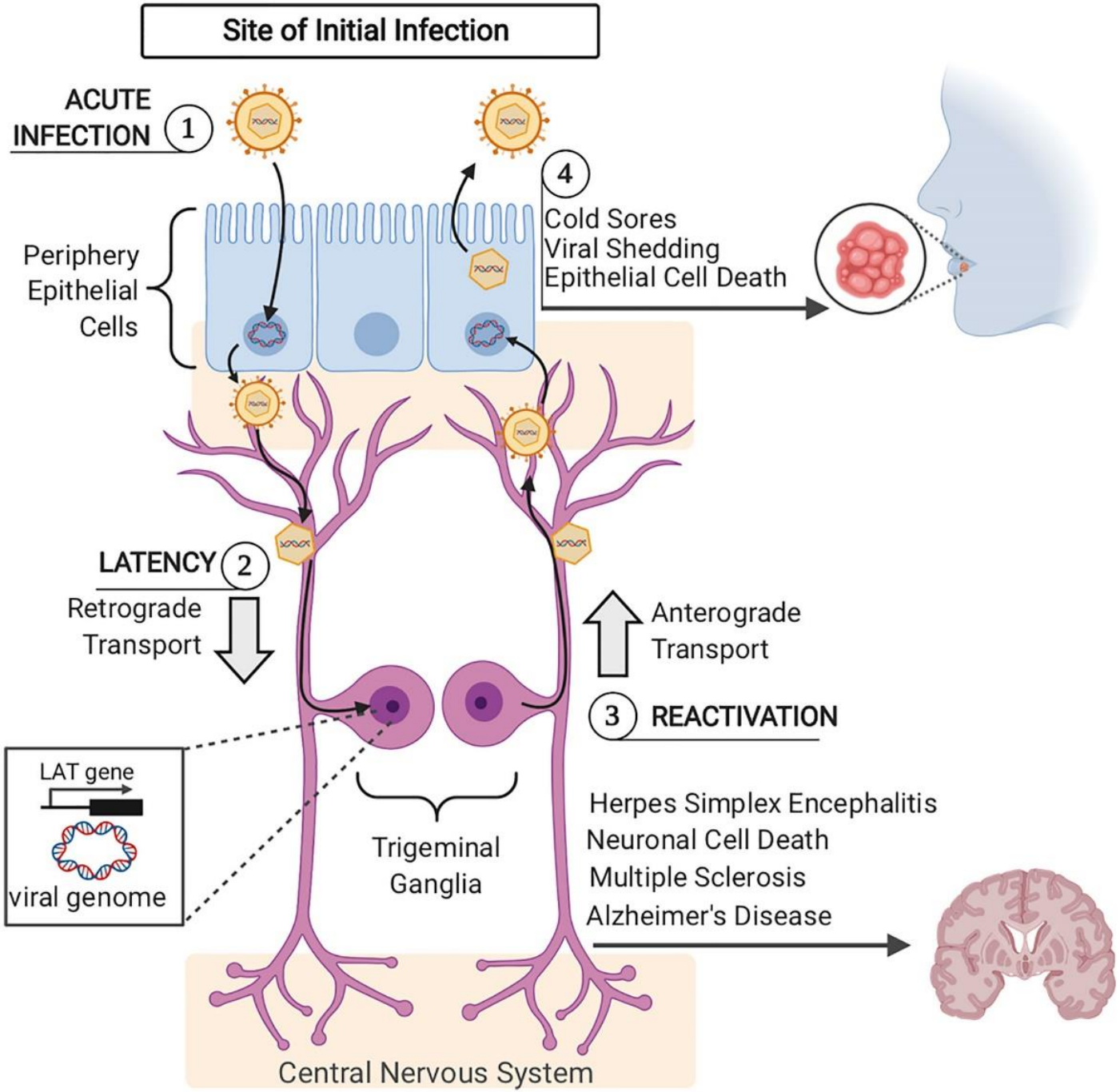
gingivostomatitis

Pathologies

- Oral Herpes
  - Primary infection HSV-1 in childhood, most often asymptomatic
  - Gingivostomatitis in children
  - Latent infection in trigeminal ganglion
- Reactivations
  - Symptomatic or not
  - Cold sore= cluster of unilateral vesicles on the vermilion border of the lip
  - Excretion of the virus in saliva
- triggered by
  - Overexposure to sunlight
  - Febrile illnesses
  - Physical or emotional stress
  - Immunosuppression
  - Unknown stimuli



Latency establishment and reactivation

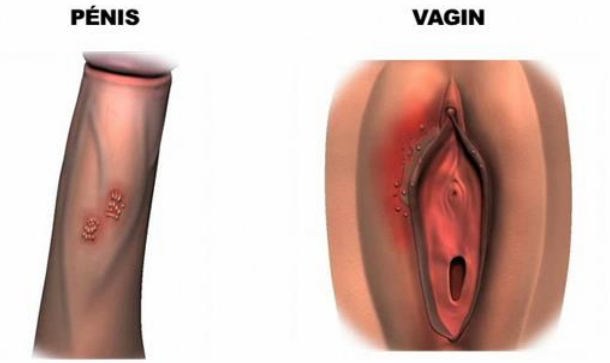


REVIEW article
Front. Immunol., 31 May 2021
Sec. Molecular Innate Immunity
Volume 12 - 2021 | https://doi.org/10.3389/fimmu.2021.644664

Pathologies

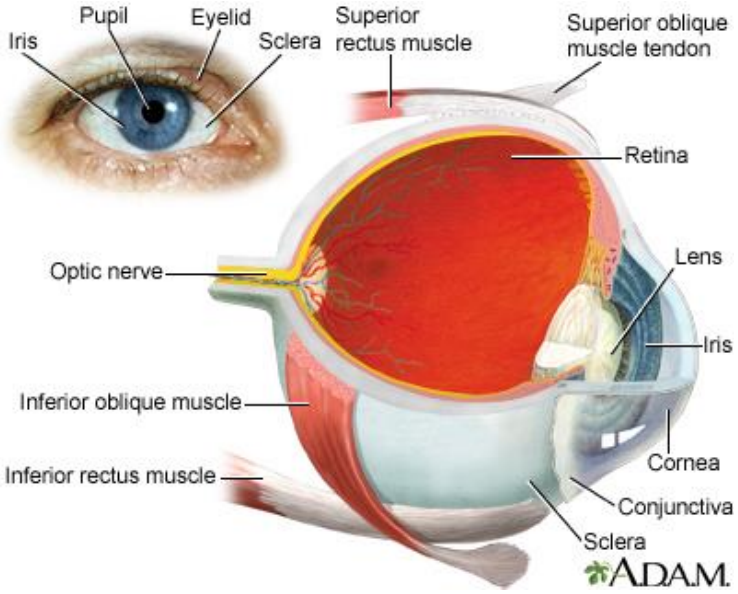
- Genital herpes
  - Most common ulcerative sexually transmitted disease in developed countries
  - can be caused by HSV-1 or HSV-2
  - 2/3 of the cases unapparent primary infection
  - 1/3 infection with painful vesicles on external genital organs, fever, lymphadenopathy
  - HSV-1 more and more involved in genital herpes
  - Latent infection in sacral ganglions

- Reactivations
  - Symptomatic or not
  - HSV-2 gives highly recurrent infections
  - Excretion of the virus in genital secretions



Pathologies

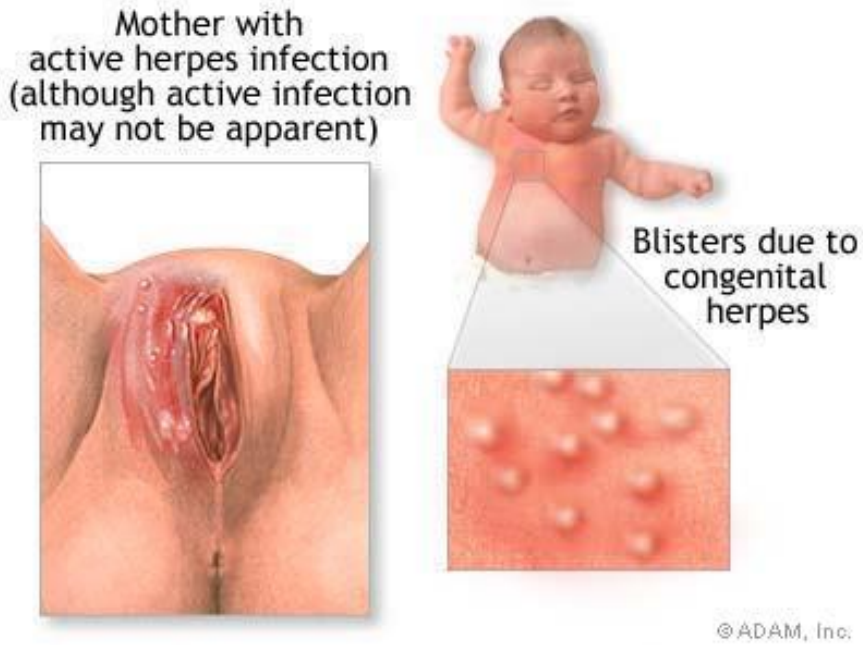
- Herpes keratitis
  - Kerato-conjunctivitis : most often HSV-1
  - Primary infection or recurrence, unilateral
  - Usually affects the corneal surface
  - Risk of corneal scarring and bacterial infections
  - major cause of unilateral blindness worldwide
- Meningoencephalitis
  - Age-independent but a peak at 50 years
  - Elevated fever
  - headaches, loss of consciousness, hallucinations, and partial paralysis and seizures
  - First cause of viral encephalitis
  - Severe without treatment
  - High mortality rate or severe neuro psychic sequelae



temporal lobe damage

Pathologies

- Neonatal Herpes
  - Rare but extremely serious, about 20 cases per year in France per 700,000 births
  - MF Transmission , 90% during delivery
  - High mortality rate or severe neuro psychic sequelae

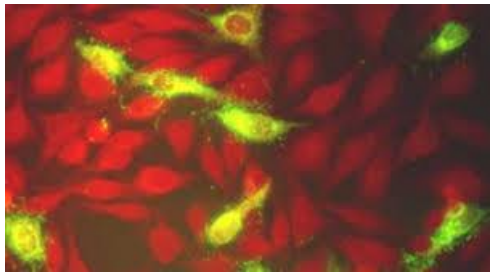


- Infection in immunocompromised patients
  - Extensive lesions
  - Possible dissemination to various organs

Photo H Agut

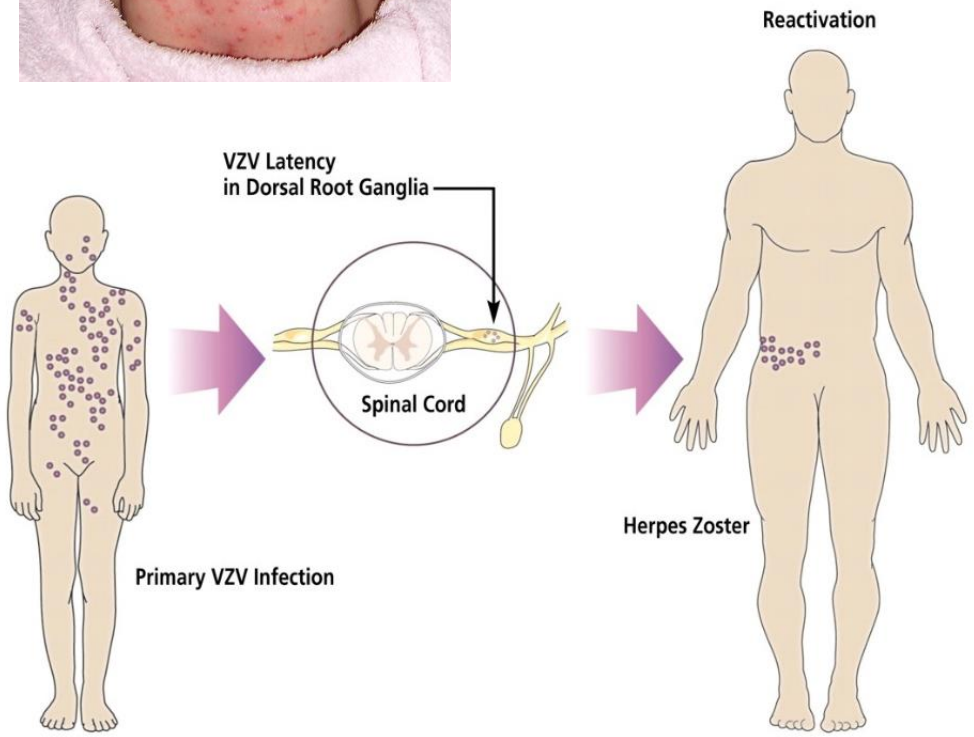
Diagnosis

- Often clinical based on characteristic lesions
- Virological confirmation for genital herpes and severe forms
- Essentially direct techniques
  - Detection of viral genome by PCR (in vesicles, in CSF for encephalitis)
  - Immunocytodiagnosis IF or IHC
    - Detection of viral antigens by labeled specific antibodies
  - Isolation of the virus by inoculation of permissive human fibroblasts
    - cytopathic effect
- Indirect (Serology) : useful for epidemiology studies



Varicella Zoster Virus (VZV)

- Same process than herpes
- Primary infection : chickenpox
  - Migration of the virus through the axons to sensory ganglion neurons (dorsal root ganglia)
- Reactivation : herpes zoster shingles
  - Rash in a territory innervated by sensory nerves (reactivation limited to one lymph node)



Epidemiology

- Prevalence: > 95% of the adults in temperate climates
- Children in tropical climates acquire varicella at older ages and a higher proportion of young adults remain susceptible
- highly contagious
- Transmission mode: from vesicle fluid (skin lesions) and especially from respiratory secretions from subjects with chickenpox
- strictly interhuman, direct, respiratory
- No shingles outbreaks but can transmit chickenpox
- Maternal fetal transmission

Pathologies : chickenpox

- usually childhood infection
- Incubation lasts 14 days
- Contagiousness

  highly contagious
  - Begins 1 to 2 days **before** rash
- Clinical signs
- Rash, intensively itchy
  - Macular eruption, papules
  - teardrop vesicles
  - Crusts, scars if scratching
  - Moderate fever, mild headache
  - lesions develop in crops so that they are in various stages of development in any affected region
- Mild illness, rarely severe, rare systemic complications
  - Secondary bacterial infection
  - Pneumonia in adults
  - neonates, and immunocompromised patients

Shingles = Herpes zoster recurrence

- Viral reactivation of latent infection in sensory nerve ganglia
  - begins with lancinating pain along the affected dermatome
  - Unilateral vesicular eruption (usually crops of vesicles on an erythematous base)
  - usually in the thoracic or lumbar region, ophthalmic herpes zoster

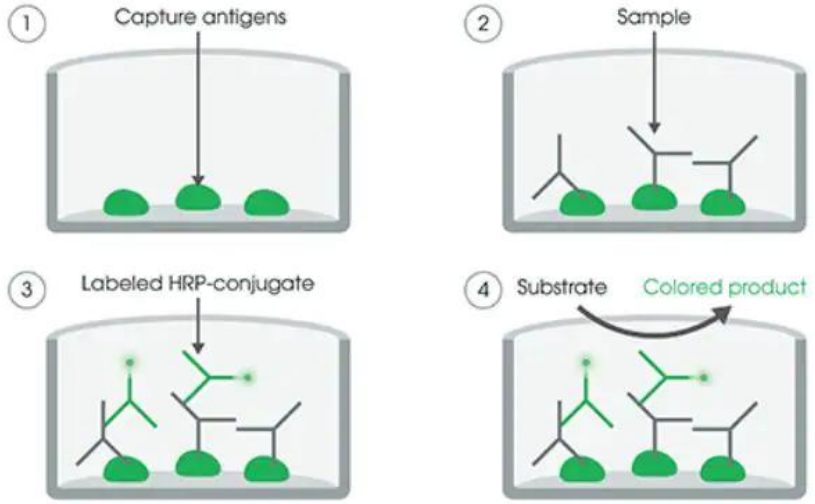
- frequently occurs in older and HIV-infected patients

more frequent and severe in immunocompromised patients

- Complications
  - Post herpetic neuralgia
  - Ophthalmic herpes zoster
  - may disseminate to visceral organs in immunocompromised patients

Diagnosis

- Essentially clinical
- Direct diagnosis
  - Detection of viral genome by PCR to diagnose complicated forms (cerebrospinal fluid, blood, amniotic liquid…)
  - Detection of viral Ag by IF
  - (Isolation in cell culture)
- Indirect diagnosis
  - Serodiagnosis (ELISA)



Human Cytomegalovirus HCMV

Natural history of HCMV infection

- Very common virus
- Approximately 60% of adults in developed countries have IgG against CMV
  - 50% in France
  - 80 to 100% in developing countries
  - Prevalence depends on the socio-economic standards of a country
- Primary infection
  - Usually asymptomatic or mild, not very specific symptoms
- reactivations and reinfections are usually asymptomatic
- Transmission from person to person
  - Saliva, contact with body secretions
  - Sexual transmission
  - Congenital : in utero
  - Iatrogenic transmission
- Most frequent congenital infection
- Most frequent opportunistic infection in immunosuppressed persons

Infections in immunosuppressed patients

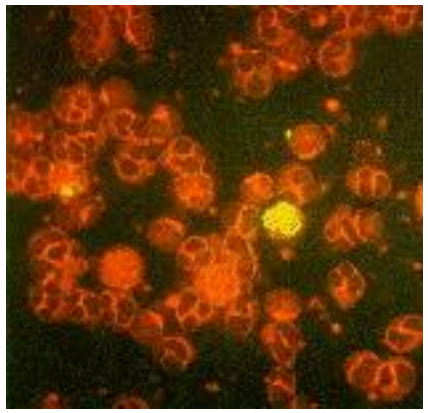
- Post transplant immunosuppressed patients
  - Solid organ and hematopoietic stem-cell transplantation
  - Most frequent and most serious opportunist infection (morbidity and mortality)
  - Symptoms appear 2 to 3 months after the allograft
  - favors acute or chronic graft rejection and bacterial or fungal infections
  - Damage of the transplanted organ (HCMV-positive donor)
  - Pneumonitis, gastrointestinal tract (colitis, esophagitis), liver (hepatitis) and eye (retinitis)
- AIDS (<100 CD4/µl)
  - Retinitis, colitis
  - Rare nowadays in HIV-infected patients receiving highly active antiretroviral therapy

Congenital HCMV infections

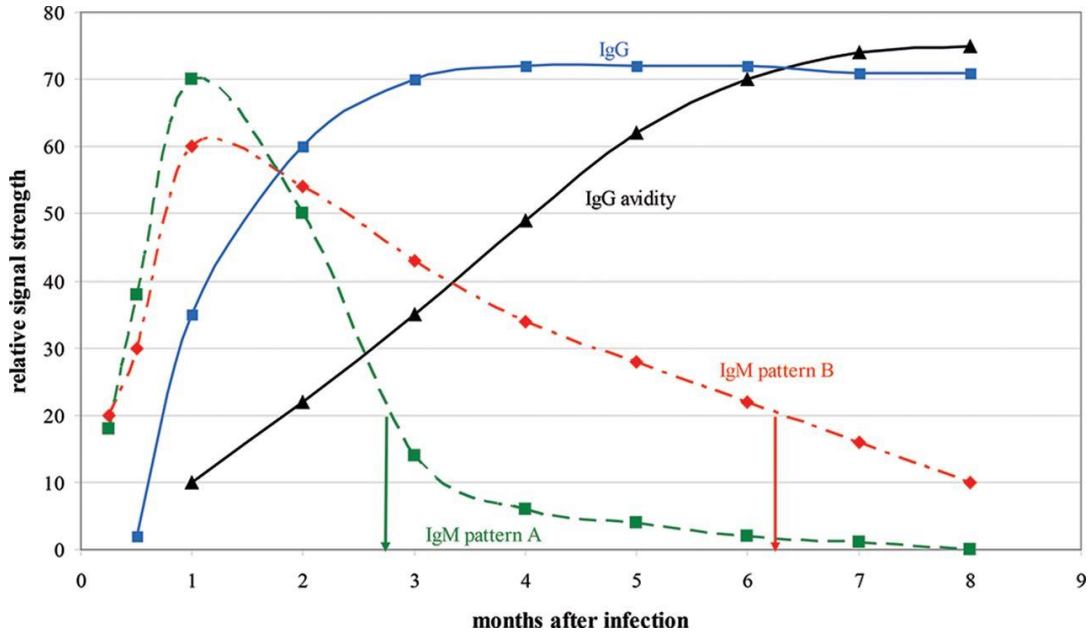
- incidence is between 0.3 and 1.2%
- Primary infection of the mother during pregnancy (intrauterine transmission rate of 40–50%)
- Reactivation in preconceptionally HCMV-seropositive mothers (transmission below 5%)
- About 7–10% of HCMV-infected infants develop disease sometimes permanent neurological damage (mental retardation, impaired hearing, deafness)
- Fatal in about 10% of cases
- breast milk-associated postnatal HCMV transmission, (premature newborns of seropositive mothers)
- In mature newborns, the infection is usually symptom-free

Diagnosis

- Direct diagnosis
  - Detection of CMV DNA
    - Real time PCR
  - (Antigen detection of the virus)
    - Rapid pp65 antigenemia assay pp65 in leucocytes in blood
  - (Cell culture)
- Indirect diagnosis
  - detection of IgM and IgG antibodies
    - measured in 2 serum samples (2 weeks)
    - avidity determination of the IgG antibodies
    - Seroconversion
  - detection of T cell responses against CMV



pp65 antigenemia



Human Herpesvirus 6 HHV6

- Two closely related species, HHV-6A and HHV-6B
- Prevalence of HHV6 over 90% (HHV6B)
  - infection typically before the age of 3
- childhood disease: roseola infantum
- most often in children aged 6 to 24 months
- Begins with a sudden high fever, lasts for 3 days
- Fever suddenly declines and is followed by a rash
- Essentially clinical diagnosis
- No vaccine, no treatment
- Viral reactivation in allogenic HSCT patients (pneumonitis, encephalitis…)
- Detection of HHV6 DNA in blood

Epstein Barr virus (EBV)

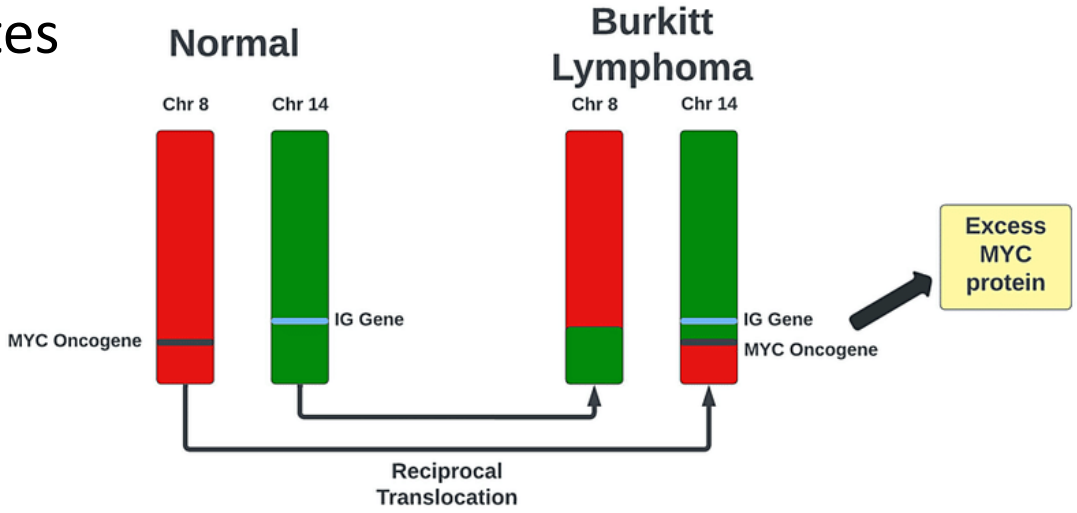
- History
  - Burkitt lymphoma in 1958
  - Epstein and Barr first detected EBV in cells of this lymphoma in 1964
  - EBV and infectious mononucleosis IM: 1968 Henle laboratory
- First human oncogenic virus discovered
  - associated with several malignancies of epithelial origin (carcinoma) and lymphocytic origin (lymphoma)
- Infectious Mononucleosis (IM)
  - febrile illness with hyper-expansion of both lytic and latent antigen-specific T cell responses
- Epidemiology
  - Transmission from person to person : saliva
  - Prevalence: about 90% of adults worldwide
    - more frequent during childhood
    - Adolescence or adulthood : IM in 50% of the cases

EBV Pathologies

- Primary infection in immunocompetent person
  - Asymptomatic in 90% of the cases
  - Infectious mononucleosis in 10% of the cases
    - lymphocytosis and atypical mononuclear cells
    - Fever, sore throat, fatigue, and tender lymph nodes, splenomegaly
  - During acute infection, primarily infects and replicates in the oropharynx
  - Establishment of latency in circulating memory B cells
- Recurrence in immunocompetent person
  - Generally unapparent
- Latency in immunocompetent person. no impact in general
  - Lymphoproliferations
  - carcinoma
- Infection in immunocompromised patient : malignant lymphomas
  - Cell-mediated immunity unable to control infected B cells proliferation

EBV pathologies: malignant infections

- Burkitt lymphoma: children (6 - 10) East Africa
  - Cancerous proliferation of a B lymphocyte clone
  - Chromosomic translocation 8:14 and dysregulation c-myc oncogene
  - Malaria : cofactor in endemic area
  - Maxillary tumour
- Nasopharyngeal carcinoma: adult South China
  - Genetic predisposition and food cofactors
  - Epithelial cancer cells infiltrated with lymphocytes
  - Associated with EBV in 100% of the cases
- Other tumours associated with EBV
  - Hodgkin lymphoma
  - T lymphomas



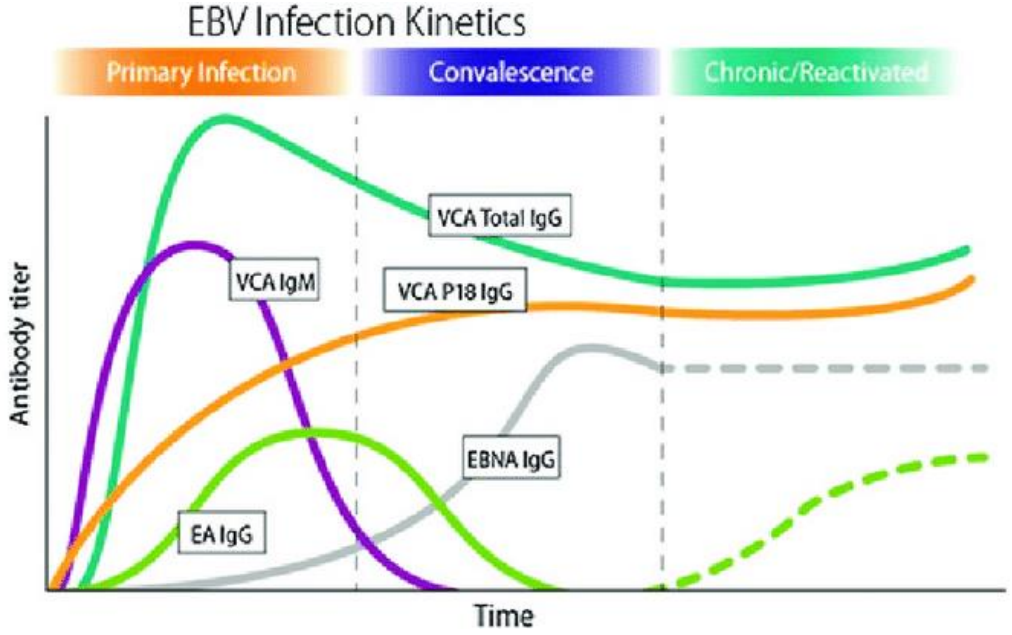
Pathologies in immunocompromised patients

- In solid organ and HSC recipient
  - Frequent reactivation, generally asymptomatic
  - Increased viral excretion in saliva
  - Major risk of lymphoma PTLD = Post Transplant Lymphoproliferative Disease
  - Poor prognosis
- During AIDS
  - oral hairy leukoplakia related to severe ID
  - Aggressive Non Hodgkin's lymphomas (NHL)

Indirect diagnosis

- IM diagnosis
  - With lymphocytosis + atypical mononuclear cells
- Determination of immune status in the context of organ transplantation
- Time-dependent antibody response
  - Antibodies against viral antigens
    - Methods : IF, ELISA
    - VCA IgM
    - VCA IgG
    - EBNA IgG

•Primary infection
•Past infection
•Absence of infection

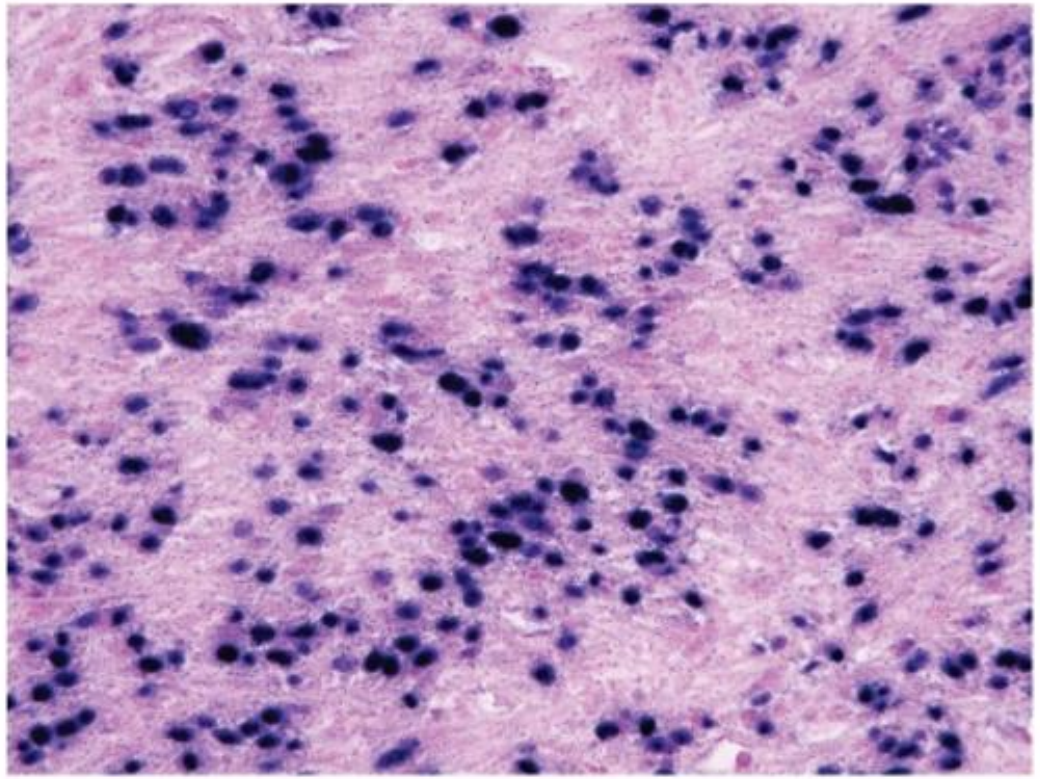


VCA IgM	VCA IgG + EA IgG	EBNA IgG	Interpretation
-	-	-	Negative EBV status
+	-	-	Early primary infection [2]
+	+	-	Acute primary infection
-	+	+ [1]	Past infection
-	-	+	Isolated EBNA IgG [2]
-	+	-	Isolated VCA/EA IgG [2]
+	+	+	Indeterminate [2]

VCA = viral capsid Antigen - EBNA Epstein Barr Nuclear Ag (a latent protein)

Direct diagnosis

- Diagnosis of lympho-proliferations B induced by EBV
  - Detection of EBV genome by PCR in circulating B cells
  - *in situ* hybridization (EBER) in biopsies
  - Isolation of virus (rare not in routine)



Human Herpesvirus 8 (HHV-8)

- also known as Kaposi sarcoma-associated herpesvirus, KSHV
- Responsible of Kaposi sarcoma in the context of immunodeficiency
- occur primarily in patients living with HIV (PLHIV)
- still one of the most frequent cancers in PLHIV with non-hodgkin lymphoma
  - 42,000 new KS cases and 20,000 deaths estimated in 2018 worldwide
- angioproliferative tumor involving skin
  - more common in men than in women (MSM)
- Transmission
  - Low prevalence in the USA (less than 5%)
  - common in the Mediterranean, the Middle East, and Africa highest in sub-Saharan Africa (reaches 50% in Uganda)
  - horizontal transmission throughout childhood
  - STD
- Multicentric Castelman disease
- Primary effusion lymphoma (PEL)