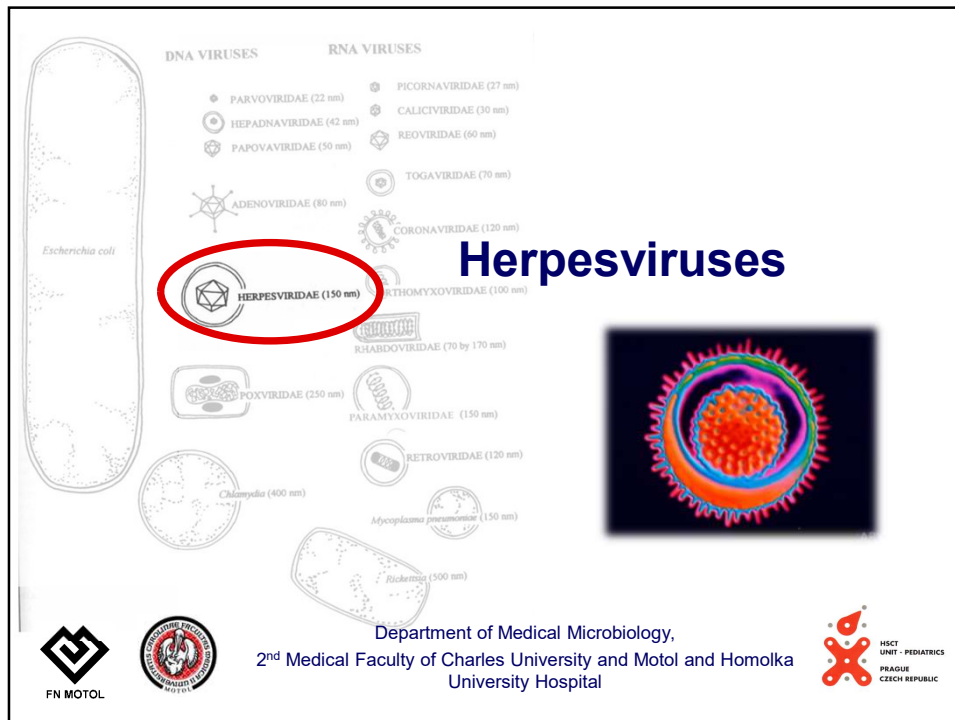
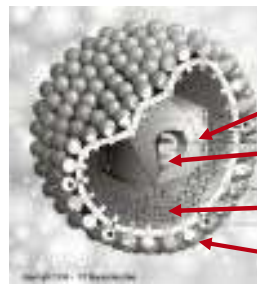




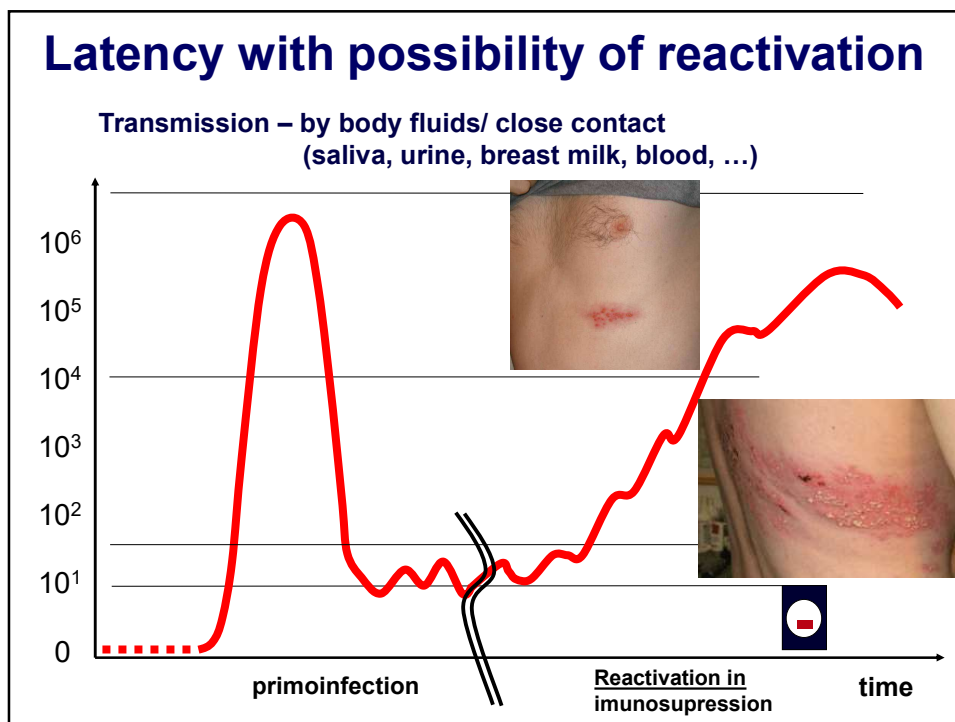
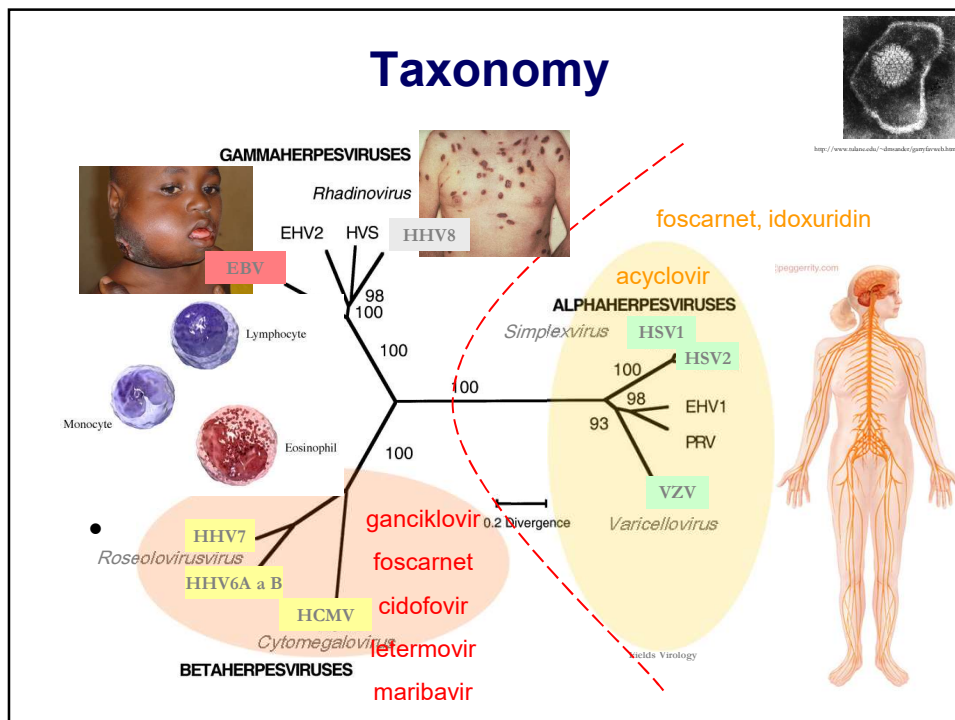
Herpesviruses

- from Greek „creep“
- enveloped ds DNA viruses
- genome of length 125-240 kb
- icosahedral capsid
- diameter of capsid of approx. 100 nm



capsid
DNA
tegument
glycoproteins







Impact of herpesviruses?

Indicative disease for HIV re-classification to AIDS stage (WHO criteria):

1. pneumocystis pneumonia
2. toxoplasma encephalitis
3. esophageal, tracheal, bronchial or lung candidiasis
4. **Chronic anal herpes simplex or herpetic bronchitis, pneumonia or esofagitis**
5. **CMV retinitis**
6. **generalized CMV infevtion (excluding liver and spleen)**
7. **progresive multifocal leukoencefalopatia**
8. repeating salmonela bacteriemia
9. repeating pneumonia within 1 year
10. chronic intestinal cryptosporidiosis
11. chronic intestinal isosporosa
12. extrapulmonary cryptococcus infection
13. disseminated or extrapulmonary histoplasmosis
14. disseminated coccidioidomycosis
15. tuberculosis
16. disseminated or extrapulmonary atypic mycobacteriosis
17. **Kaposhi sarkoma**
18. **malignant lymphoma (Burkitt's lymphoma, imunoblastic and primary cerebelar lymphoma)**
19. **Invasi carcinoma of cervix**
20. HIV encefalopatia
21. wasting syndrom



α herpesviruses

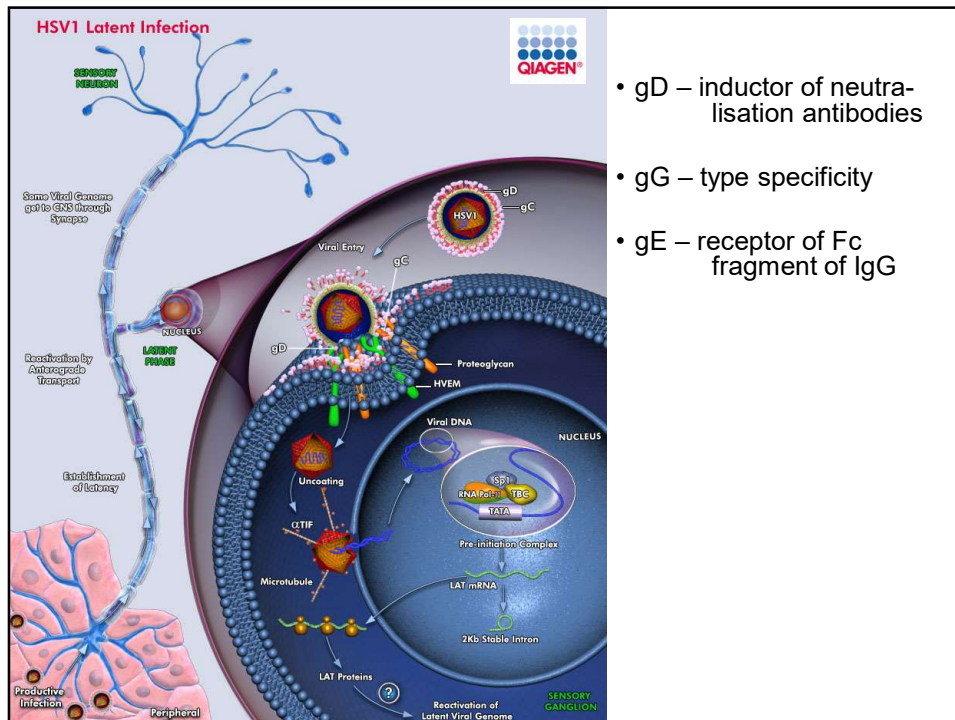
- closely related to nervous tissue – sensoric ganglia
- rapid cytolytic cycle
- infection of mucous tissue

Virus	Taxonomy	Primary infection	Recurrent infection / Reactivation	Place of latency
HSV-1	Human herpesvirus HHV-1	Gingivostomatitis, keratoconjunctivitis, encephalitis	Herpes labialis, esophagitis, benign crbl. ataxia, faryngotonsitis, pneumonitis, hepatitis	Sensoric ganglia n. V
HSV-2	HHV-2	Herpes genitalis, Neonatal herpes, encephalitis	Herpes genitalis, esophagitis, pneumonitis, hepatitis	Sacral sensoric ganglia
VZV	HHV-3	Varicella - Chicken pox	Herpes Zoster (Shingles), encephalitis/cerebelitis, pneumonitis, hepatitis	Sensoric ganglia n. V Dorsal spine ganglia

In allogeneic HSCT setting less frequently in case of acyclovir prophylaxis; reactivation of HSV without ACV prophylaxis in 80% of patients

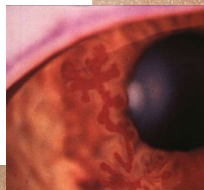
Detection: serology and PCR

Therapy: acyclovir, foscarnet, idoxuridin ...



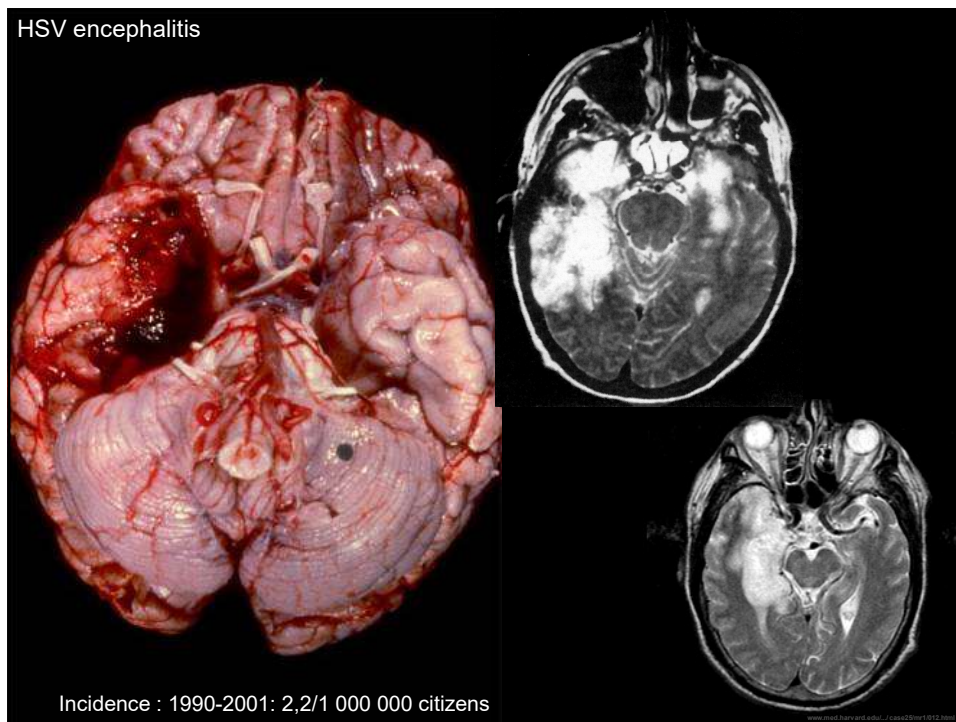
Pathological impact of HSV and VZV

- Intraepidermal vesicles
- no destruction of the basal membrane

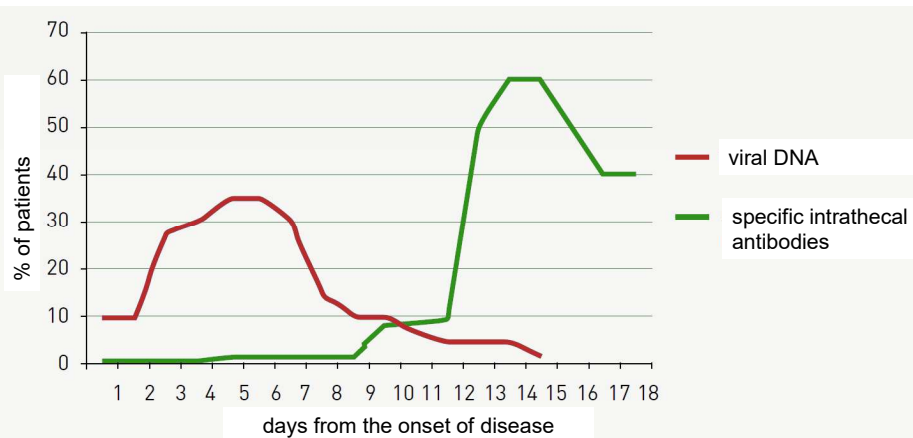


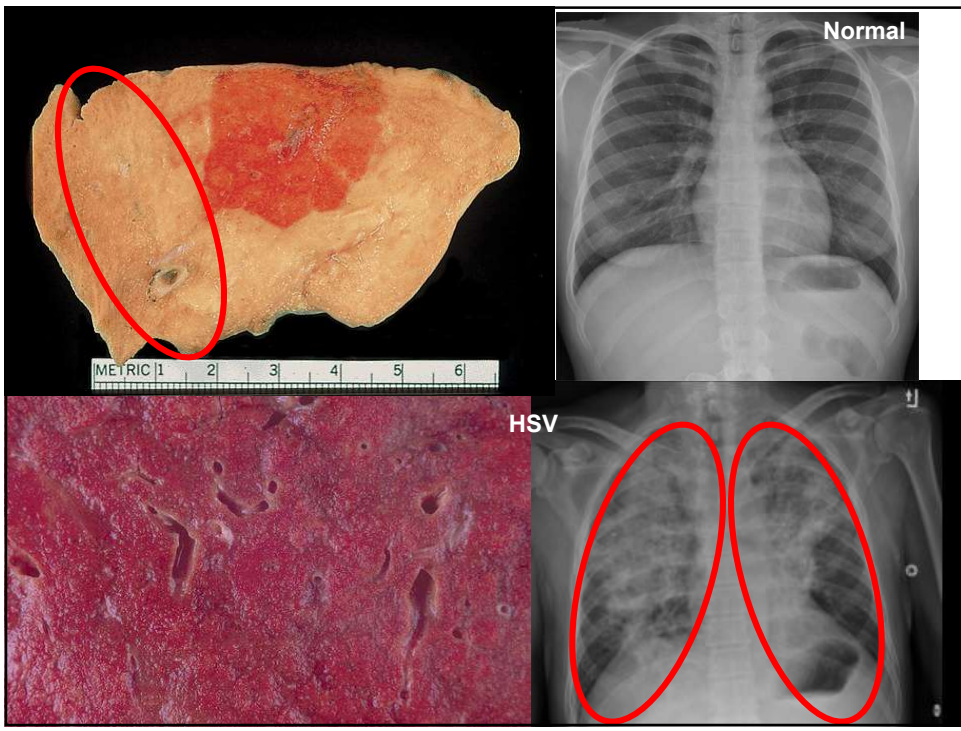
Varicella – chicken pox





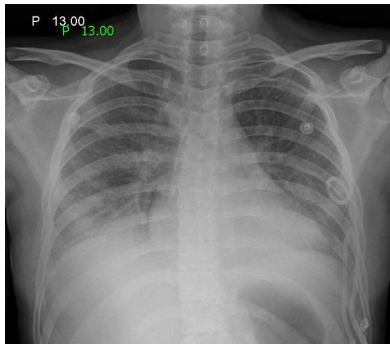
Antibody response to viral infection and detection of virus in CSF



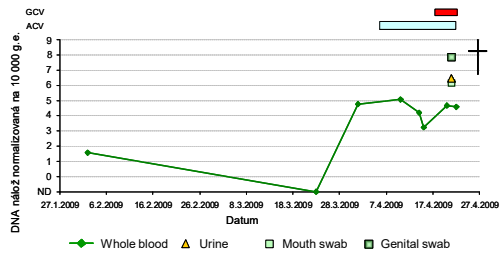


Different impact and destruction in different organs

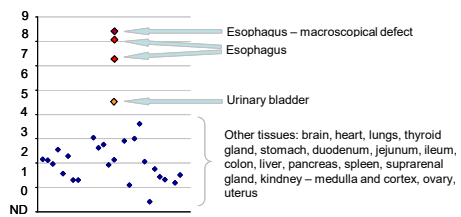
HSV (girl treated for ALL)



HSV pneumonia



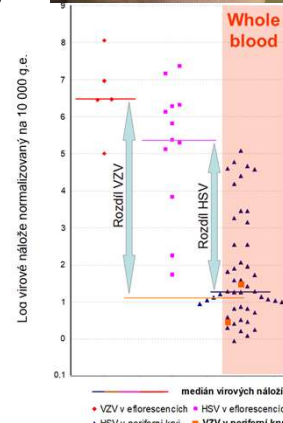
HSV quantity detected in the tissue at the autopsy



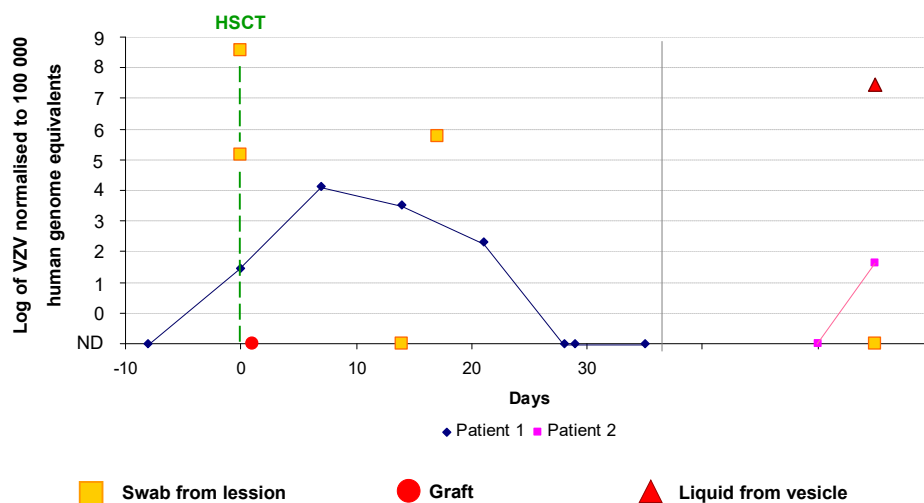
Varicella – Zoster Virus

- Incubation period 10-21 days
- Infectious especially in catarrhal stage
- Proliferation in respiratory system
dissemination to LN, to primary viremia, proliferation
- Proliferation in liver and spleen – secondary viremia (after 10-15 days fever) – dissemination to dermis
- In 90% benign
- Secondary infections of vesicles (usually *S. aureus*, *S. pyogenes*)
- infection of mucous tissue

Primary infection	Recurrent infection / Reactivation	Place of latency
Varicella - Chicken pox	Herpes Zoster (Shingles), encephalitis/cerebellitis, pneumonitis, hepatitis Postherpetic neuralgia	Sensoric ganglia n. V Dorsal spine ganglia (C3-L2)



Difference in materials VZV – chicken pox at D+0



Neonatal HSV infection



- **Primary infection during pregnancy** (STORCH/TORCH)
- **Neonatal infection**
 - during delivery, or at the end of pregnancy
 - rare, often lethal



Type of infection	Specification
Skin, eye and oral herpes	Localised infection, mucous and skin lesions without infections of inner organs
Disseminated infections	Especially herpetic hepatitis
Herpetic encephalitis	CNS infection with seizures and tremor

β herpesviruses

- closely granulocytes, monocytes, endothelial cells, fibroblasts...

Virus	Taxonomy	Primary infection	Recurrent infection / Reactivation	Place of latency
CMV	HHV-5	99% asymptomatic, slight hepatitis Mononucleosa-like syndrome Congenital CMV disease	Esp. IS and AIDS patients; Pneumonia, GIT (necrotising pseudomembranous colitis, esophageous ulcers), retinitis, encephalitis,...	Granulocytes, mononuclear cells
HHV-6B	HHV-6	Six disease, exanthema subitum, Roseola infantum	Encephalitis (Limbic), pneumonitis, hepatitis	Mononuclear cells, Lymphocytes CD4+
HHV-7	HHV-7	Asymptomatic, Roseola infantum	Encephalopathy, post-infectious myeloradiculoneuritis, epilepsy, hepatitis, hypersensitive syndrome	Lymphocytes CD4+
HHV-6A	HHV-9	Orphan virus		

Detection: serology and PCR

Therapy: ganciclovir, foscarnet, cidofovir, maribavir, letermovir...

Pathological impact of CMV

In immunocompetent

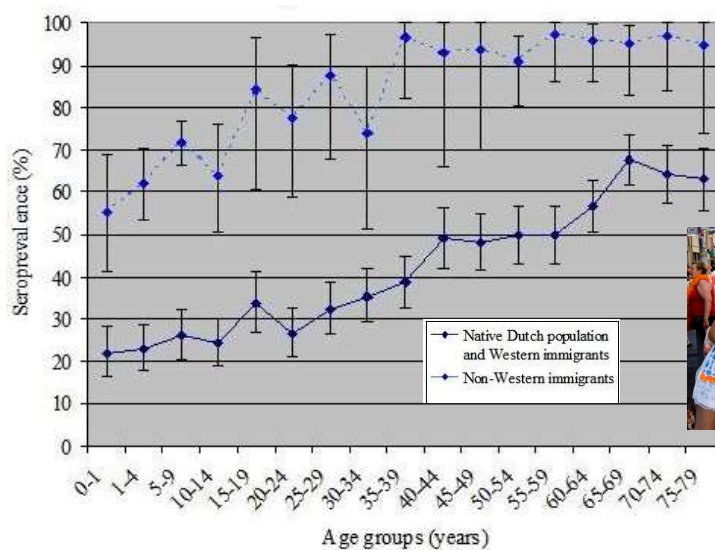
Asymptomatic in 95% of children
mononukleosis like sy.
In pregnant woman teratogenic
Associations with malignant
glioma, ca. of breasts
Possible association with
Alzheimer's disease

In immunocompromised mainly

trombocytopenia, pneumonitis,
hepatitis, encefalitis, retinitis, colitis,
esofagitis, pankreatitis,
vasculitis, malaise, vomiting, arthralgia,
myalgia



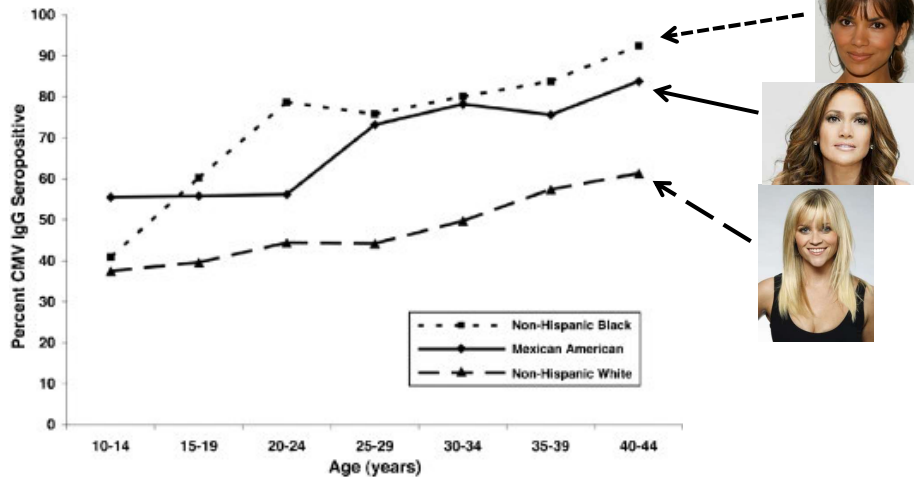
CMV seroprevalence



Korndewal et al. European Society for Paediatric Infectious Disease 2012
<https://www.eurospid.eu/en/ESPID-COVID-19-2020-2021>

CMV seroprevalence

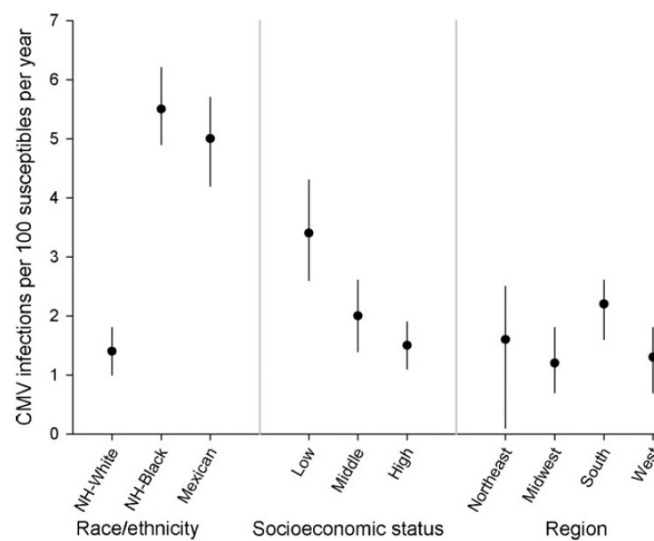
- 60-90% of healthy adult population <http://www.tulane.edu/~dmsander/garryfavweb.html>
- increases with age and decrease in developed countries



Cannon JCV 2009

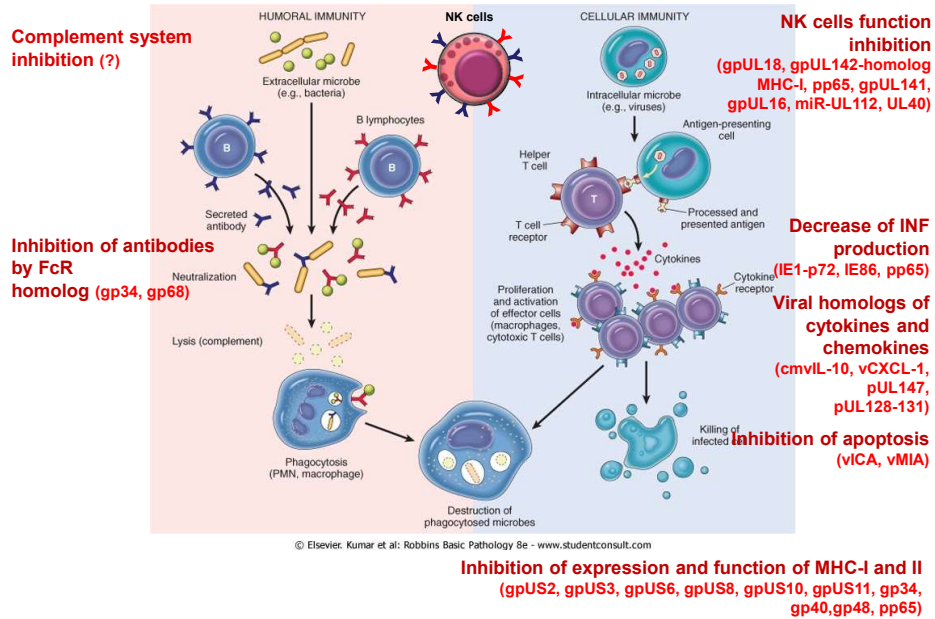
Incidence of CMV primoinfection

CMV Force of Infection by Race/Ethnicity, SES, and Region



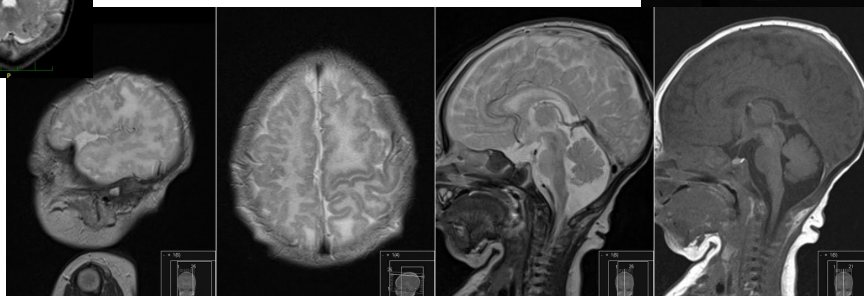
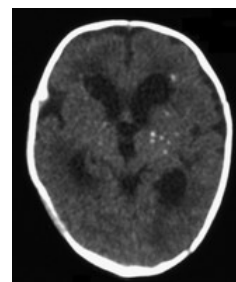
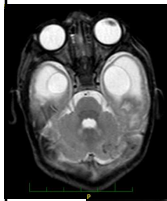
Cannon JCV 2009

How CMV manipulates with immunity?



Teratogenic impact of CMV

- In primoinfection in pregnancy or reactivation
 - TORCH (Toxoplasmosis, O – Other infections, Rubella, CMV, HSV-2)
 - Brain destruction, hepatopathy, problems in blood count
 - Cause of sensorineural hearing loss in about 30-50% clinically symptomatic children and 8-12% of asymptomatic children.



Symptoms and impact of cCMV



Placental infection

- swelling of the placenta – worse diffusion characteristics
- smaller cotyledon development – smaller placental surface

IUGR

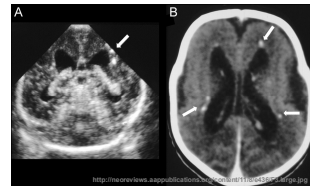
Fetal infection

- bone marrow suppression **petechia, „blueberry muffin baby“**
- CMV end-organ infection
- vasculitis – especially eyes and a CNS

Neurologic problems/seizures
Brain calcification/ cavity

CMV excretion to urine

Premature delivery



Symptoms and impact of cCMV

Asymptomatic

90% of children with cCMV



Symptomatic



<http://medictrivia.files.wordpress.com/2010/07/blueberry-baby1.jpg>

Congenital CMV infection (cCMV)

According to CDC

Symptoms of congenital CMV at delivery

Premature birth
Hepatopathy (Hepatomegaly, Jaudice)
Pulmonary signs (Pneumonitis)
Splenomegaly
Petechia and purpura
IUGR
Neurological seizures

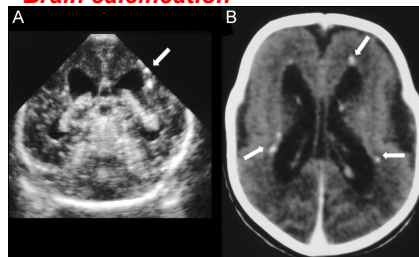


Blueberry muffin baby characterized by purpura as a sign of extramedullary hematopoiesis.

Long term effects of cCMV – Permanent outcomes

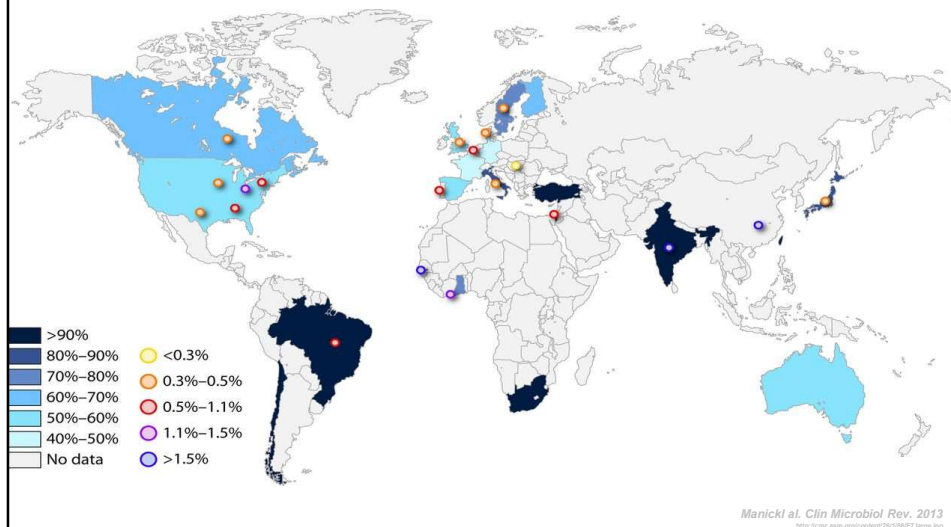
Sensoneural hearing loss (SNHL)
Visual loss
Mental disorder
Microcephaly
Motorical problems (coordination)
Neurological seizures (epilepsy)
Rarely death

Brain calcification

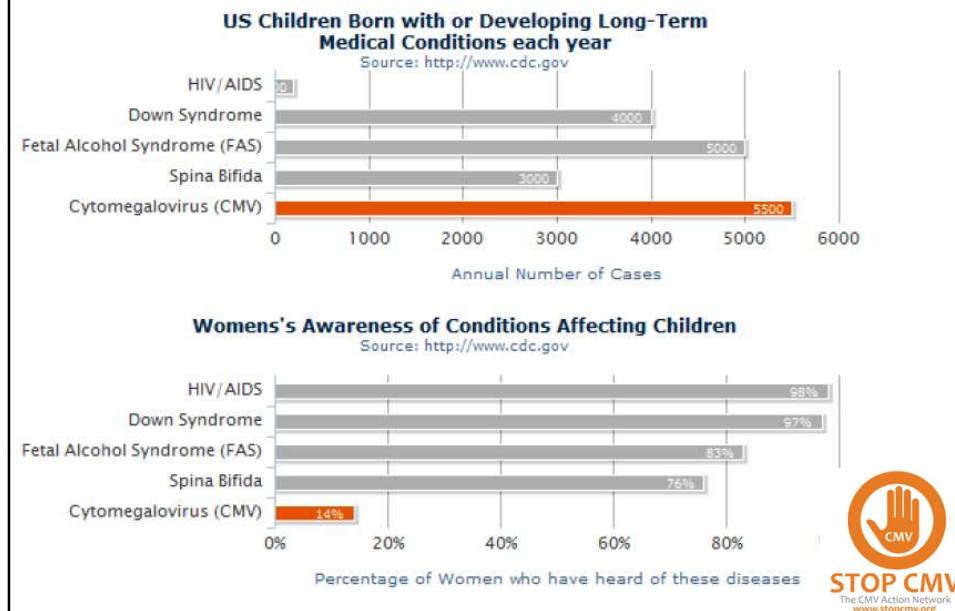


What is the frequency of cCMV?

There is 370,000 children born every day in the world, representing 134 millions/year.
Average frequency approx. 1,5% of living birth – 2.01 millions of children with cCMV/year.
In Europe and Czech Republic is estimated frequency 0.5-1% cCMV of living newborns.



What is a knowlegde about cCMV and its impacts?



Patological activities of HHV-6

HHV-6

Recently 2 distinct viral species

HHV-6 A

Unknown
„Orphan virus“



HHV-6 B

Immunocompetent host

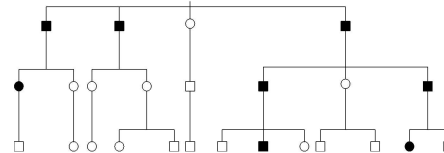
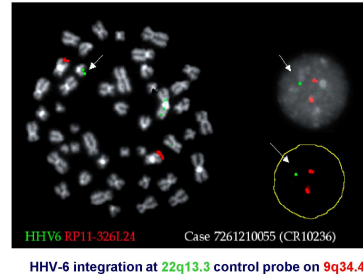
- Sixth disease
- Febrile seizures
- Encephalitis

Immunocompromised host

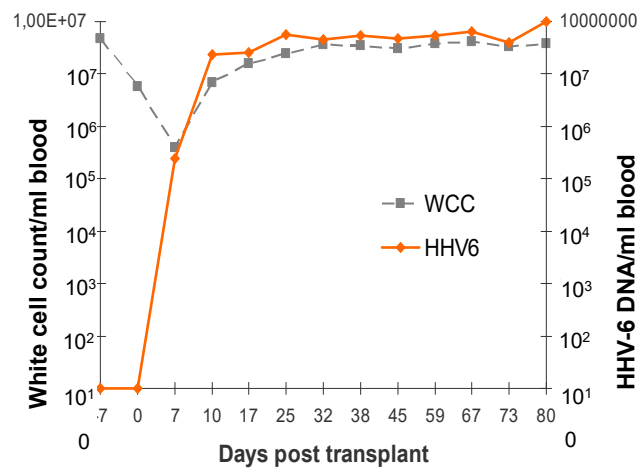
- Encephalitis
- Hepatitis
- Pneumonitis
- Pericarditis
- Delayed engraftment after HSCT

Chromosomally integrated HHV-6 (ici-HHV-6)

- Viral DNA integrated into human chromosomes
 - Inherited from parents to child
 - Viral DNA is present in every body cell (e.g. hair roots, nails)
 - Ratio of viral DNA : human DNA = 1:1
- Described frequency in population between 0.2-2.9%
(Tanaka-Taya 2004, Ward 2007)
- Both variants (A or B) integrates
- No clear observed reactivation ici-HHV-6 to active infection in vivo
- In vitro reactivations are doubtful

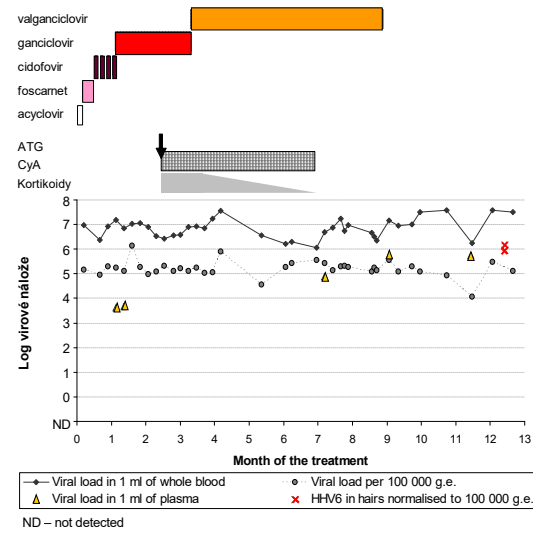


HHV6 DNA in blood after HSCT donor with ici-HHV-6



Clark et al., JID 2006

Chromosomally integrated HHV-6 (ici-HHV-6)



Patient with SAA
50 years
After start of the IS therapy– partial response only
Dependent of thrombocyte infusion
G-CSF therapy
Died due to peracute sepsis of *St. aureus*.

Detection of high HHV-6 DNA quantity is NOT NECESSARY an active infection.
Detection in hair, or nails detects Ci-HHV-6 safely.

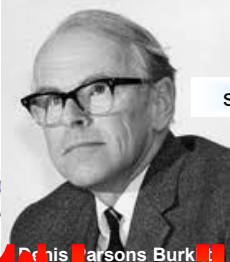
γ herpesviruses

- closely lymphocytes, epithelial cells, endothelial cells...

Virus	Taxonomy	Primary infection	Recurrent infection / Reactivation	Place of latency
EBV	HHV-4	asymptomatic Infectious mononucleosis Chronic active EBV infection	Esp. IS and AIDS patients; hairy leucoplakia, encephalitis, pneumonia, hemolytic anemia EBV associated malignancy (Nasopha. ca., Burkitt's ly., HD, post-tx. EBV-LPD)	B lymphocytes, epitelial cells
KSHV (Kaposi sarcoma herpesvirus)	HHV-8	Usually asymptomatic	Kaposi sarcoma Primary efuse lymphoma Castelman disease	Endothelial cells

Detection: serology and PCR
Therapy (EBV): symptomatic, anti-CD20, IFN-α, specific EBV lymphocytes

EBV discovery



surgeon

1958
„A sarcoma involving the jaws of African children.” *British Journal of Surgery*

1961
„The Commonest Children's Cancer in Tropical Africa — A Hitherto Unrecognised Syndrome.”

1963 - 1. kultivace viru

1964 – Publikováno v *Lancet*:
„Cultivation in vitro of human lymphoblasts from Burkitt's malignant Lymphoma”



Burkitt's lymphoma high incidence




Michael Anthony Epstein
(*1921)
Patolog, specialista na elektronovou mikroskopii

1st described human oncogenic virus

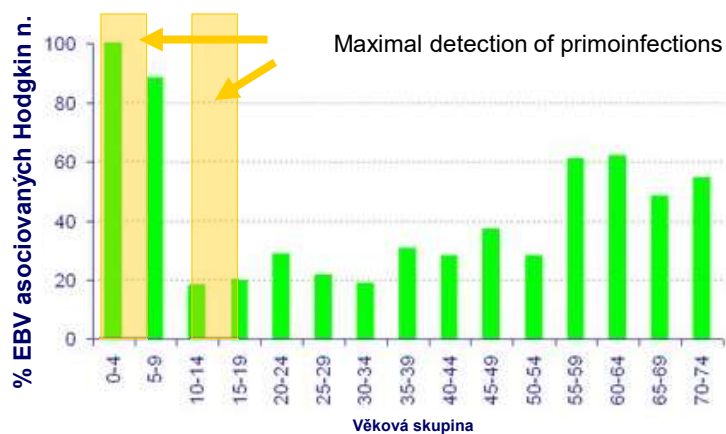
Yvonne M. Barr
(*1932)

Bert Geoffrey Achong
(1928-1996)

Transmission and epidemiology

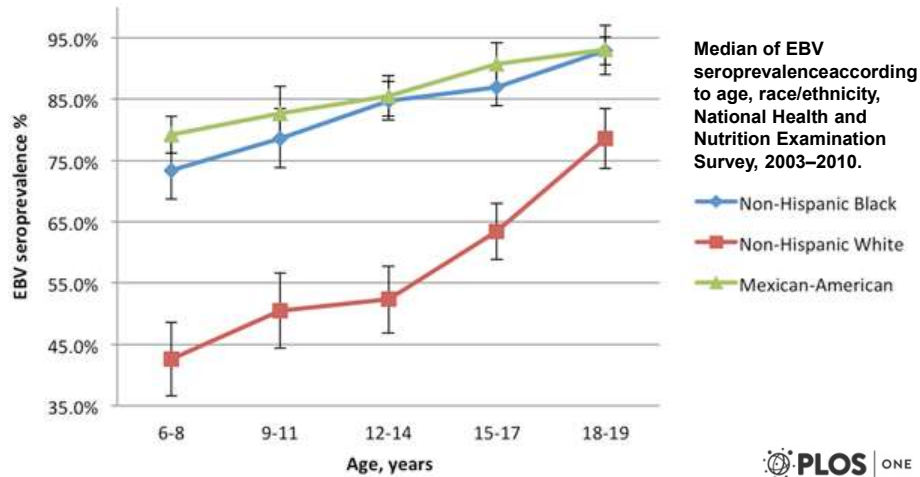
- Transmission through saliva by oral route
- 80 - 90% adult population is seropositive

(in developing countries, it is 90% of children older 2 yrs)

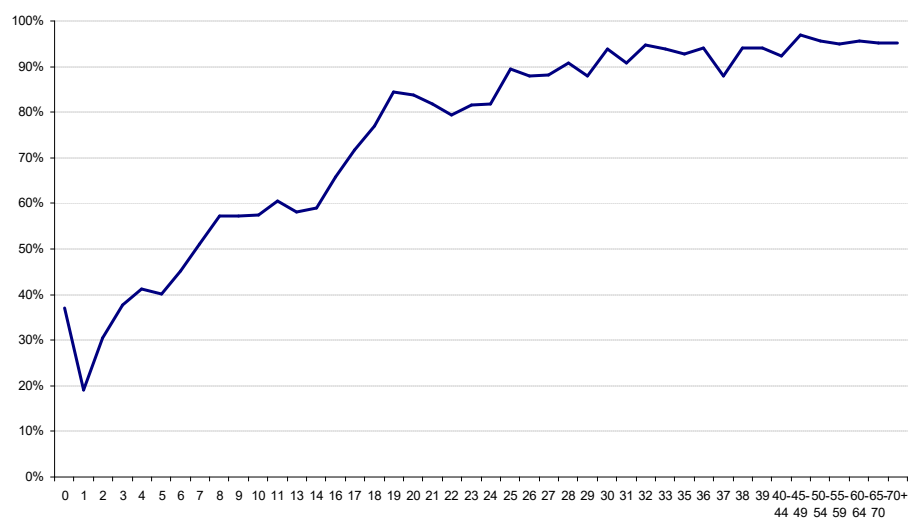


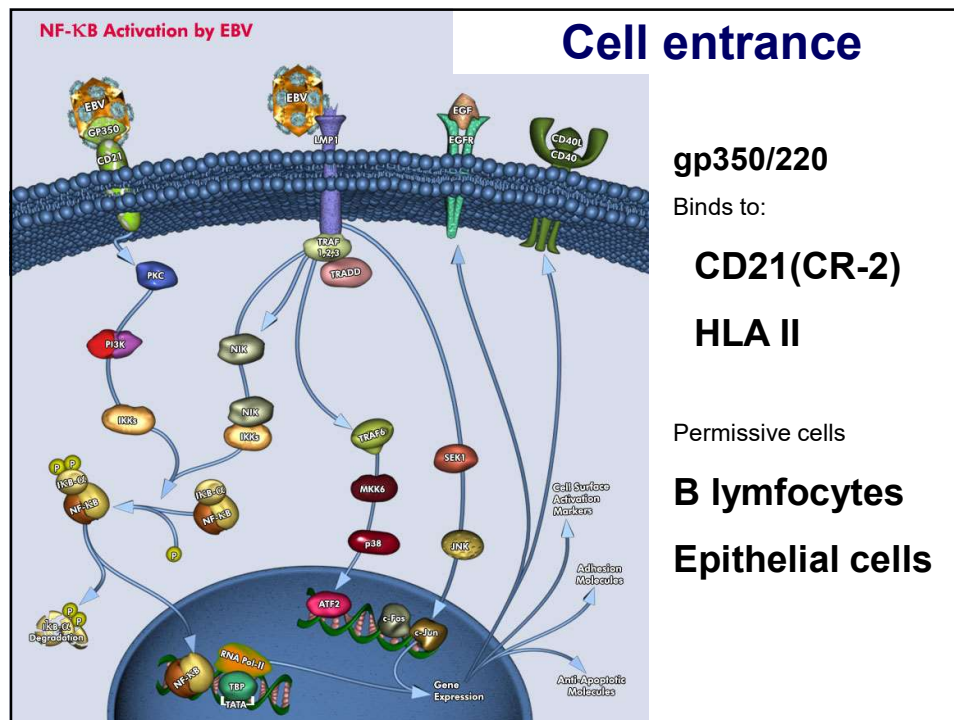
Transmission and epidemiology

- Transmission through saliva and oral route
- (permissive cells: B lymphocytes and epithelial cells)
- 80 - 90% of adults population is seropositive



Transmission and epidemiology of EBV in Motol UH

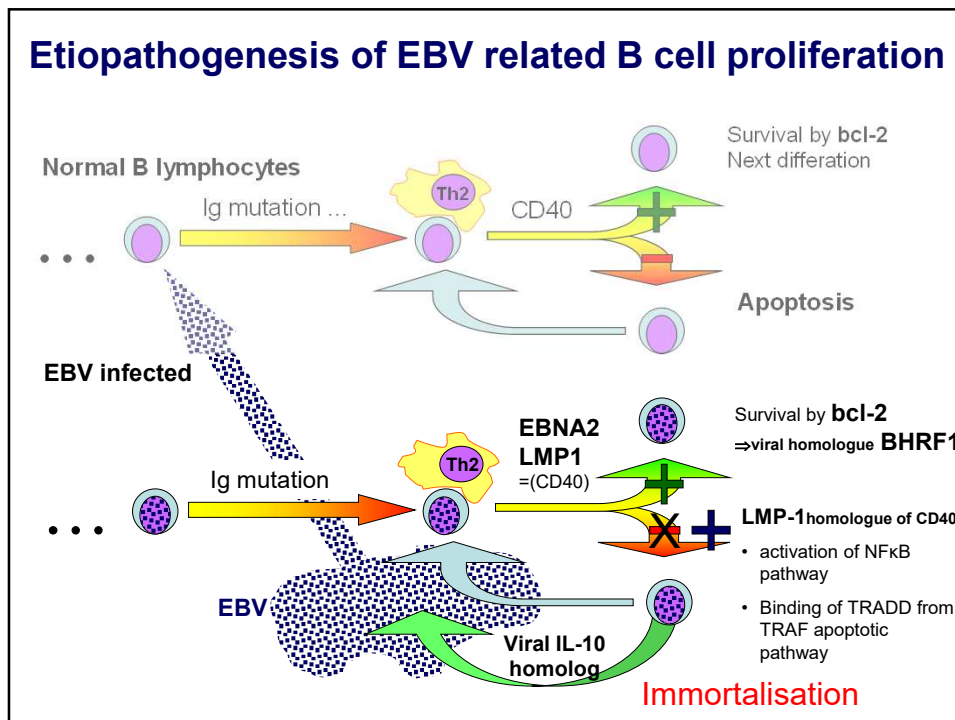
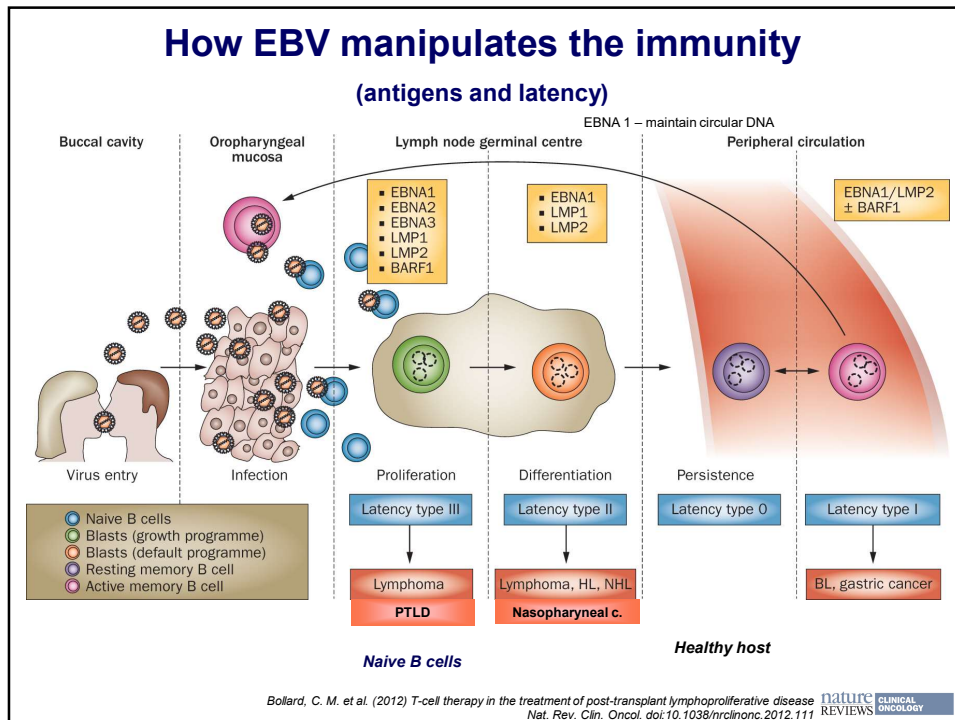




How EBV manipulates the immunity /proliferation?

EBNA-1	Sequence-specific DNA-binding protein to EBV element; sequence-nonspecific chromosome association protein; transactivator of viral latent genes and host genes; responsible for episome replication, segregation and persistence of viral genome; <u>involved in p53 degradation and oncogenesis</u>
EBNA-LP	Transcriptional coactivator of EBNA-2-dependent viral and cellular gene transcription; primarily indirectly associates with host DNA sites located at or near the transcriptional start; associates with cellular transcriptional (co)factors and EBNA-2; <u>dismisses repressor complex from promoter or enhancer sites</u> ; is essential for EBV-mediated B-cell transformation
EBNA-2	Together with EBNA-LP cooperatively activates viral and cellular gene transcription for transformation; primarily indirectly associates with host DNA sites located at the enhancer or intergenic region; associates with cellular transcriptional (co)factors and EBNA-LP; <u>is critical for EBV-mediated B-cell transformation</u>
EBNA-3A	A coactivator of EBNA-2, EBNA-3A and EBNA-3C associations with RBPJ inhibit RBPJ recruitments to DNA; downregulate cMyc transcription and block EBNA-2 activation effects; <u>and induce CDKN2 and chemokines</u> . Induces G1 arrests, which is essential for EBV-mediated B-cell transformation
EBNA-3B	A coactivator of EBNA-2; dispensable for B-cell transformation; <u>viral tumor suppressor</u> ; and upregulates CXCL10. EBNA-3B-knockout induces DLBCL-like tumors
EBNA-3C	Coactivates with EBNA-2 host <i>CXCR4</i> and <i>CXCL12</i> genes; induces CDKN2, chemokines and aurora kinase B; mediates RB degradation; attenuates H2AX expression and overcomes EBV-infection-mediated DNA damage response; promotes cell proliferation; <u>induces G1 arrests</u> ; essential for EBV-mediated B-cell transformation
LMP-1	Mimics the constitutively active form of CD40, a major EBV-encoded oncogene; activates NF-κB, JNK and p38 pathways; is critical for EBV-mediated B-cell transformation, a major EBV-encoded oncogene; activates NF-κB, JNK and p38 pathways; and induces EMT of NPC and acquisition of CSC-like properties
LMP-2A	Mimics constitutively active, antigen-independent BCR signaling through constitutive activation of the ERK/MAPK pathway224; blocks antigen-dependent BCR signaling; <u>induces B-cell lymphoma in transgenic condition</u> ; is important but not essential for <i>in vitro</i> primary B-lymphocyte growth transformation; rescues the LMP-1-generated impairment in germinal center in the response to antigen in animals; confers resting B cells sensitive to NF-κB inhibition and apoptosis; suppresses differentiation and promotes epithelial cell spreading and motility in epithelial cells; and enriches cancer stem cell-like population
EBER	Most abundant EBV-encoded noncoding RNAs; <u>augments colony formation and induces growth</u> ; confers cells resistance to PKR-dependent apoptosis; induces cytokines and modulates innate immune response; binds to La, PKR, L22, PRR and RIG-I; and EBER-mediated RIG-I activation likely contributes to EBV oncogenesis. EBER blockades of PKR-mediated phosphorylation of eIF2α results in blockage of eIF2α-mediated inhibition of protein synthesis and resistance to IFNα-induced apoptosis
miRNAs	Transcribed from BART and BHFR1; validated targets include Bim, BRUCE, CXCL11, DICER1, PUMA; has a role in sustaining latently infected cells. <u>BHFR1 miRNA and BART miRNAs interfere with apoptosis</u> . The miR-BART15-3p promoted apoptosis 331

Experimental & Molecular Medicine (2015) 47,



Infectious mononucleosis

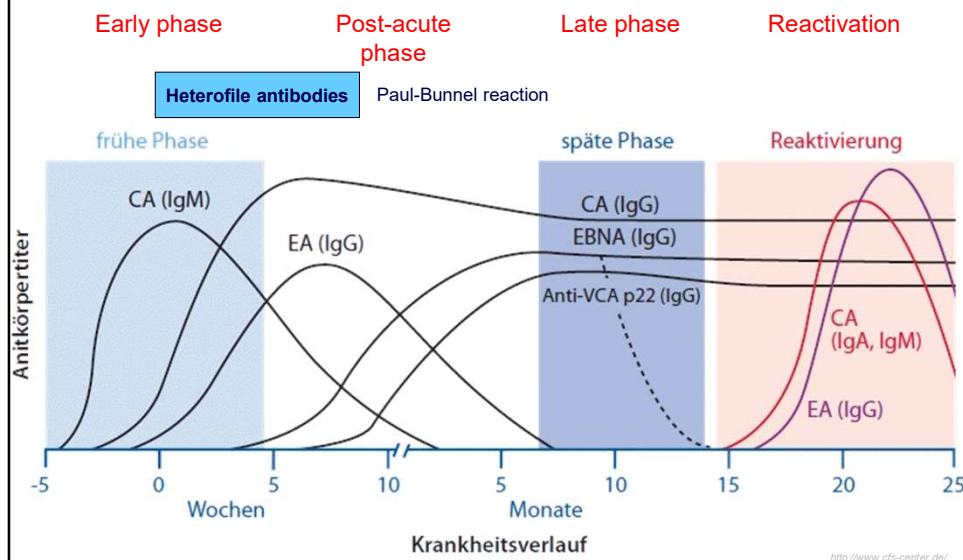
- „Kissing disease“
- Proliferation affects spleen, liver and lymph nodes
- Tiredness lasting for weeks, increased temperature and fevers (often approx. 39 °C), pharyngitis and swelling of the lymph nodes (submandibular and cervical); hepatosplenomegaly, hepatopathy, swelling of the eye lashes and face, malaise
- Incubation period 4-6 weeks
- At the beginning seems like „tonsillitis“
- Transmission by saliva
- Treatment approx. 6 months
Relax and diet (2-3 months);
Subsequently it is necessary to have some relax in physical activity



<http://home.teleport.com/~bobbi/InfectiousMononucleosis.htm>

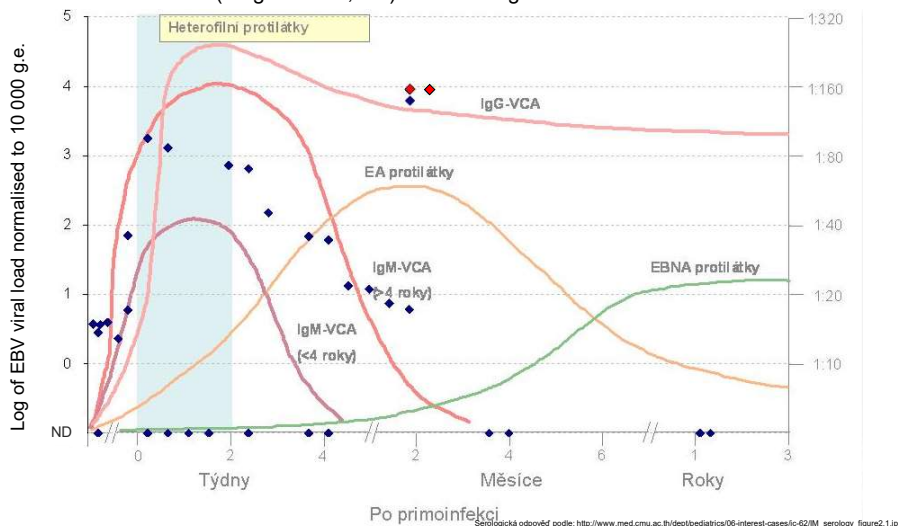
Diagnostics

Basic diagnosis of EBV is indirect – serological.



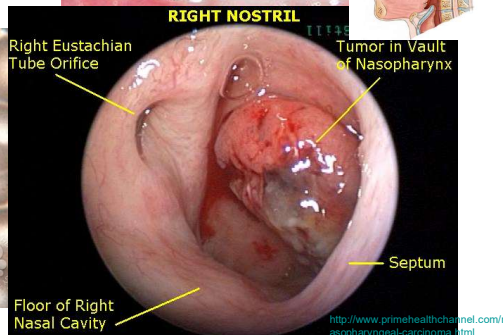
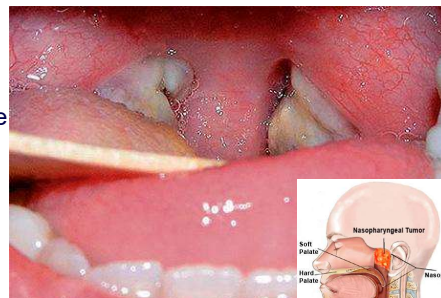
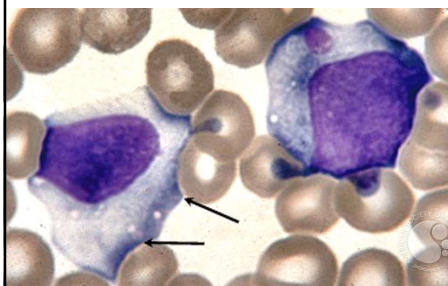
Viral load in patients with dg. B27 - IM

- Positive – 26 patients (62%)
 - 50 samples positive (65%); median of positivity 110 (range 11-157,670) in 100 000 g.e.



Pathological activities of EBV

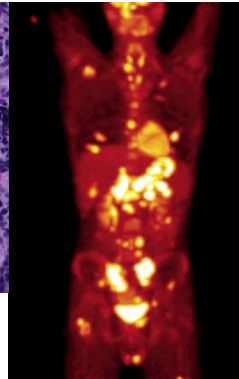
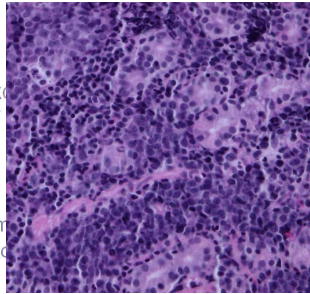
- **Immunocompetent host**
 - Infectious mononucleosis
 - Chronic active EBV infection
 - X-banded lymphoproliferative disease
 - Malignant diseases
 - Hodgkin disease
 - Burkitt's lymphoma
 - non-Hodgkin T/NK lymphoma
 - Nasopharyngeal carcinoma
 - Gastric carcinoma
 - Angioblastic T lymphoma



Patological activities of EBV

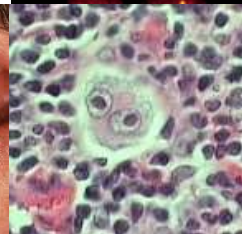
• Imunokompetentní hostitel

- Infekční mononukleóza
- Chronická aktivní EBV infekce
- X-vázaná lymfoproliferativní
- Maligní onemocnění
 - Hodgkinova nemoc
 - Burkittův lymfom
 - non-Hodgkinský T/NK lymfom
 - Nasopharyngeální karcinom
 - Karcinom žaludku
 - Angioblastický T lymfom



• Immunocompromised host

- Hairy leukoplakia
- Above listed malignant diseases
- Post-transplant lymphoproliferative disease (EBV-LPD)
- Encephalitis/myelitis
- Pneumonia
- Hepatopathy/hepatitis

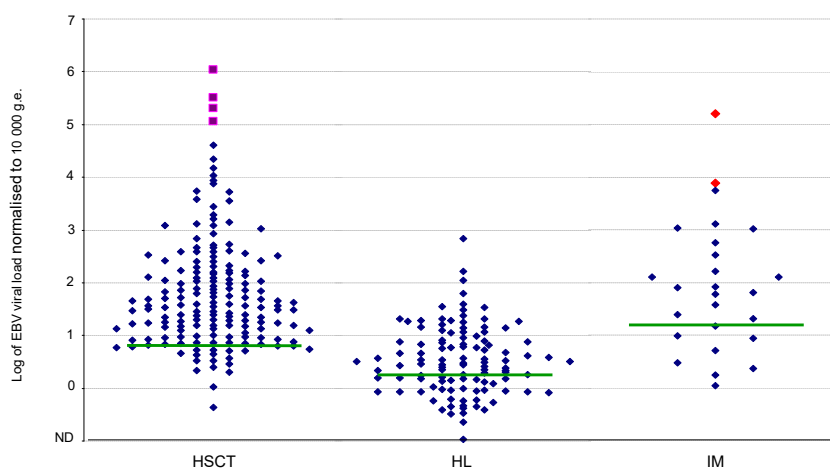


<http://www.ksom.edu/faculty/chamberlain/Website/lectures/lecture/aids.htm>

www.med-ed.virginia.edu/courses/path/immes/wcd/hodgkin

Direct detection - PCR

- Detection in peripheral blood (plasma, whole blood), possibly in the tissue
- In HL and IM, EBV is detected in peripheral blood in low quantity.
- Median of detected quantity in whole blood increased from HL → HSCT → IM



Chronic active EBV infection

Infected T lymphocytes and NK cells

Signs often connected with prolonged presence of interferons in the organism.

Diagnostic criteria of a case definition for SCAEBV [15]

Category	Criteria
Clinical	Intermittent fever, lymphadenopathy, and hepatosplenomegaly.
Hematologic	Anemia, thrombocytopenia, lymphocytopenia or lymphocytosis, neutropenia, and polyclonal gammopathy.
Virological	Elevated antibody titers and positivity for antibodies to EBV-related antigens (VCA IgG, ≥ 5120 ; VCA IgA, positive; EA [D] IgG, ≥ 640 ; EA [D] IgA, positive; and EA [D] and EA [R] IgG, ≥ 640) and/or detection of EBV genomes in affected tissues.
Other	Chronic illness that cannot be explained by other known disease processes.

F. Sánchez et al. / Annals of Diagnostic Pathology 12 (2008) 368–371

Chronic active EBV infection

	T-cell type (n = 16)	NK-cell type (n = 12)	P
Symptoms			
Fever, > 1 d/wk (%)	67	25	.04
HMB (%)	13	75	.002
Splenomegaly (%)	73	100	.08
Large granular lymphocytosis (%)	13	83	.0004
Calcification in basal ganglia (%)	7	33	.10
Laboratory data			
IgG (mg/dL, mean $\pm$ SD)	2213 $\pm$ 1104	1682 $\pm$ 464	.11
IgE (IU/mL, mean $\pm$ SD)	282 $\pm$ 298	2774 $\pm$ 3774	.04
VCA IgG (geometric mean titer)	2405	446	.01
EA IgG (geometric mean titer)	831	119	.02
EBNA (geometric mean titer)	30	45	.24
Viral load			
PBMC (copies/ μ g DNA, mean $\pm$ SD)	10 ^{4.1} $\pm$ 0.5	10 ^{4.4} $\pm$ 0.4	.09
Plasma (copies/mL, mean $\pm$ SD)	10 ^{2.9} $\pm$ 1.1	10 ^{2.4} $\pm$ 2.1	.49

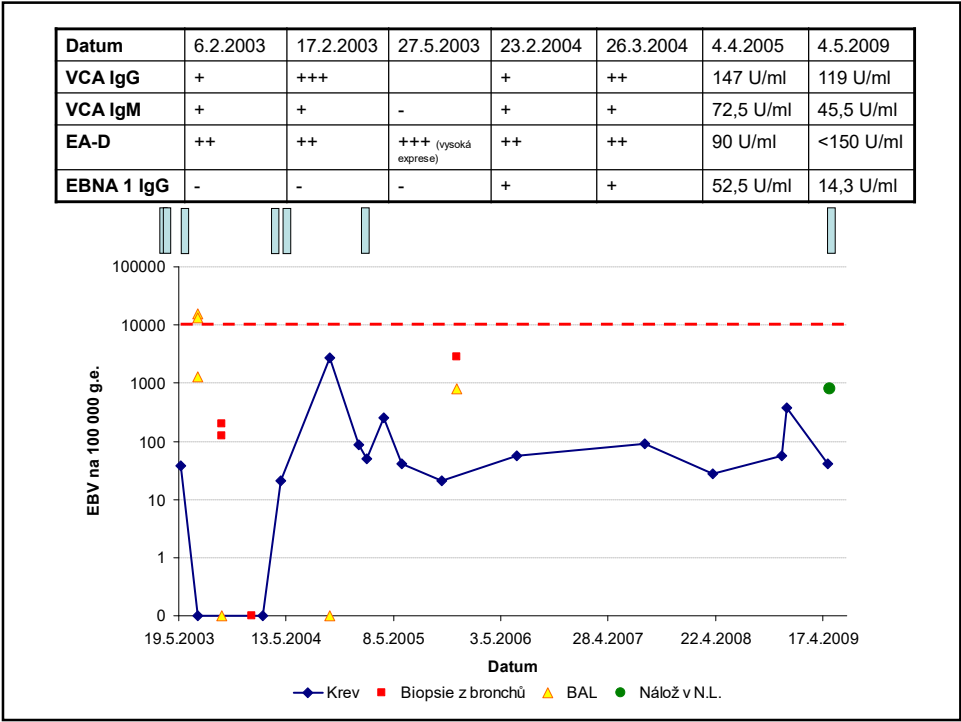
HMB indicates hypersensitivity to mosquito bites; VCA, viral capsid antigens; EA, early antigens; EBNA, EB nuclear antigens; PBMC, peripheral blood mononuclear cells. Fisher exact test was used to compare symptoms between groups. Student *t* test was used to compare the mean copy numbers of EBV-DNA or laboratory data. Bold letters indicate statistically significant results.

Table 2. Clinical features of 30 patients with chronic active Epstein-Barr virus infection

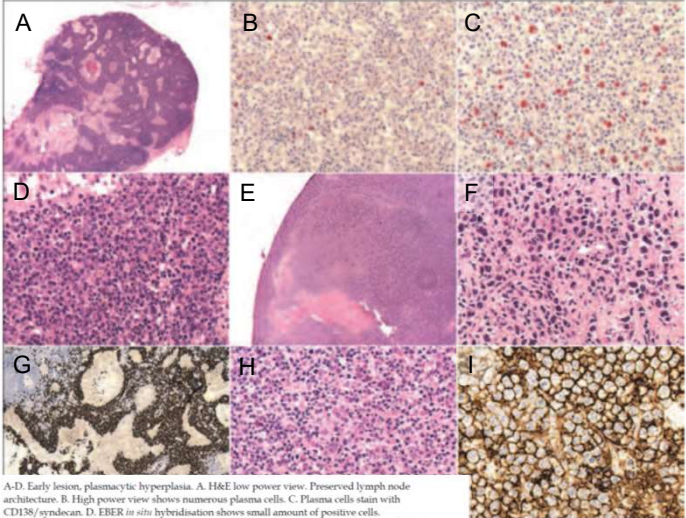
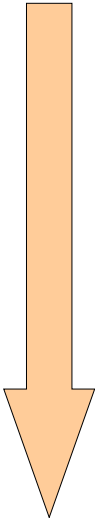
Symptoms and signs	(%)	Life-threatening complications	(%)
Fever	100	Hemophagocytic syndrome	21
Liver dysfunction	90	Coronary artery aneurysm	21
Splenomegaly	90	Hepatic failure	18
Lymphadenopathy	50	Malignant lymphoma	16
Thrombocytopenia	50	Interstitial pneumonia	12
Anemia	48	Central nervous system involvement	7
HMB	43	Sepsis	7
Skin rash	28	Pulmonary hypertension	4
Calcification in basal ganglia	18	Intestinal perforation	4
Oral ulcer	18	Myocarditis	4
Hydroa vacciniforme	14		

HMB indicates hypersensitivity to mosquito bites.

Kimura et al. Blood 15 July 2001, Vol. 98, No. 2

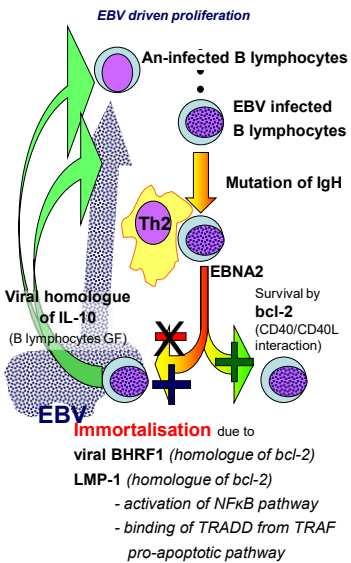


EBV associated posttransplant proliferative disease (EBV-LPD)



A-D. Early lesion, plasmacytic hyperplasia. A. H&E low power view. Preserved lymph node architecture. B. High power view shows numerous plasma cells. C. Plasma cells stain with CD138/syndecan. D. EBER *in situ* hybridisation shows small amount of positive cells.
E-G. Polymorphic PTLD. E. Low power view shows disturbed lymph node architecture. F. Higher power shows a polymorphic infiltrate composed of plasma cells, lymphocytes (small, medium-sized, large and Reed-Sternberg-like). G. EBER ISH shows numerous positive cells.
H-I. Monomorphic PTLD. H. Diffuse proliferation of large atypical cells. I. CD20 staining shows their B-cell origin (courtesy to Prof. Thomas Tousseyn).

Etiopathogenesis and classification EBV-LPD



World Health Organization Classification of Post-transplant Lymphoproliferative Disorder (PTLD)

Category	Subtype
Early lesions	Plasmacytic hyperplasia Infectious mononucleosis-like lesion
Polymorphic PTLD Monomorphic PTLD (classify according to lymphoma they resemble)	B-cell neoplasms - Diffuse large B-cell lymphoma - Burkitt lymphoma - Plasma cell myeloma - Plasmacytoma-like lesion - Other ^a T-cell neoplasms - Peripheral T-cell lymphoma NOS - Hepatosplenic T-cell lymphoma - Other
Classical Hodgkin lymphoma-like PTLD	cT1-2 gr 3 cT3-4

<http://www.cancernetwork.com/oncology-journal/lymphoma-risk-and-response-after-solid-organ-transplant>

Different symptoms of poly-, oligo- and monoclonal proliferation.

Mononucleosis-like syndrome

(fever, sore throat, myalgia, tonsillar hypertrophy and cervical lymphadenopathy, hepatopathy (bilirubinemia))

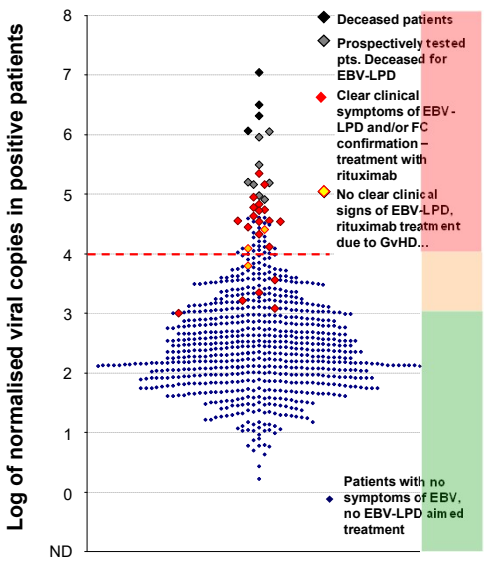
Tumorous form

(Symptoms secondary to the presence of lymphoid tumors: pain, obstruction, perforation, GI bleeding, respiratory distress, etc.)

Disseminated disease

(Proliferating B cells in blood and bone marrow, high fever and/or multi-organ failure)

Prospective testing – maximal quantity



➤ **Very high risk level reactivation**
 $> 10^4$ NVCs – ≈ 50 IU/ng
 suspected and/or correlated with signs of EBV-LPD - 102 samples from 26 children (7.1%) and 22 adults (3.2%)
EBV-LPD was confirmed in 22 (45%)

➤ **Risk level reactivation**
 $> 10^3$ and $< 10^4$ NVCs – $\approx 5-50$ IU/ng
 687 samples from 95 (26%) children and 104 adults (15%)

➤ **Benign level reactivation**
 $< 10^3$ NVCs $\approx 5 < \text{IU/ng}$
 low quantities with no clinical signs of EBV were detected in majority of samples from 275 children and 481 adults.

EBV-LPD diagnosis

Diagnosis of neoplastic EBV-LPD should fulfill at least 2 of the following criteria:

- Change and/or destroy of the cell tissue culture by lymphoproliferative process
- Presence of monoclonal, or oligoclonal proliferation proven with cell and/or viral markers
- Evidence of EBV infection in many cells (e.g.. DNA, RNA, protein...

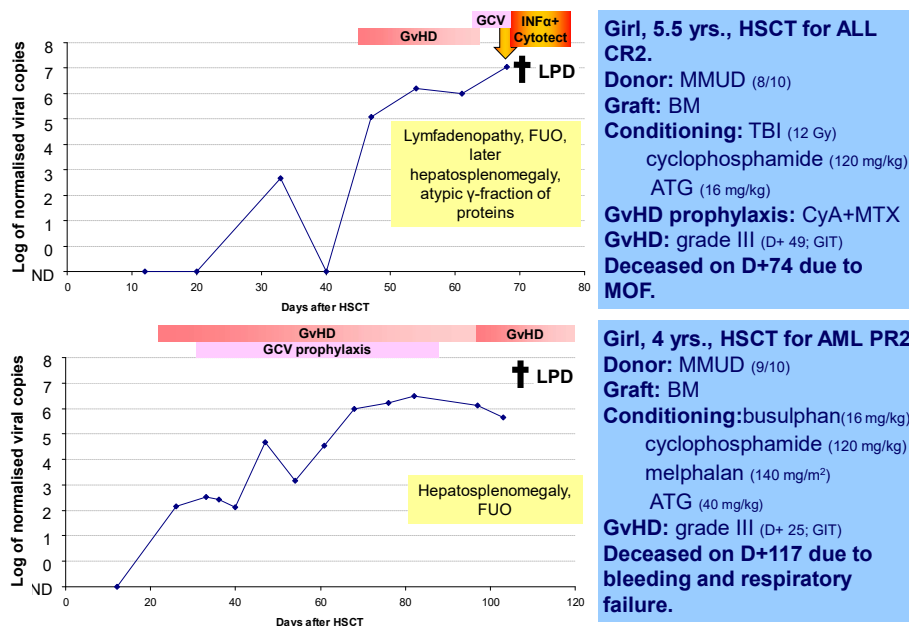
EBV DNA detection in whole blood is not enough.

Dle definice EBMT IDWP, 2007

- Clinical symptoms
- Image methods
- Immunology (Flow cytometry, Ig levels, clonality)
- Histology N.L. (detecting the presence of EBV)
- Direct detection of virus
 - EBV load (based mainly on NA detection)
 - Sample type: plasma, whole blood, MNC
 - Different methods of PCR – most frequently quantitative real-time PCR

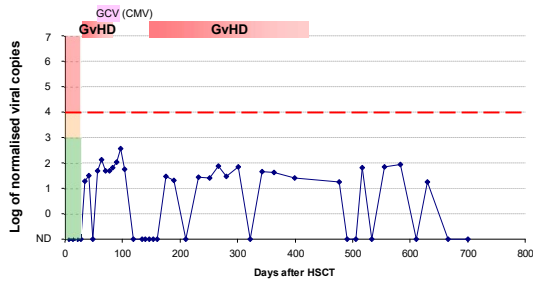


Retrospectively tested patients

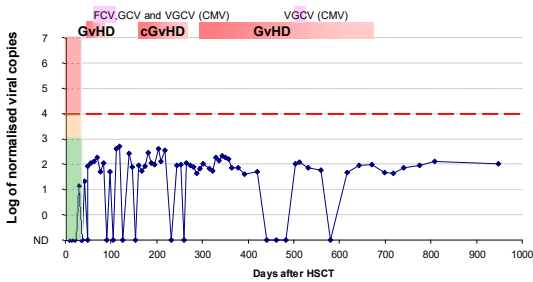


Benign level reactivation

< 10³ NVCs ≈ 5 < IU/ng

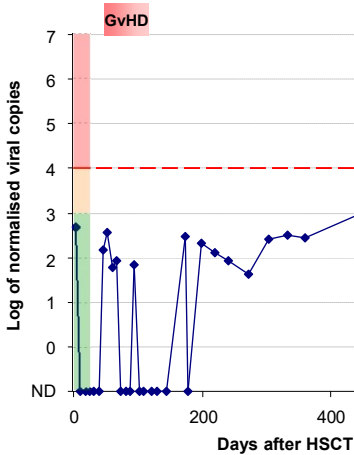


Boy, 13 yrs., HSCT for MDS-RAEB
Donor: MMUD (8/10)
Graft: PBSC
Conditioning: busulphan (16 mg/kg)
cyclophosphamide (120 mg/kg)
melphalan (140 mg/m²)
ATG (40 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade II (D+28;GIT)
Other: BKV-HC(D+40), CMV(D+55)
Outcome: alive, no clin. problems



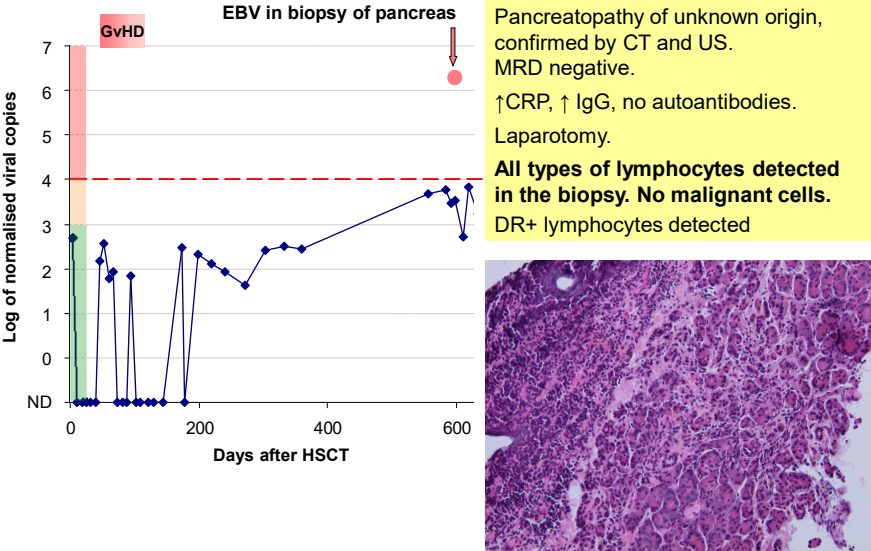
Girl, 13 yrs., HSCT for SAA
Donor: MMUD (9/10)
Graft: BM
Conditioning: TBI (5,4 Gy)
cyclophosphamide (200 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade II (D+40;GIT)
Other: CMV(D+46), lung affection of unknown
ethiology, steroid DM
Outcome: alive, no clin. problems

Localised EBV-LPD (NHL)

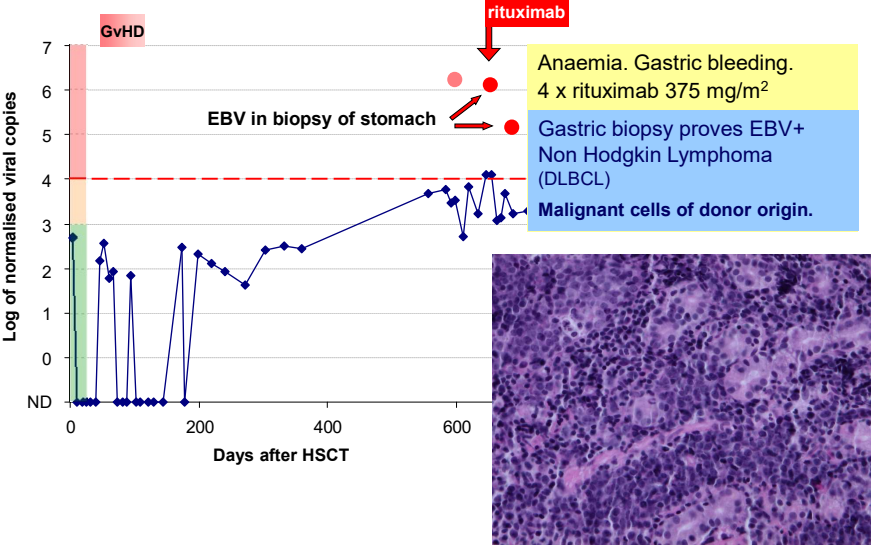


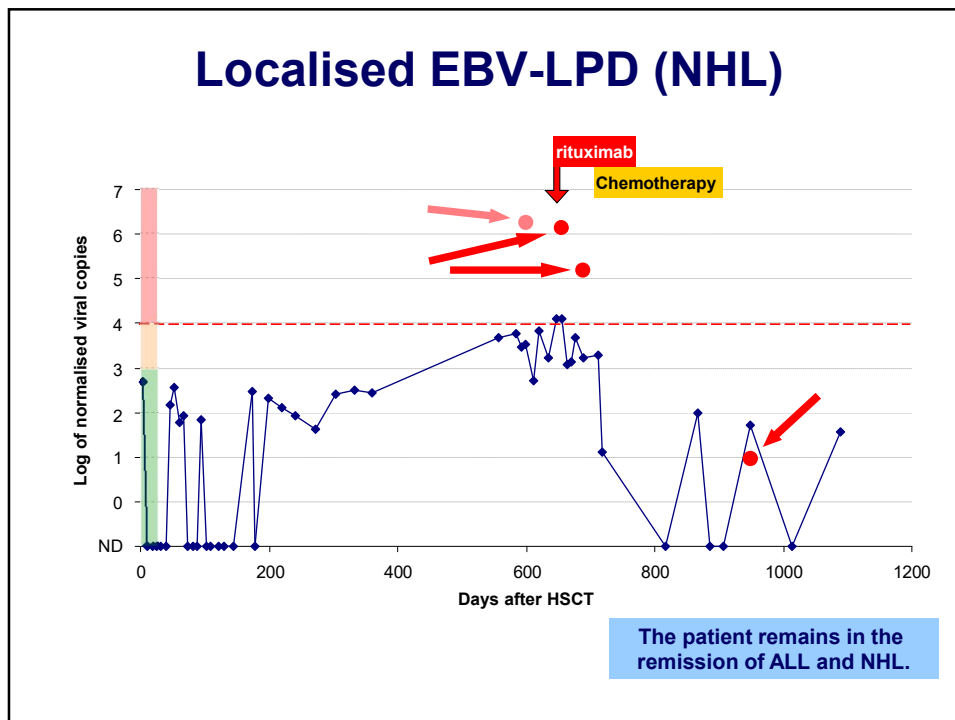
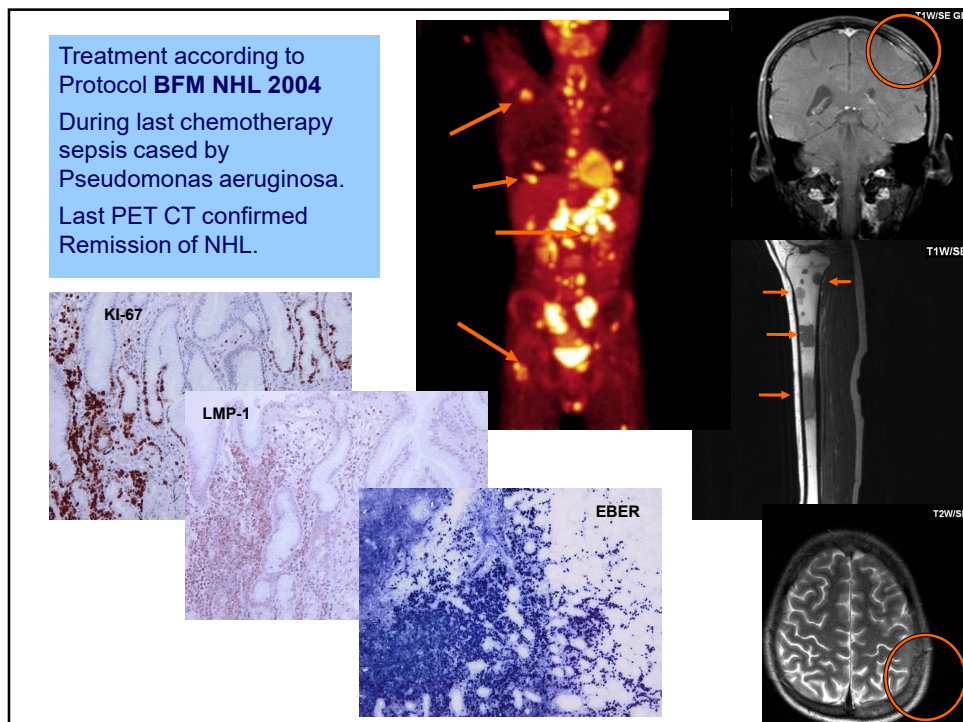
Boy, 15 yrs., HSCT for ALL in 1. CR
CMV, EBV, HSV seronegative
Donor: MSD (brother)
Graft: PBSC
Conditioning: TBI (12 Gy)
etoposid (60 mg/kg)
GvHD prophylaxis: CyA
Engraftment: D+14
Chimaerism: CC D+28
D+95 – 20% autologous (negative MRD)
D+130 – 1% autologous (negative MRD)
GvHD: grade II (D+28;GIT, skin)
Th: MP (1 mg/kg; until D+74) + CsA (until D+102)
D+280 – Herpes zoster (ACV)

Localised EBV-LPD (NHL)

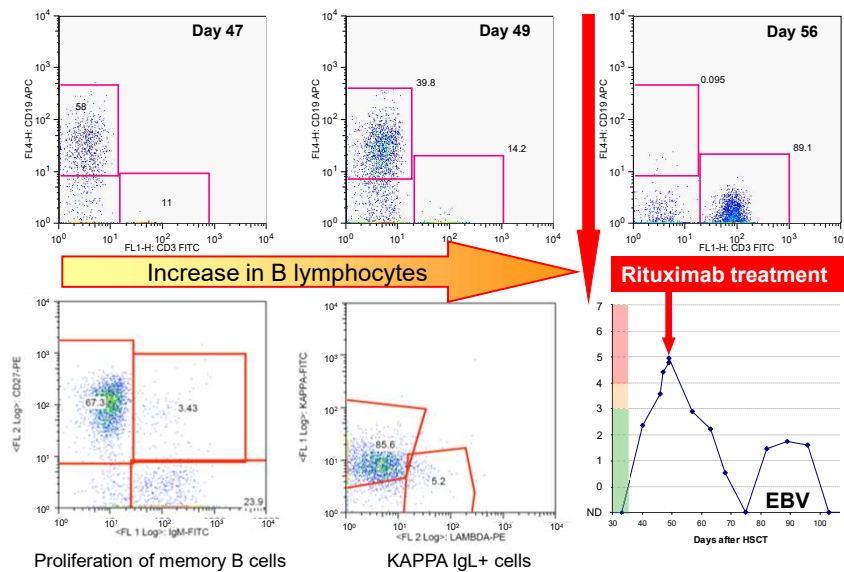


Localised EBV-LPD (NHL)





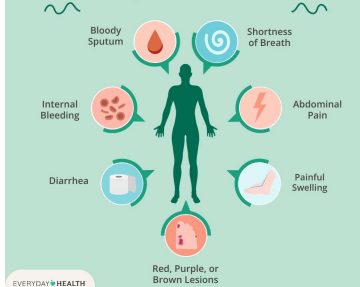
Flow cytometry EBV-LPD confirmation



HHV-8



Symptoms of Kaposi Sarcoma



- Kaposi sarcoma
- Primary exudative lymphoma
- Castelman's disease

Castelman disease

