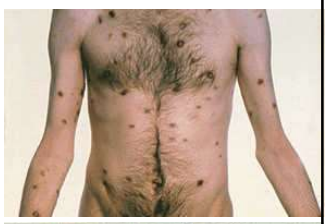
Petr Hubáček

Herpesviruses

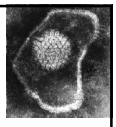
Why herpesviruses (DNA) viruses?

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

1. pneumocystis pneumonia
2. toxoplasma encefalitis
3. esofageal, 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. tuberkulosis
16. disseminated or extrapulmonary atypic mycobacteriosis
17. **Kaposhi sarkoma**
18. **malignant lymfoma (Burkitt‘s lymfoma, imunoblastic and primary cerebelar lymfoma)**
19. Invasi carcinoma of cervix
20. HIV encefalopatia
21. wasting syndrom

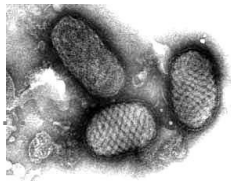


DNA viruses



ds DNA

- ***Herpesviridae*** → α HSV, VZV; β CMV, HHV-6A, HHV-6B and 7; γ EBV, HHV-8
- ***Adenoviridae*** → Adenoviruses (group A-F)
- ***Polyomaviridae*** → BKV, JCV, WUV, KIV, SV40...
- ***Papillomaviridae*** → Papillomaviry
- ***Poxviridae*** → Variolla virus, vaccinia, molluscum contagiosum
- ***Hepadna*** → HBV

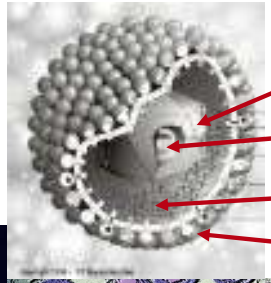


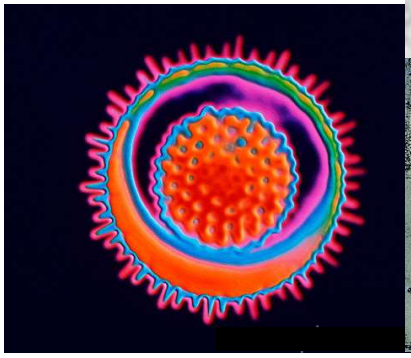
ss DNA

- ***Parvoviridae*** → Parvovirus B19, lidský bocavirus (HBoV)
- ***Anelloviridae*** → TTV, TTMV, TTMDV

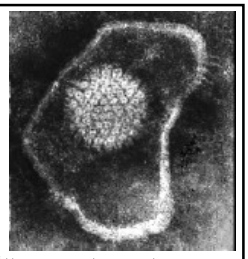
Herpesviruses

- Enveloped ds DNA viruses
- Genome of length 125-240 kb
- Icosahedral capsid
- Diametre of capsid of approx. 100 nm

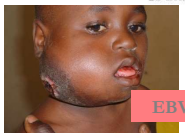


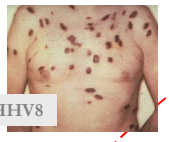


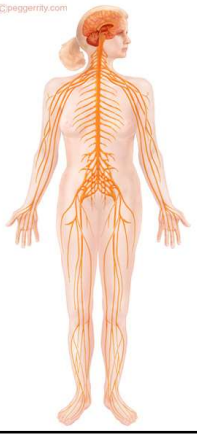
Taxonomy



http://www.tulane.edu/~dmsander/garryfavweb.html

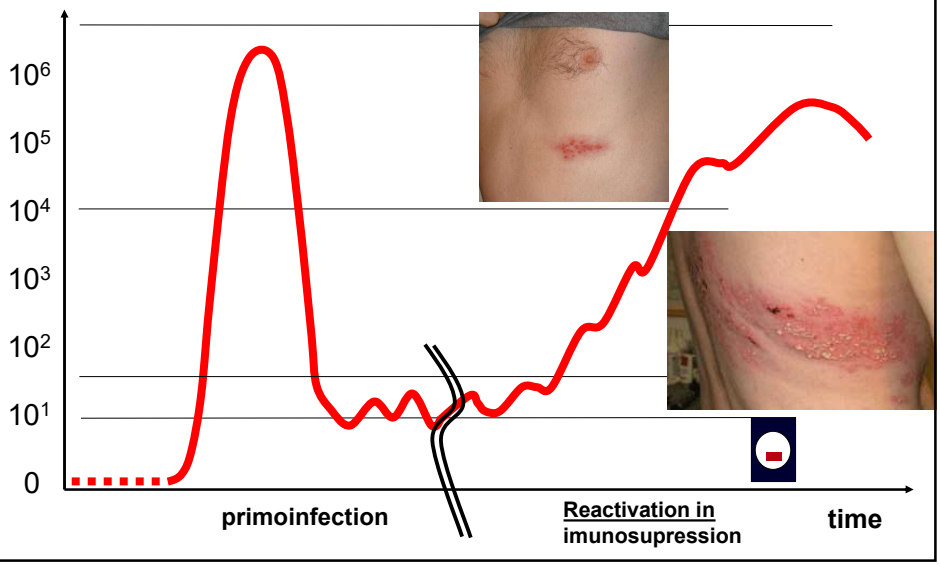


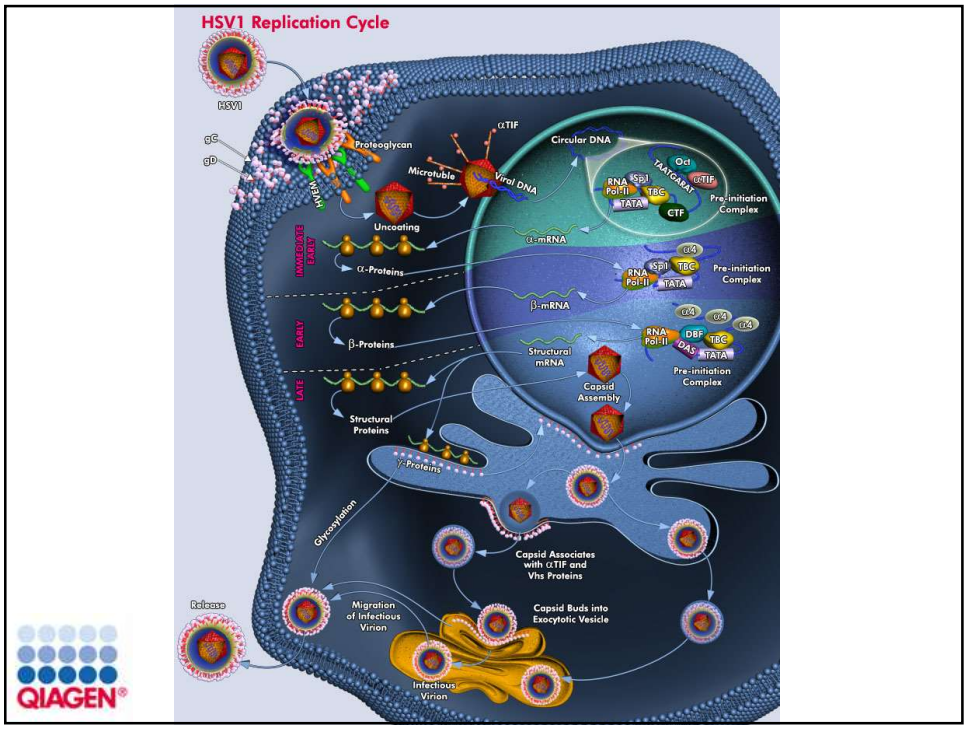


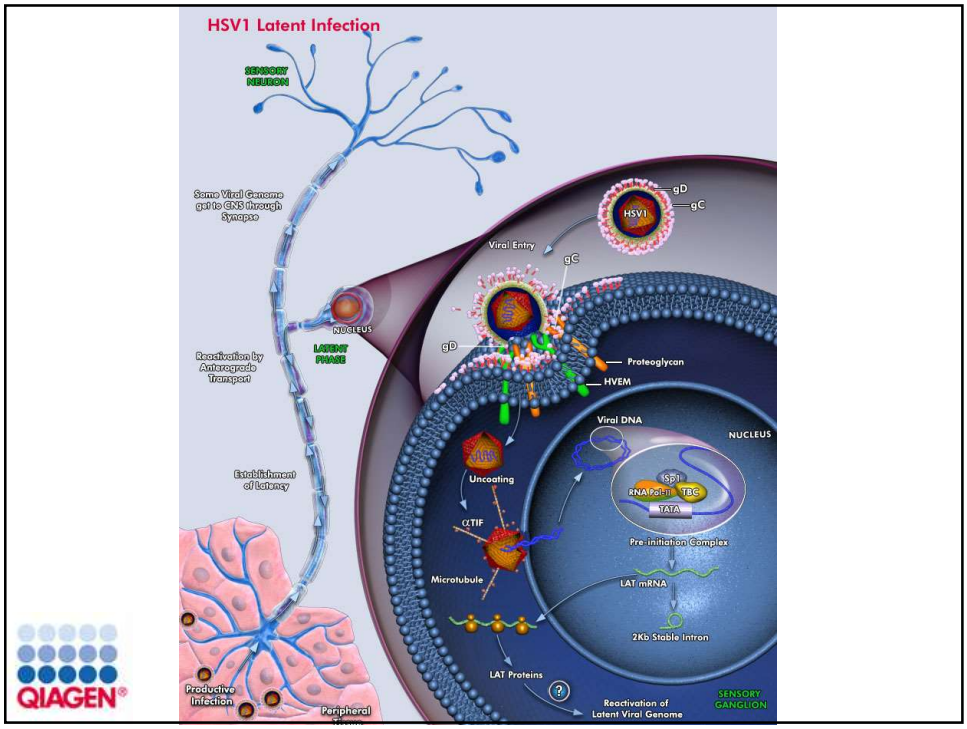


Latency with possibility of reactivation

Transmission – by body fluids (saliva, urine, breast milk, blood, …)




HSV1 Replication Cycle
HSV1
gC
gD
Proteoglycan
HVEM
Microtubule
αTIF
Viral DNA
Circular DNA
Uncoating
IMMEDIATE EARLY
α-Proteins
α-mRNA
Oct
TAATGARAT
RNA Pol II
Sp1
TBC
TATA
CTF
Pre-initiation Complex
β-mRNA
β-Proteins
EARLY
Structural mRNA
Capsid Assembly
LATE
Structural Proteins
γ-Proteins
Glycosylation
Capsid Associates with αTIF and Vhs Proteins
Migration of Infectious Virion
Capsid Buds into Exocytotic Vesicle
Release
Infectious Virion
QIAGEN

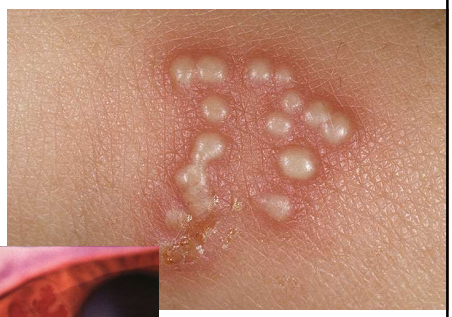

HSV1 Latent Infection
SENSORY NEURON
Some Viral Genome get to CNS through Synapse
NUCLEUS
LATENT PHASE
Reactivation by Anterograde Transport
Establishment of Latency
Productive Infection
Peripheral
HSV1
gD
gC
Viral Entry
Proteoglycan
HVEM
Viral DNA
NUCLEUS
Uncoating
αTIF
Microtubule
Sp1
RNA Pol II
TBC
TATA
Pre-initiation Complex
LAT mRNA
2Kb Stable Intron
LAT Proteins
Reactivation of Latent Viral Genome
SENSORY GANGLION
QIAGEN

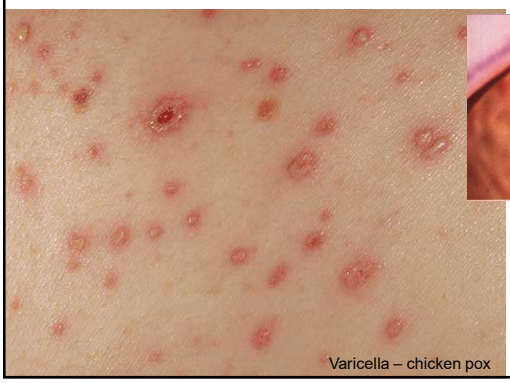
Pathological impact of HSV and VZV

HSV – herpes simplex, benign crbl. ataxia, gingivostomatitis, faryngotonsititis, **encefalitis, pneumonie, hepatitis**

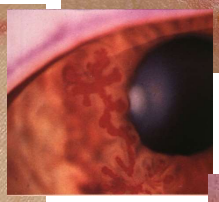
VZV – varicella, herpes zoster, encefalitis, pneumonie, hepatitis

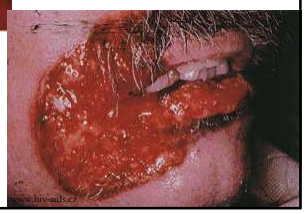
– *In allogeneic HSCT setting less frequently in case of acyclovir prophylaxis; reactiovation of HSV without ACV prophylaxis in 80% of patients*



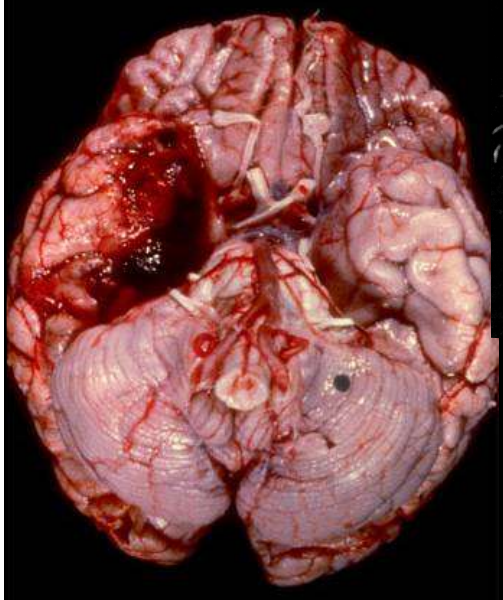


Varicella – chicken pox

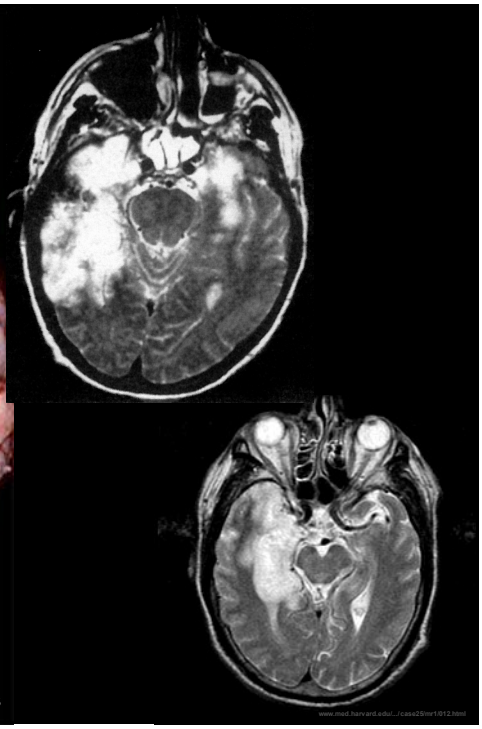




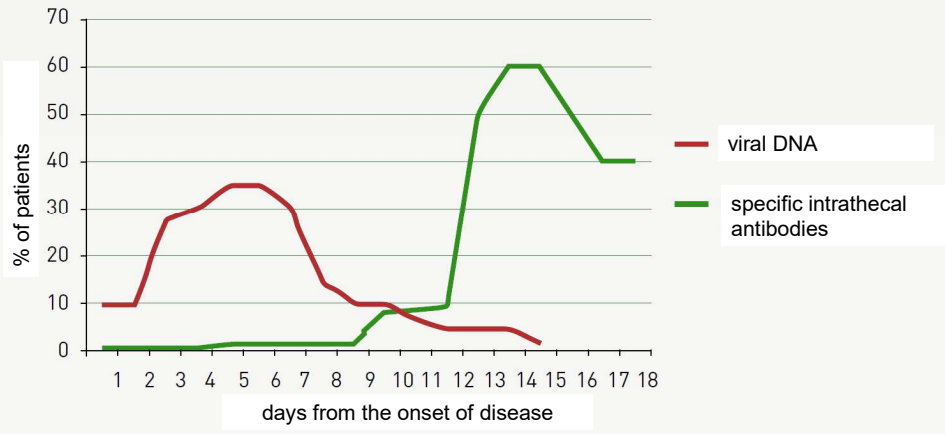
HSV encephalitis

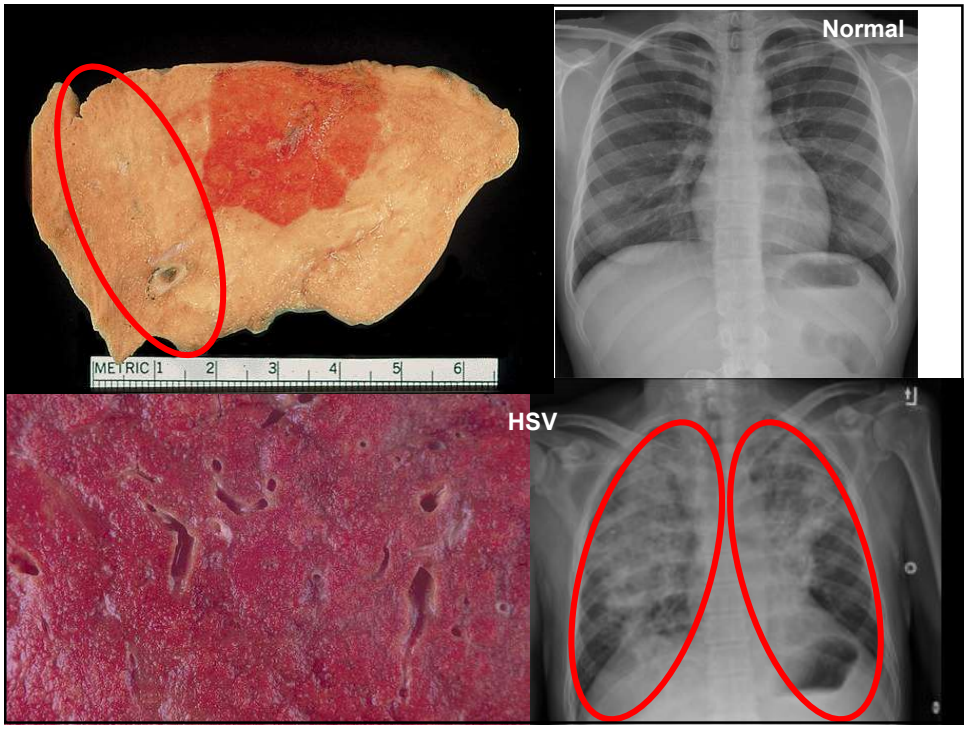


Incidence : 1990-2001: 2,2/1 000 000 citizens

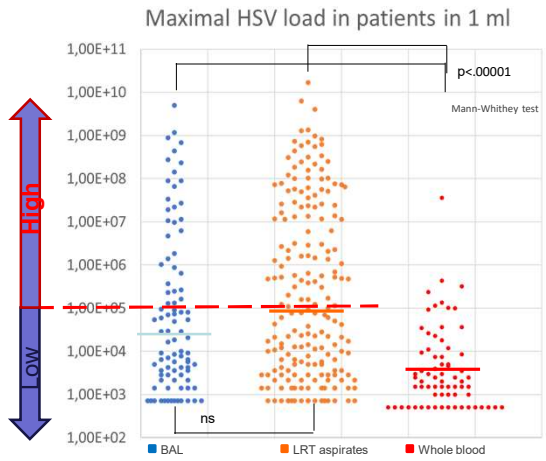


Antibody response to viral infection and detection of virus in CSF





HSV in the samples



254 pts positive in LRTs

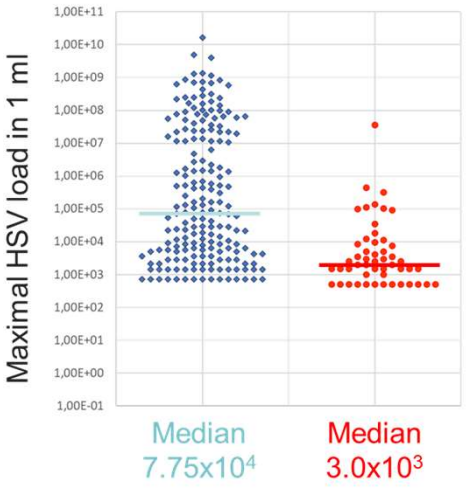
WBs from 69 pts

HSV in the samples and patients

- 928 patients have boths LRT and whole blood samples tested

	Whole blood +	Whole blood -	
LRTs +	42	**150**	**192**
LRTs -	9	727	739
	51	877	928

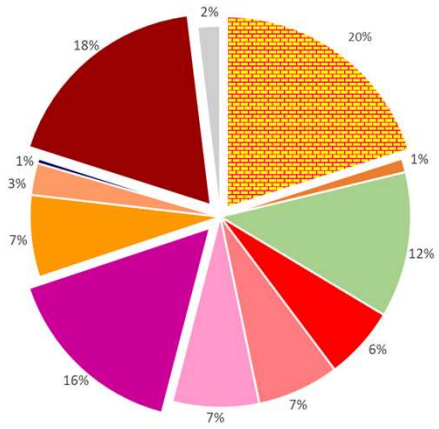
χ^2 test $p < 0.00001$



Mann Whitney test $p < 0.00001$

HSV in critically ill patients

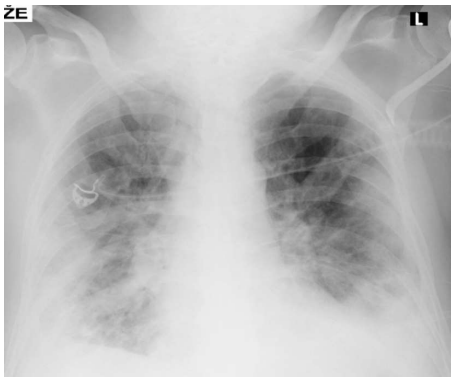
Primary diagnosis in patients positive for HSV from DARICM (n=265)



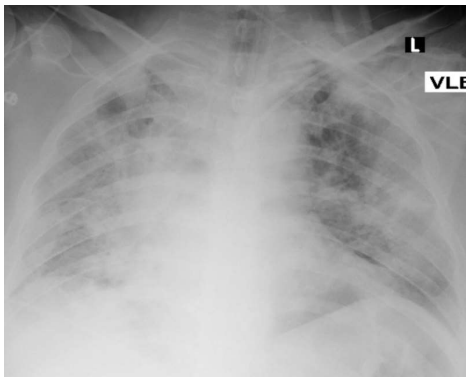
	Deceased pts. in different category
COVID pneumonia	33%
Immunosupresive patient	33%
Lung transplant recipient	50%
Malignant disease	38%
Chest surgery	28%
Surgery - other than chest	16%
Sepsis	34%
Respiratory infection	44%
Pneumonia	14%
HSV encephalitis	0%
Trauma	38%
Other	60%

HSV in critically ill patients – X-ray

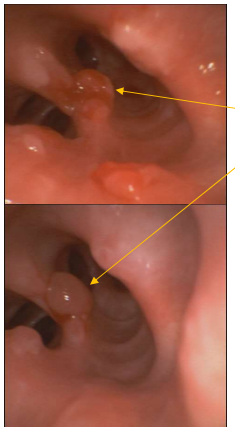
HSV



COVID-19

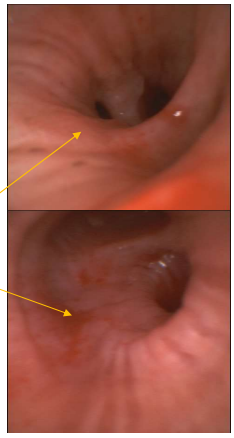


HSV in critically ill patients -
Bronchoscopic picture



Sometimes herpetiformic vesicles

Frequently fragile, swollen mucosa with tendency to bleeding.



March 1979

Cytomegalovirus The Troll of Transplantation

Henry H. Balfour Jr, MD

Arch Intern Med. 1979;139(3):279-280. doi:10.1001/archinte.1979.03630400011006

Pathological impact of CMV

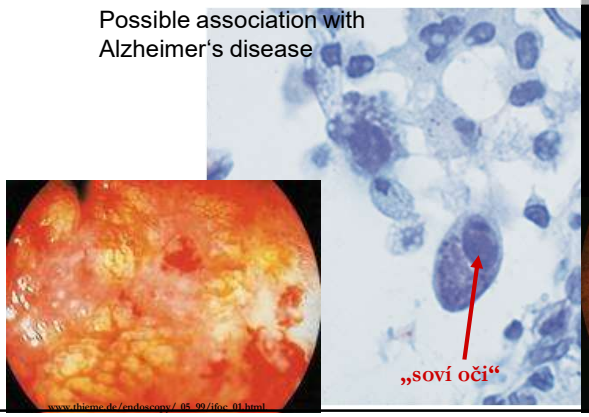
In immunocompetent

- 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, vomitting, artralgia, myalgia



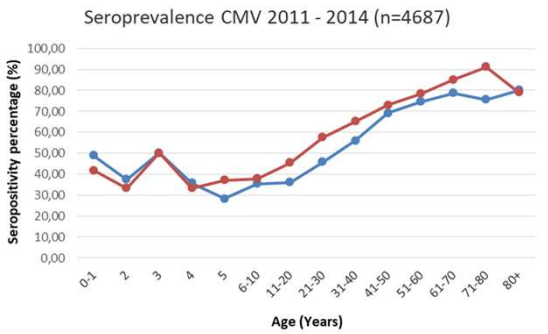
General posibilities of viral dg.

INDIRECT (Antibody detection)

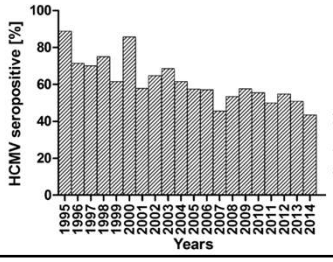
- ELISA
- CLIA…

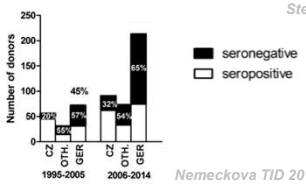
Detection of IgM and IgG antibodies.

IgG avidity became high after approx. 3 months.



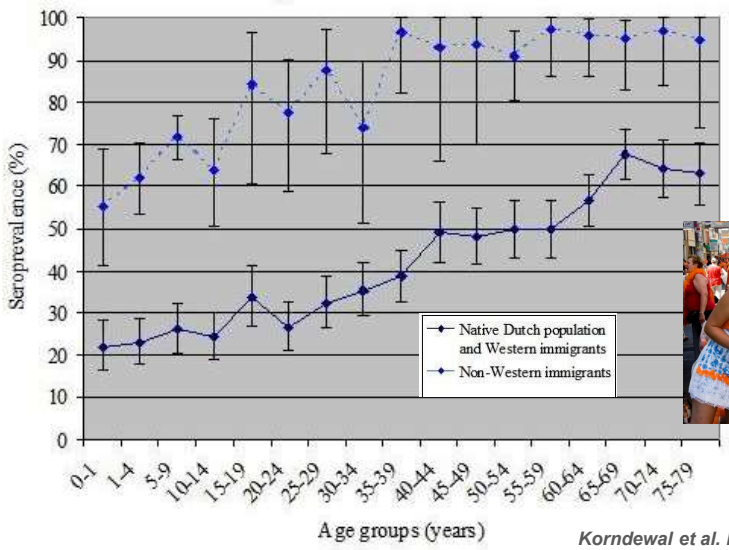
Stepanova et al. CMV workshop 2017





Nemeckova TID 2017

CMV seroprevalence

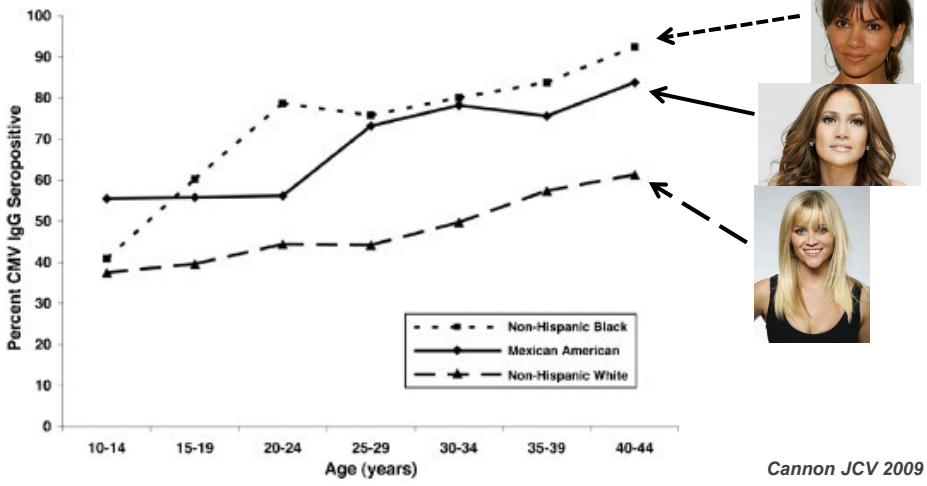


Korndewal et al. European Society for Paediatric Infectious Disease 2012

https://www.abstractserver.com/ESPID2012/pictures/p_435_00079.jpg

CMV seroprevalence

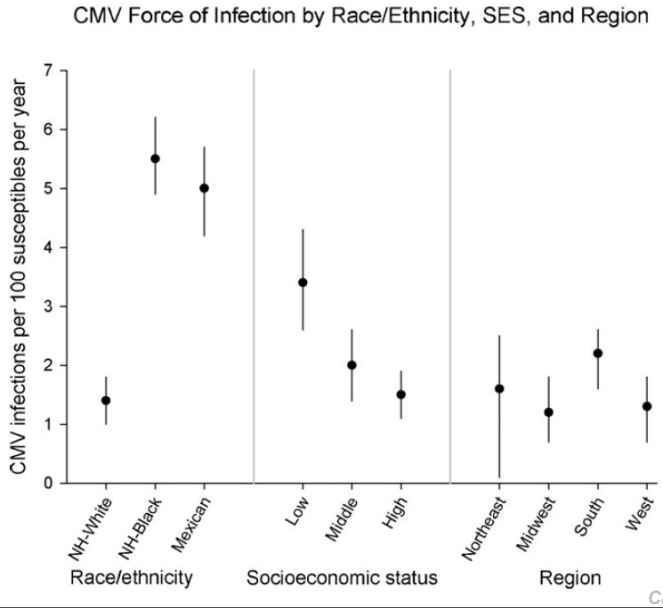
- 60-90% of healthy adult population http://www.tulane.edu/~dmsander/garryfavweb.html
- increases with age and decrease in developed countries



Cannon JCV 2009

https://encrypted-tbn3.gstatic.com/images?q=tbn:ANd9GcRpA_jZXafN6tARnBRXk6Mh32MDm7OAdNLwYoi2X8kgQF7gLtHzgg

Incidence of CMV primoinfection

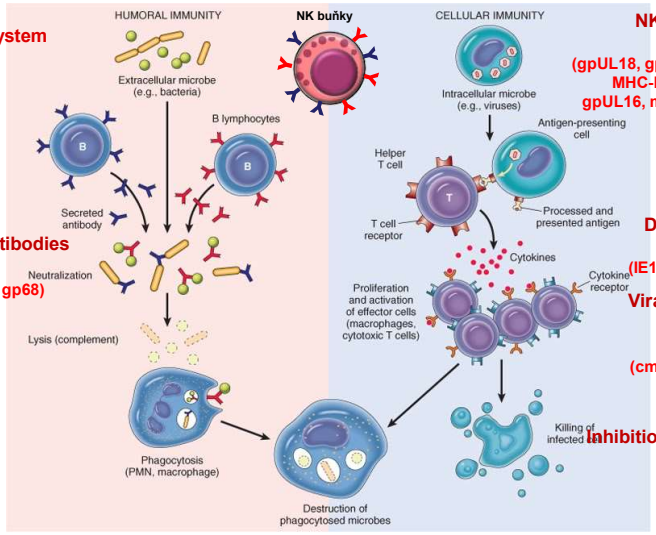


Cannon JCV 2009

How CMV manipulates with immunity?

Complement system inhibition (?)

Inhibition of antibodies by FcR homolog (gp34, gp68)



NK cells function inhibition (gpUL18, gpUL142-homolog MHC-I, pp65, gpUL141, gpUL16, miR-UL112, UL40)

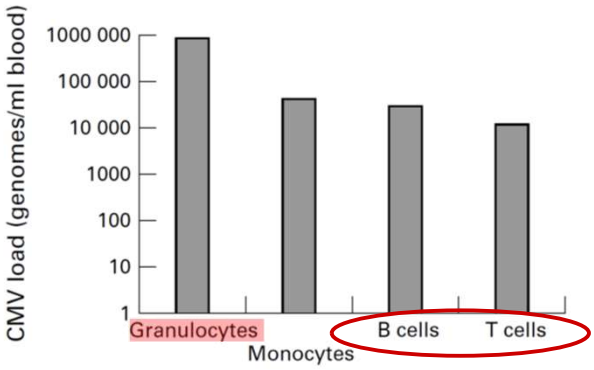
Decrease of INF production (IE1-p72, IE86, pp65)

Viral homologs of cytokines and chemokines (cmvIL-10, vCXCL-1, pUL147, pUL128-131)

Inhibition of apoptosis (vICA, vMIA)

Inhibition of expression and function of MHC-I and II (gpUS2, gpUS3, gpUS6, gpUS8, gpUS10, gpUS11, gp34, gp40,gp48, pp65)

CMV manipulates immunity



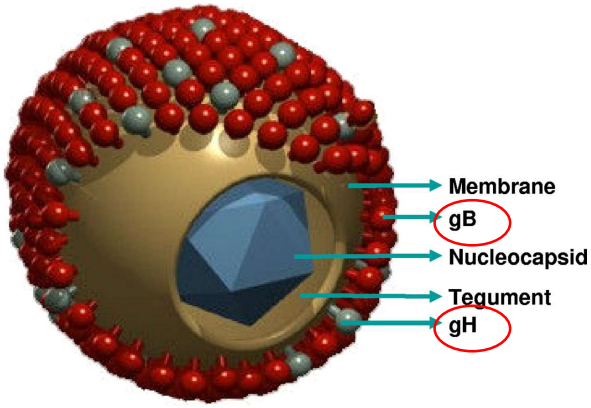
Renal transplant recipients

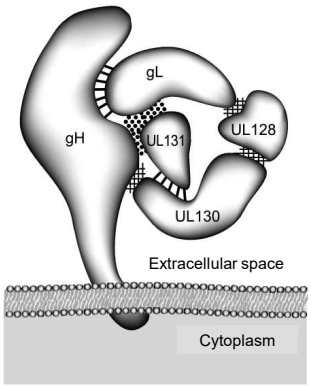
Emery V J Clin Pathol 2001;54:84–88

Direct and indirect effect of the infection

e.g. decrease of PLT level in active CMV infection

Not only one CMV - genotypes



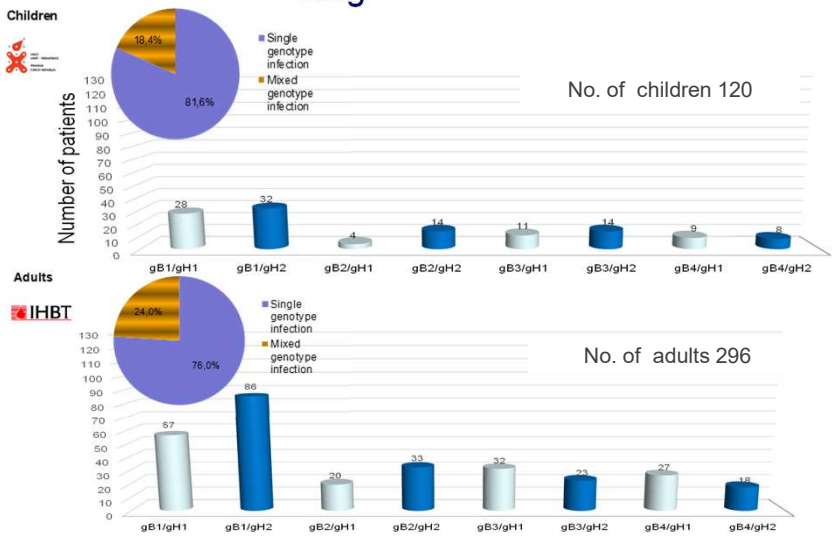


Tania Crough, and Rajiv Khanna Clin. Microbiol. Rev. 2009;22:76-98

Most frequently according to the binding surface glycoproteins – gB, gH, gO, gN…

CMV genotypes (gB1-4/gH1,2)

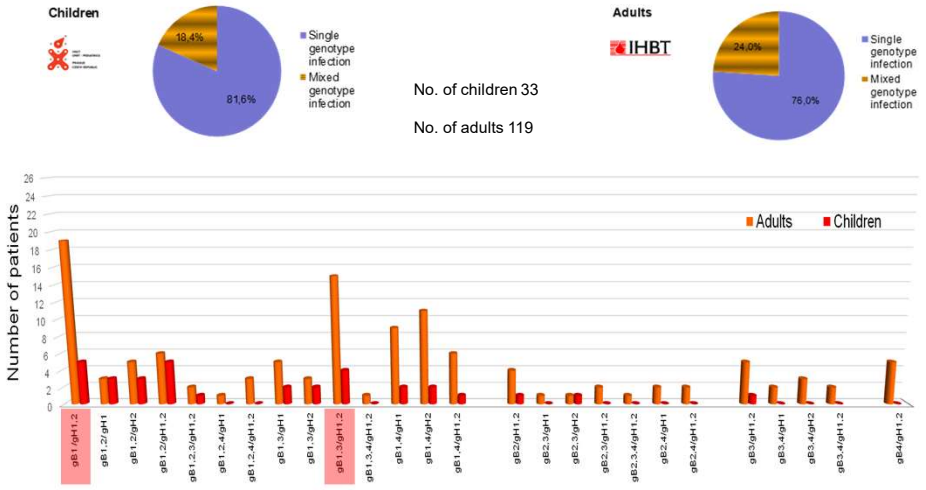
single infections



Most frequently detected genotypes in “single strain” infection were gB1gH2 (detected in 390 samples from 32 children (26.7%) and 86 adults (28.4%) and gB1gH1 (detected in 296 samples from 28 children and 57 adults).

Hubacek et al. EBMT 2015 P581

CMV genotypes mixed infections

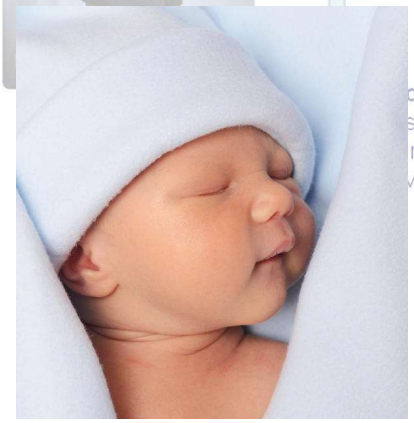


Most frequently detected strains in "mixed strains" infections were **gB1gH1gH2** (in 45 samples from 19 adults and 5 children) and **gB1gB3gH1gH2** (in 44 samples from 15 adults and 4 children).

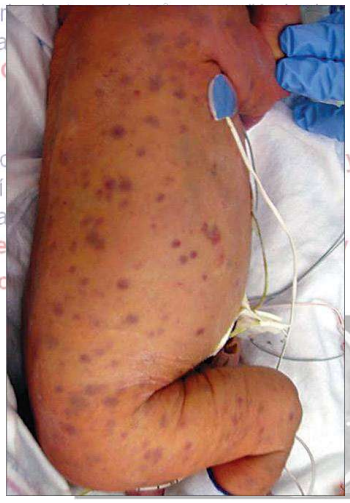
Hubacek et al. EBMT 2015 P581

Symptoms and impact of cCMV

Asymptomatic
90% of children with cCMV



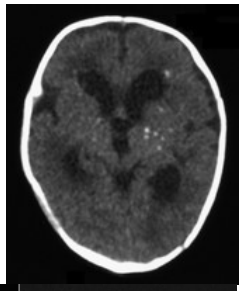
Symptomatic

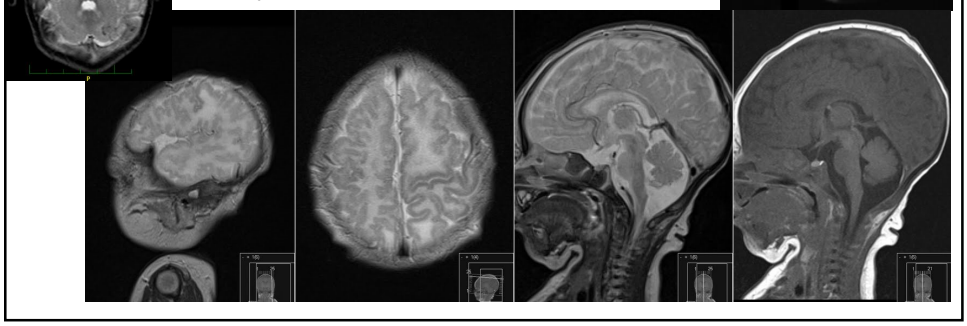


http://medicotrivia.files.wordpress.com/2010/07/blueberry-baby1.jpg

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 sensoneural hearing loss in about 30-50% clinically symptomatic children and 8-12% of asymptomatic children.





Pathofysiology of cCMV symptoms

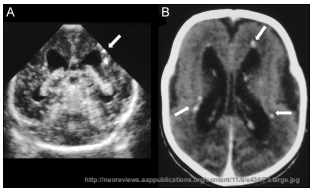
Placental infection
– swalling of the placenta – worse difussion characteristics
- smaller cotymedon development – smaller placental surface
IUGR

Fetal infection
- bone marrow supression **petechia, „blueberry muffin baby"**
- CMV end-organ infection
- vasculitis – especially eyes and a CNS
Neurologic problems/seisures
Brain calcification/ cavity

Sympt

CMV excretion to urine

Premature delivery



Symptoms and impact of cCMV

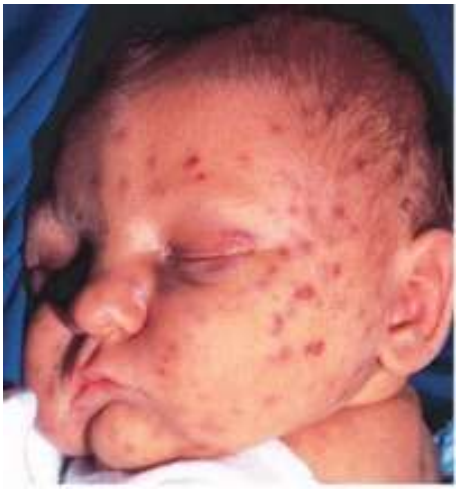
According to CDC

Symptoms of congenital CMV at delivery

Premature birth
Hepatopathy
Pulmonary signs
Splenomegaly
IUGR
Neurological seizures

Long term effects of cCMV

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



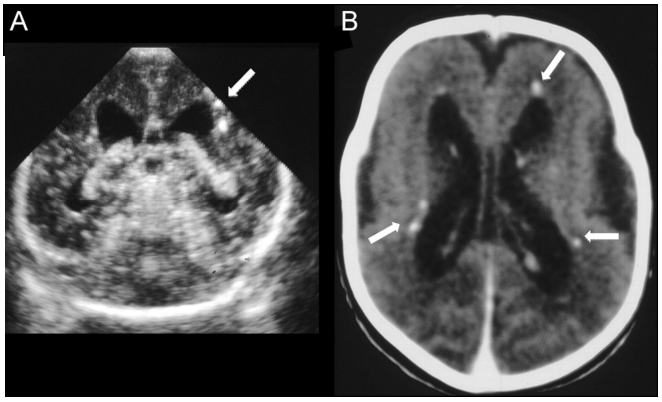
Blueberry muffin baby characterized by purpura as a sign of extramedullary hematopoesis.

Symptoms and impact of cCMV

Transient Outcomes

- Hepatomegaly
- Splenomegaly
- Jaundice
- Petechia and purpura
- Pneumonitis
- Fetal growth retardation
- Seizures

Brain calcification



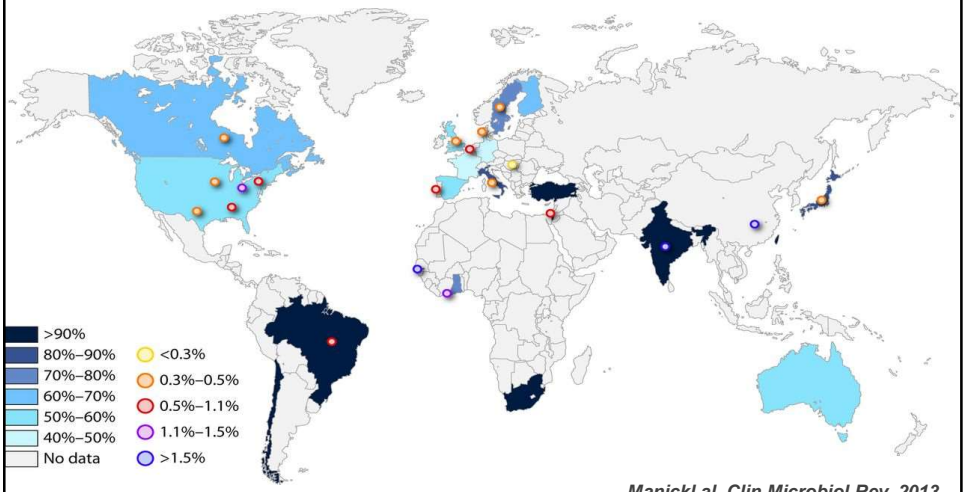
http://neoreviews.aappublications.org/content/11/8/e436/F3.large.jpg

Permanent Outcomes

- Microcephaly
- Vision loss
- Hearing loss
- Mental retardation
- Motor disabilities
- Seizures
- Death

What is the frequency of cCMV?

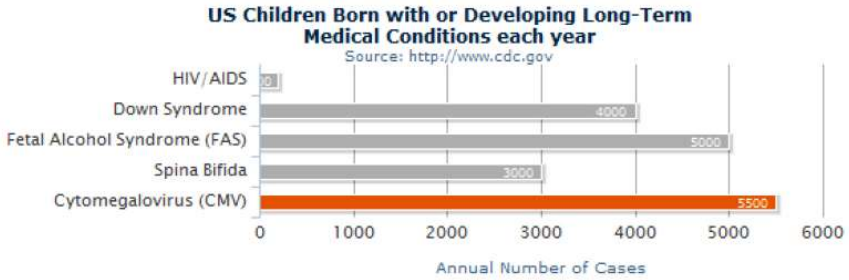
There is 370,000 children born every day in the world, representing 134 millions/year.
Average frequency approx. 1,5% of living birth – 2.01 millions of children with cCMV/year.
In Europe and Czech Republic is estimated frequence 0.5-1% cCMV of living neoborns.

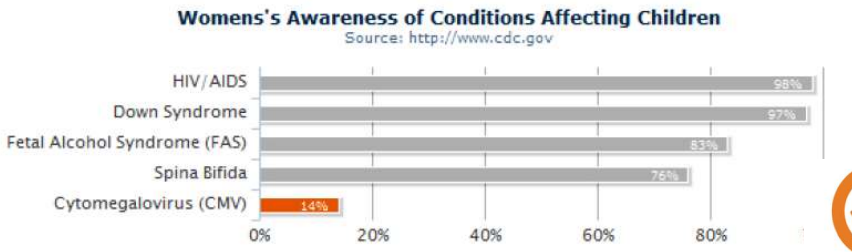


Manickl al. Clin Microbiol Rev. 2013

http://cmr.asm.org/content/26/1/86/F7.large.jpg

What is a knowlegde about cCMV and its impacts?





STOP CMV
The CMV Action Network
www.stopcmv.org

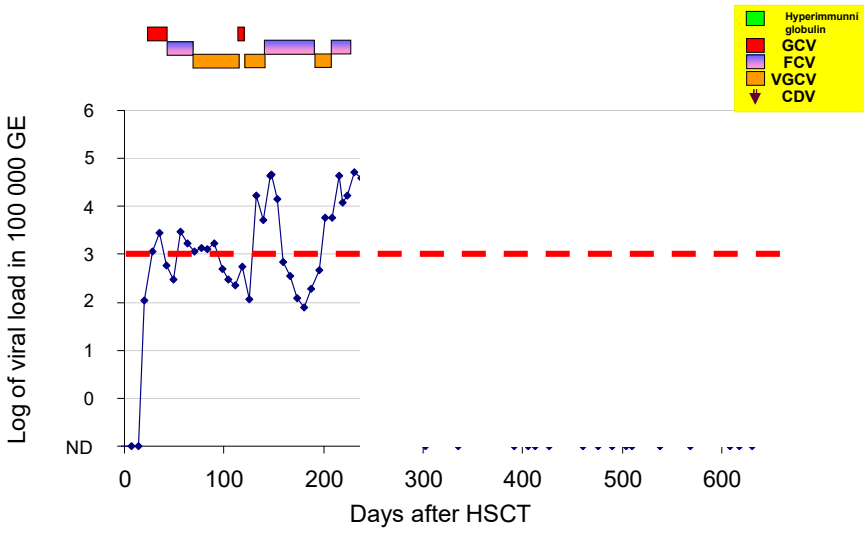
Patient after allogeneic HSCT

Girl, 16.5 yrs of age at HSCT
Allogeneic HSCT for AML M2 (AML1/ETO+) in 2nd CR
MMUD – 7/10
Conditioning: Busulphan, Cyclofosfamid, Melphalan, ATG
Graft: Periferal stem cells
CD34+: 11,12 x 10^6 /kg; CD3+: 302,1 x 10^6 /kg; NC: 12,09x10^8 /kg
GvHD profylaxis: MTX and CsA
CMV status donor/recipient: D-/R+

Non-CMV complications:
D+16 haemorrhagic cystitis –hyperhydration
D+61 –GvHD grade II (skin and GIT)
therapy : steroids 1 mg/kg
D+377 – Herpes zoster – acyclovir treatment
D+440 – Laser coagulation of retinal bleeding
(not proven, suspected, active CMV retinitis)

Patient after allogeneic HSCT

D+ 29 – first CMV treatment

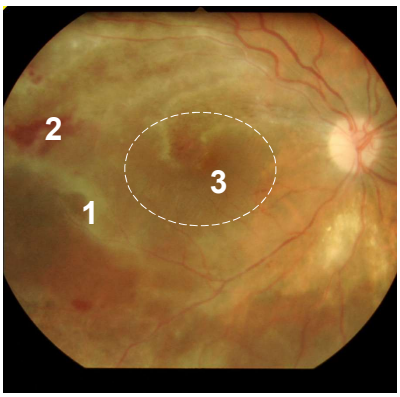


Patient after allogeneic HSCT

D+230 – during foscarnet treatment patient presented diplopy, headache, vomitting and sleepness.

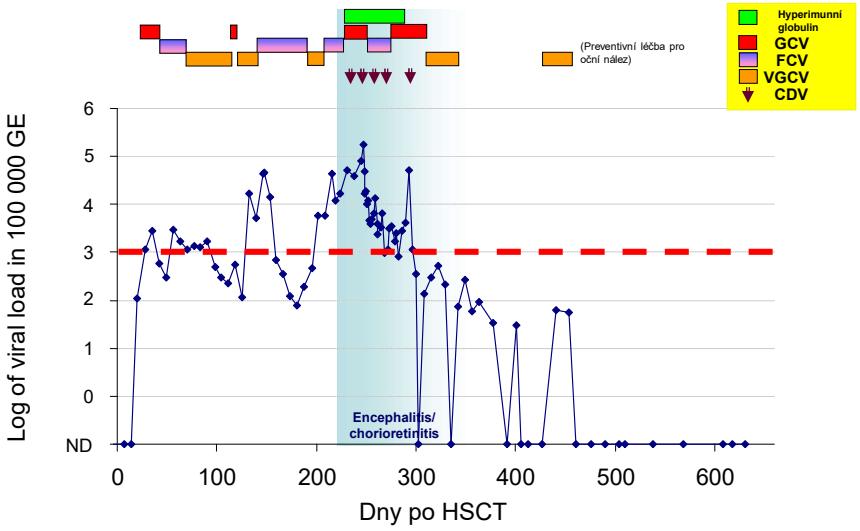
CMV detected in CSF (approx. 2 600 000 copies / ml) and increase of viral load in peripheral blood.

Results confirmed **encephalitis and** bilateral **chorioretinitis**.

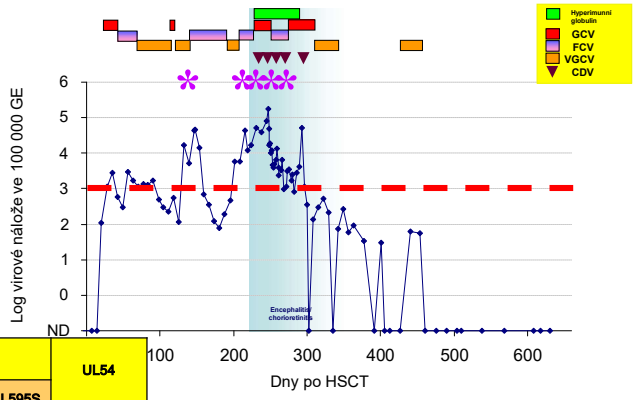


1 – retinal fibrotisation
2 – intraretinal bleeding
3- epiretinal pseudomembrane

Patient after allogeneic HSCT



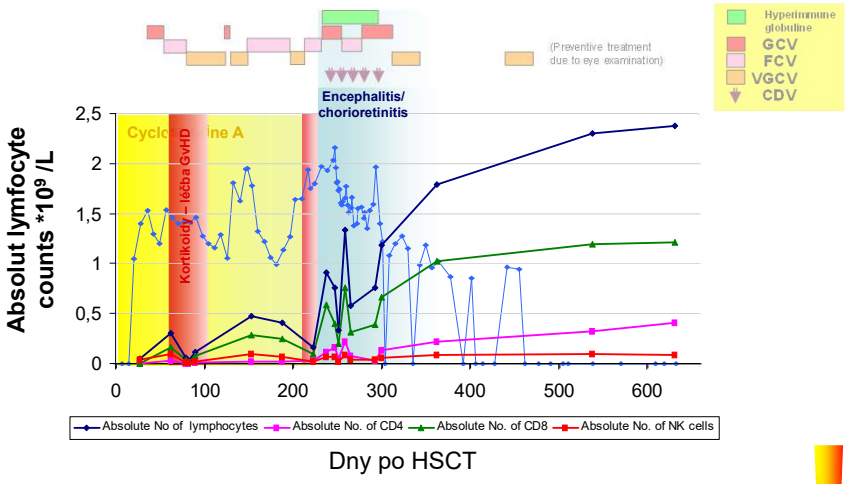
Ganciclovir resistance



Den	Materiál	Gen UL97		UL54
		Mutace A594V	Mutace L595S	
146	*Krev*	WT+mutation	WT+mutation	ND
209	*Krev*	WT	Mutatiom	ND
231	*Krev*	WT	Mutatiom	ND
234	*CSF*	WT+mutation	Mutatiom	ND
238	*Krev*	ND	ND	Mutatiom
247	*Krev*	ND	ND	Mutatiom
248	*Krev*	WT+mutation	Mutatiom	ND
276	*Plazma*	WT	Mutatiom	ND

Possibility of resistance mutant detection is in case of long-lasted treatment usefull.

Lymfocyte counts

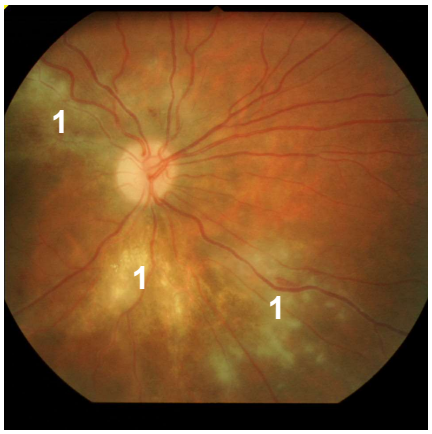


Even a short term steroid treatment leads to decrease of the lymfocyte count neccessary for infection control

Patient after allogeneic HSCT

Outcome

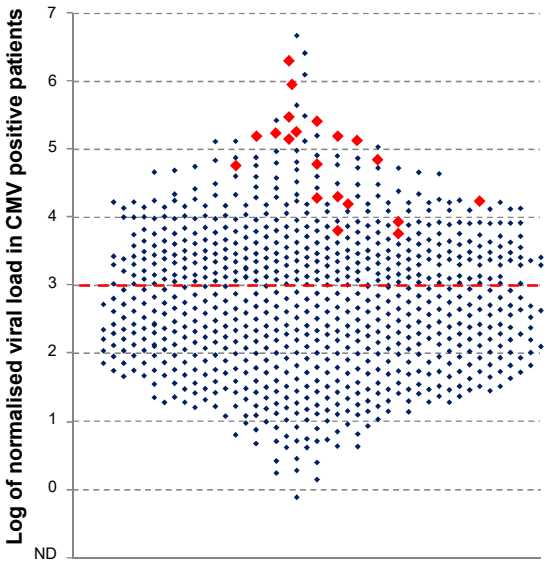
- Recently the patient is regularly controlled by ophtalmologests. ´Visus in one eye is very limited, however the second eye is healthy. In boths eyes there is limitation of peripheral visus.
- There are no signs of relaps of the primary disease, GvHD and other infections including CMV.



1 – retinal fibrotization

CMV resistance

max. quantity in whole blood



High viral load correlates with resistence development

(Mann-Whitney p<0.0001)

gB1gH1 – 5 pts..
gB3gH1 – 4 pts.
gB1gH2 – 4 pts.
gB4gH2 – 2 pts.
gB2gH1 – 1 pts.
gB2gH2 - 1 pts.
and mixed infections (**gB1,gH1,2, gB2, gH1,2 and gB1,3gH1,2**)

75% of patients with resistance deceased. Only one deceased with proven CMV pneumonia.

Clinical Infectious Diseases

INVITED ARTICLE

IDSA Infectious Diseases Society of America | hivma hiv medicine association | OXFORD

IMMUNOCOMPROMISED HOSTS: David R. Snydman, Section Editor

Definitions of Cytomegalovirus Infection and Disease in Transplant Patients for Use in Clinical Trials

Per Ljungman,[1,2] Michael Boeckh,[4,5] Hans H. Hirsch,[6] Filip Josephson,[3] Jens Lundgren,[7] Garrett Nichols,[8] Andreas Pikis,[9] Raymund R. Razonable,[10] Veronica Miller,[11] and Paul D. Griffiths[12]; for the Disease Definitions Working Group of the Cytomegalovirus Drug Development Forum[a]

Table 1. Cytomegalovirus Disease Categories and Required Quality of Evidence

Disease	Proven	Probable	Possible
Pneumonia	Yes	Yes	Yes
Gastrointestinal disease	Yes	Yes	Yes
Hepatitis	Yes	No	No
Retinitis	Yes	No	No
Encephalitis/ventriculitis	Yes	Yes	No
Nephritis	Yes	No	No
Cystitis	Yes	No	No
Myocarditis	Yes	No	No
Pancreatitis	Yes	No	No
Other end-organ diseases	Yes	No	No
Syndrome	No	Yes	No

All 3 categories require appropriate clinical symptoms and/or signs.

Definitions

Clinical Infectious Diseases

INVITED ARTICLE

IDSA | hivma

IMMUNOCOMPROMISED HOSTS: David R. Snydman, Section Editor

Definitions of Resistant and Refractory Cytomegalovirus Infection and Disease in Transplant Recipients for Use in Clinical Trials

Roy F. Chemaly,[1] Sunwen Chou,[2] Hermann Einsele,[3] Paul Griffiths,[4] Robin Avery,[5] Raymund R. Razonable,[6] Kathleen M. Mullane,[7] Camille Kotton,[8] Jens Lundgren,[9] Takashi E. Komatsu,[10] Peter Lischka,[11] Filip Josephson,[12] Cameron M. Douglas,[13] Obi Umeh,[14] Veronica Miller,[15] and Per Ljungman[16,17]; for the Resistant Definitions Working Group of the Cytomegalovirus Drug Development Forum

DEFINITIONS OF CMV INFECTION

CMV Infection

CMV infection is defined as virus isolation or detection of viral proteins (antigens) or nucleic acid in any body fluid or tissue specimen. It is recommended that both the source of the specimens tested (eg, plasma, serum, whole blood, peripheral blood leukocytes [PBLs], cerebrospinal fluid [CSF], bronchoalveolar lavage [BAL] fluid, urine, or tissue) and the diagnostic method used be described clearly.

CMV Reinfection

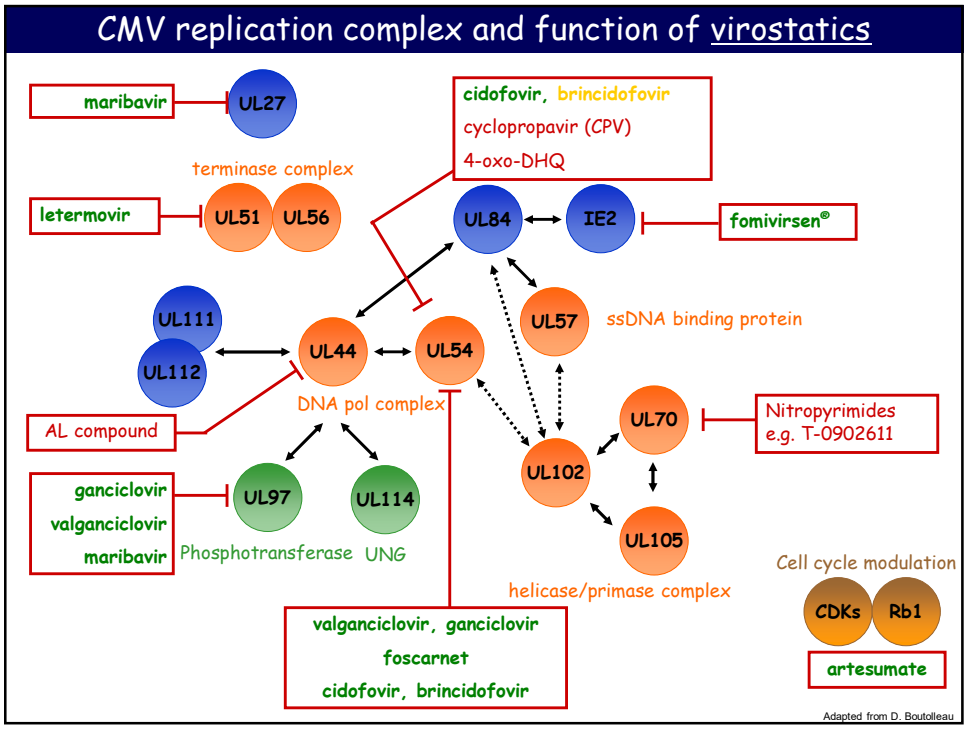
Reinfection is defined as detection of a CMV strain that is distinct from the strain that caused the initial infection.

CMV Reactivation

CMV reactivation is likely if the 2 viral strains (prior and current strain) are found to be indistinguishable either by sequencing specific regions of the viral genome or by using a variety of molecular techniques that examine genes known to be polymorphic.

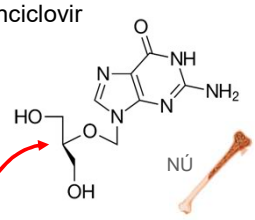
Table 1. Risk Factors for Cytomegalovirus Resistance in Hematopoietic Cell Transplant Recipients[a,b]

Risk Factor	
Host factors	
Prolonged antiviral CMV drug exposure (>3 mo)	
Previous antiviral CMV drug exposure	
Recurrent CMV infection	
Inadequate antiviral CMV drug absorption and bioavailability	
Inadequate antiviral CMV oral prodrug conversion	
Variation in antiviral CMV drug clearance	
Subtherapeutic antiviral CMV drug level	
Poor patient compliance with antiviral drug regimen	
T-cell depletion	
Haploidentical, allogeneic, or cord blood HCT	
Delayed immune reconstitution	
CMV-seropositive recipient and CMV-seronegative donor	
Treatment with antithymocyte antibodies	
Active GVHD	
Young age	
Congenital immunodeficiency syndromes	
Viral factors	
CMV viral load rise while receiving treatment (after >2 wk of adequate dosing)	
Failure of CMV viral load to fall despite appropriate treatment	
Rise in CMV viral load after initial decline while receiving appropriate treatment	
Intermittent low-level CMV viremia	
High CMV viral loads	

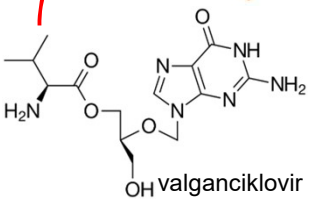


And what about the virostatics?

ganciclovir



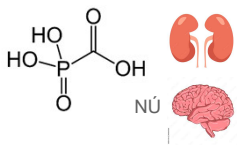
Indications:
- First line therapy
- Systemic treatment of CMV nfections
- i.v.



valganciklovir

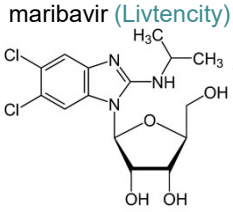
Indications:
- First line therapy
- Systemic treatment of CMV nfections
- p.o.

foscarnet



Indikace:
- 2nd line th.
- Systemic treatment of CMV nfections
- i.v.

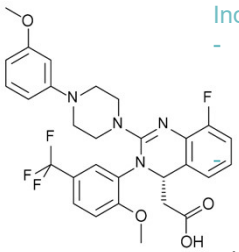
maribavir (Livtencity)



Indications:
- Systemic th. of **refractory/ resistant** CMV infection
- p.o.

NÚ -dysguezie

letermovir (Prevymis)



Indication:
- Systemic **prophylaxis** of CMV infections p.o.

NÚ –GIT, rash

Patological activities of HHV-6

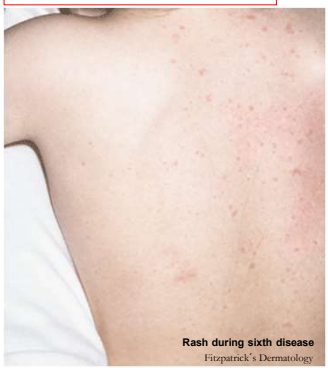
HHV-6

Recently 2 distinct viral species

HHV-6 A

Unknown

„Orphan virus“



Rash during sixth disease

Fitzpatrick's Dermatology

HHV-6 B

Immunocompetent host

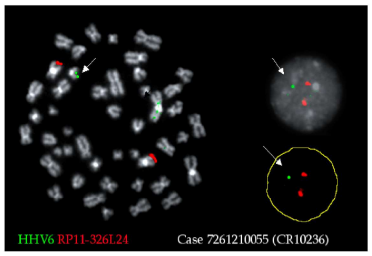
- **Sixth disease**
- **Febrile seizures**
- **Encephalitis**

Immunocompromised host

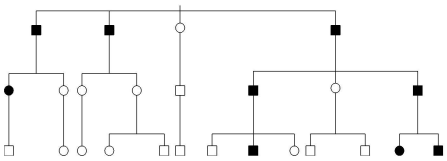
- **Encephalitis**
- Hepatitis
- Pneumonitis
- Pericarditis
- **Delayed engraftment after HSCT**

Chromosomally integrated HHV-6 (CI-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 CI-HHV-6 to active infection in vivo
- In vitro reactivations are doubtful

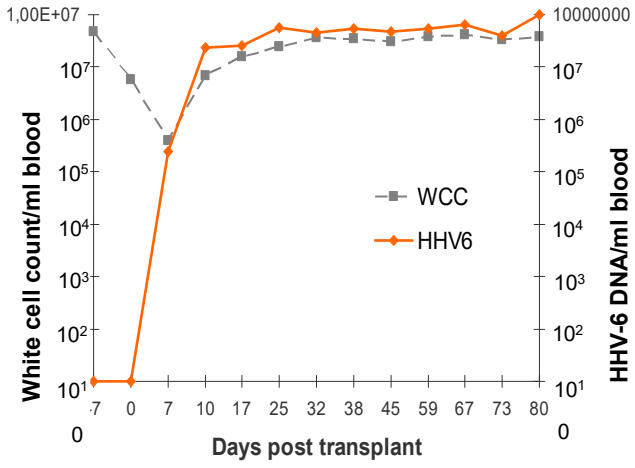


HHV-6 integration at 22q13.3 control probe on 9q34.4



HHV6 DNA in blood after HSCT

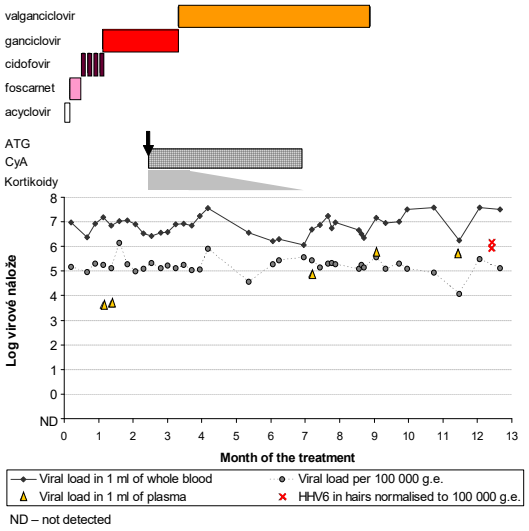
donor with Ci-HHV-6



Clark et al., JID 2006

Patient 2

Chromosomally integrated HHV-6 (Ci-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.

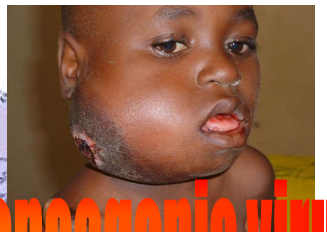
Detection in hair, or nails detects Ci-HHV-6 safely.

EBV discovery

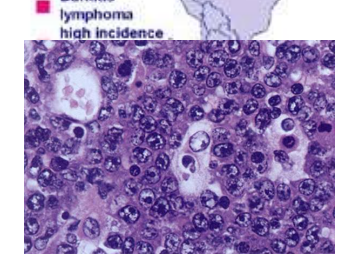
surgeon

Denis Parsons Burkitt

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



1st described human oncogenic virus



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"

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

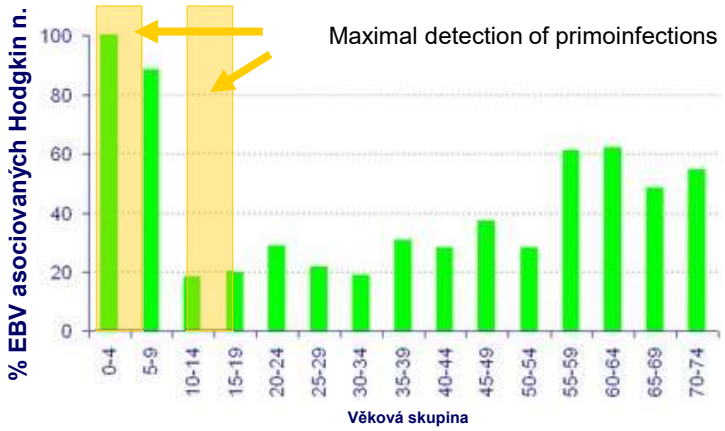
Yvonne M. Barr
(*1932)

Bert Geoffrey Achong
(1928-1996)

Transmission and epidemiology

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

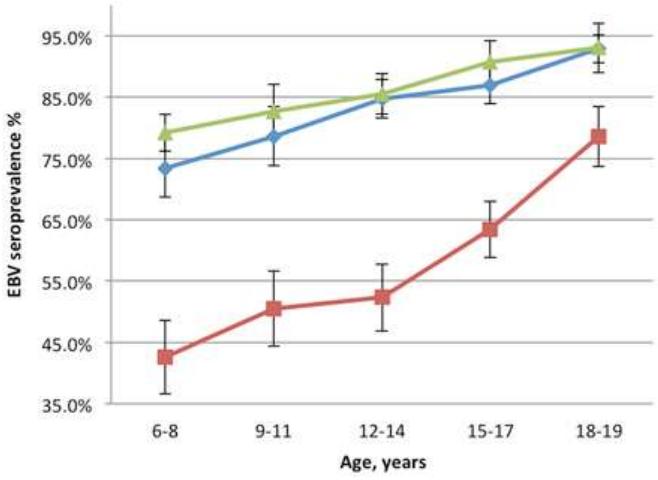
(in developing countries, it is 90% of children older 2 yrs)



Maximal detection of primoinfections

Transmission and epidemiology

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



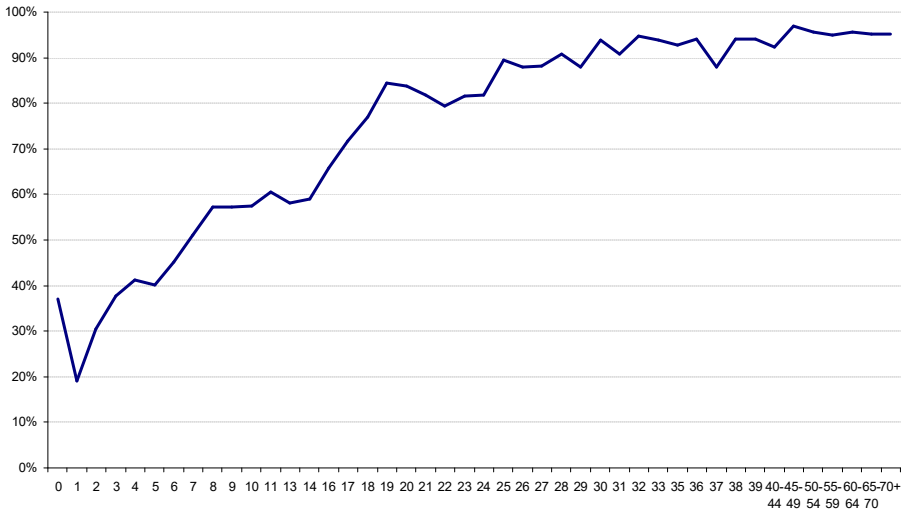
Median of EBV seroprevalenceaccording to age, race/ethnicity, National Health and Nutrition Examination Survey, 2003–2010.

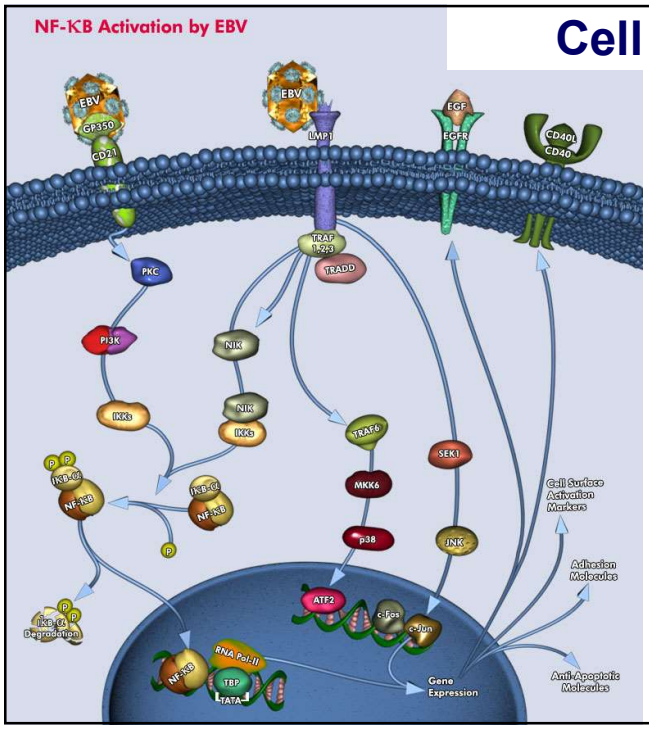
PLOS ONE

*Dowd JB, Palermo T, Brite J, McDade TW, et al. (2013) Seroprevalence of Epstein-Barr Virus Infection in U.S. Children Ages 6-19, 2003-2010. PLoS ONE 8(5): e64921. doi:10.1371/journal.pone.0064921*http://www.plosone.org/article/info:doi/10.1371/journal.pone.0064921

Transmission and epidemiology of EBV in Motol UH

FN MOTOL





Cell entrance

gp350/220

Binds to:

CD21(CR-2)

HLA II

Permissive cells

B lymfocytes

Epithelial cells

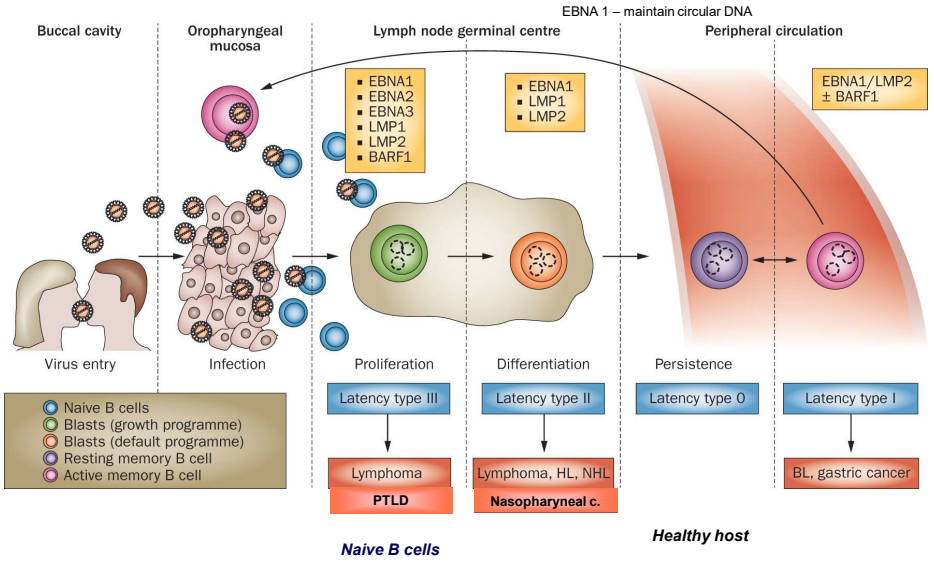
How EBV manipulates the immunity /prolipheration?

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; involved in p53 degradation and oncogenesis
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; dismisses repressor complex from promoter or enhancer sites; 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; is critical for EBV-mediated B-cell transformation
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; and induce CDKN2 and chemokines. Induces G1 arrests, which is essential for EBV-mediated B-cell transformation
EBNA-3B	A coactivator of EBNA-2; dispensable for B-cell transformation; viral tumor suppressor; and upregulates CXCL10. EBNA-3B-knockout induces DLBCL-like tumors
EBNA-3C	Coactivates with EBNA-2 host *CXCR4* and *CXCL12* 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; induces G1 arrests; 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; induces B-cell lymphoma in transgenic condition; is important but not essential for *in vitro* 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; augments colony formation and induces growth; 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 BHRF1; validated targets include Bim, BRUCE, CXCL11, DICER1, PUMA; has a role in sustaining latently infected cells. BHRF1 miRNA and BART miRNAs interfere with apoptosis. The miR-BART15-3p promoted apoptosis 331

Experimental & Molecular Medicine (2015) 47,

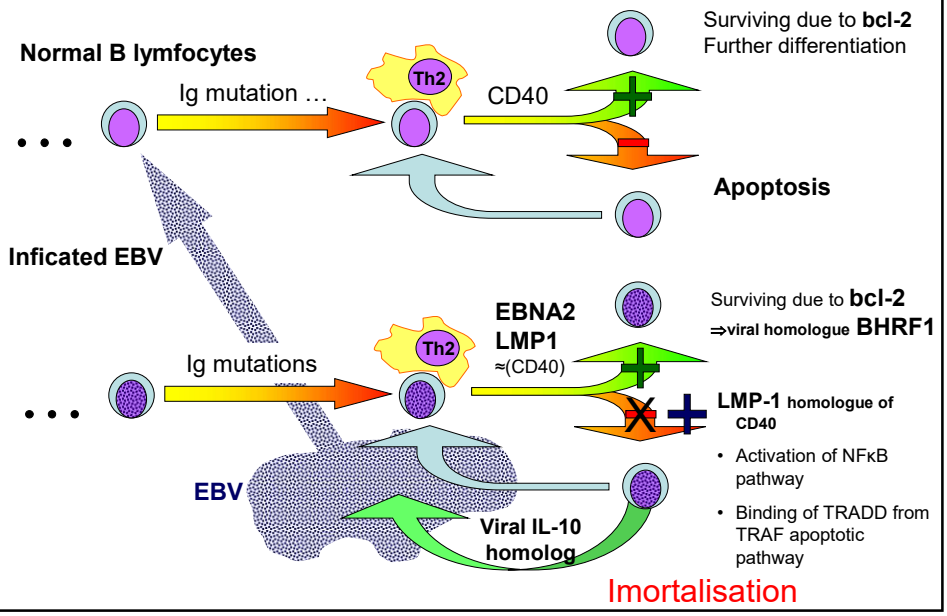
How EBV manipulates the immunity

(antigens and latency)



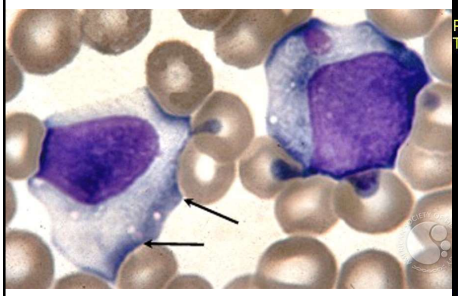
Bollard, C. M. et al. (2012) T-cell therapy in the treatment of post-transplant lymphoproliferative disease
Nat. Rev. Clin. Oncol. doi:10.1038/nrclinonc.2012.111

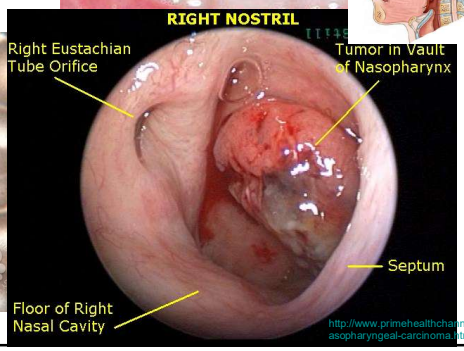
How EBV manipulates the immunity



Patological activities of EBV

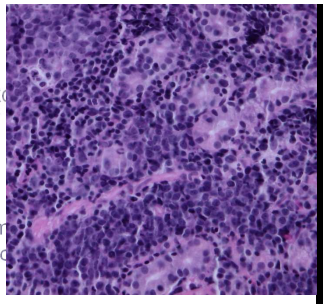
- **Immunocompetent host**
  - Infectious mononucleosis
  - Chronic active EBV infection
  - X-binded lymfoprolipherative disease
  - Malignant diseases
    - Hodgkin disease
    - Burkitt's lymphoma
    - non-Hodgkin T/NK lymphoma
    - Nasopharyngeal carcinoma
    - Gastric carcinoma
    - Angioblastic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

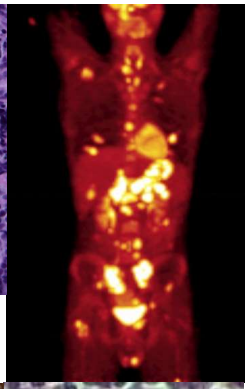




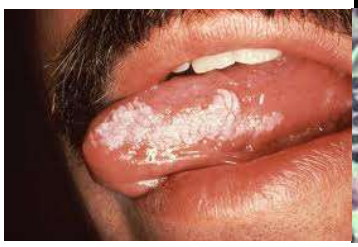
Patological activities of EBV

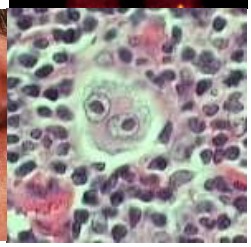
- **Imunokompetentní hostitel**
  - Infekční mononukleóza
  - Chronická aktivní EBV infekc
  - X-vázaná lymfoproliferativní
  - Maligní onemocnění
    - Hodgkinova nemoc
    - Burkittův lymfom
    - non-Hodgkinský T/NK lym
    - Nasopharyngeální karcino
    - Karcinom žaludku
    - Angioblastický T lymfom





- **Immunocompromised host**
  - Hairy leukoplaky
  - Above listed malignant diseases
  - Post-transplant lymfoproliferative disease (EBV-LPD)
  - Encefalitis/myelitis
  - Pneumonie
  - Hepatopathy/hepatitis





http://www.kcom.edu/faculty/chamberlain/Website/lectures/lecture/aids.htm

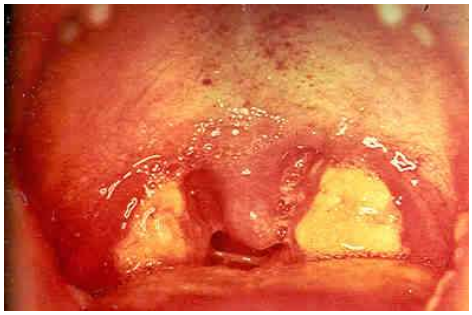
www.med-ed.virginia.edu/courses/path/innes/wcd/hodgkin

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
- Inkubation period 4-6 weeks
- At the beginning seems like „tonsilitis"
- Transmission by saliva
- Treatment approx. 6 months

  Relax and diet (2-3 months);

  Subsequently it is neccesary to have some relax in physical activity



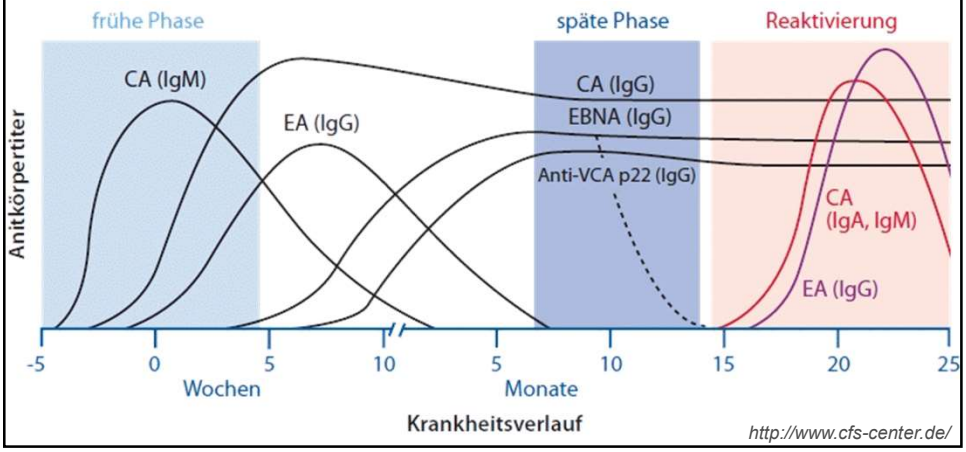
http://home.teleport.com/~bobh/InfectiousMononucleosis.htm

Diagnostics

Basic diagnosis of EBV is indirect – serological.

Early phase | Post-acute phase | Late phase | Reactivation

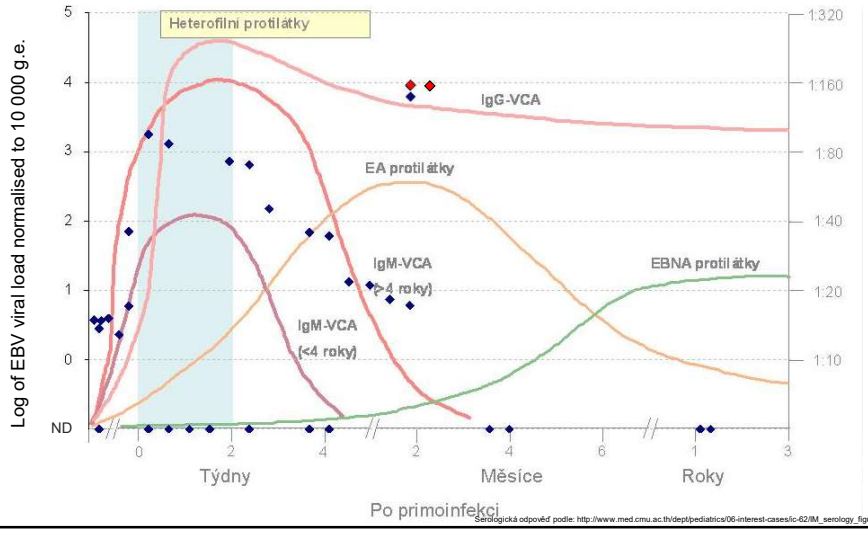
Heterofile antibodies Paul-Bunnelova reaction



http://www.cfs-center.de/

Viral load in patients with dg. B27 - IM

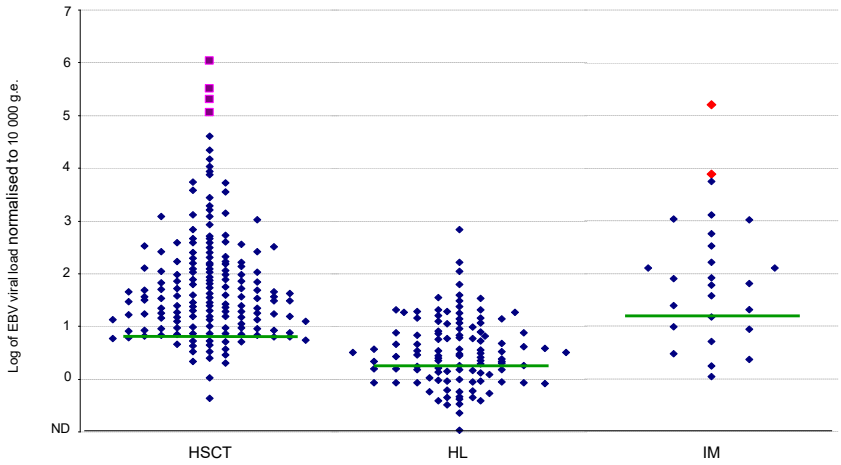
- Positive – 26 patients (62%)
  - 50 samples positive (65%); median of positivity 110 (range 11-157,670) in 100 000 g.e.



Sérologická odpověď podle: http://www.med.cmu.ac.th/dept/pediatrics/06-interest-cases/ic-62/IM_serology_figure2.1.jpg

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 lymfocytes 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 ± SD)	2213 ± 1104	1682 ± 464	.11
IgE (IU/mL, mean ± SD)	282 ± 298	2774 ± 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/μg DNA, mean ± SD)	$10^{4.1 \pm 0.5}$	$10^{4.4 \pm 0.4}$	.09
Plasma (copies/mL, mean ± 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.

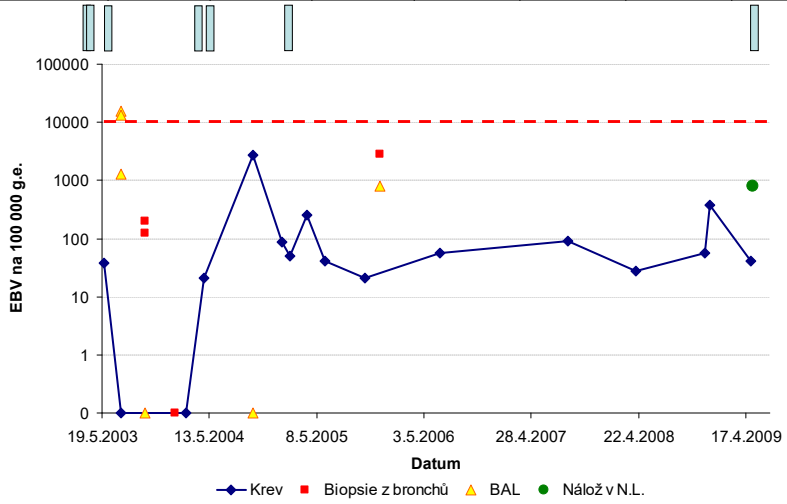
Table 2. Clinical features of 30 patients with chronic active Epstein-Barr virus infection

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 hypertention	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

Datum	6.2.2003	17.2.2003	27.5.2003	23.2.2004	26.3.2004	4.4.2005	4.5.2009
VCA IgG	+	+++		+	++	147 U/ml	119 U/ml
VCA IgM	+	+	-	+	+	72,5 U/ml	45,5 U/ml
EA-D	++	++	+++ (vysoká exprese)	++	++	90 U/ml	<150 U/ml
EBNA 1 IgG	-	-	-	+	+	52,5 U/ml	14,3 U/ml

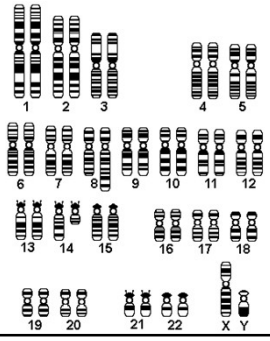


EBV load in 1 ml of biological material

Malignant impact of EBV

NHL - Burkitt lymphoma

Very agressive
Picture of the **„Sky of stars "** – „stars" are apoptotic tumor cells which are fagocyte by macrophages; „sky" – represent tumor lymfocytes
Typical fusion t(8:14) chromosome 8 with c-myc oncogen
In the equatorial Africa incidence 5-15/100,000 of children
In Europe and USA 0,2-0,3/100,000 citizens



http://mynotes4usmle.tumblr.com/post/33262736354/burkitts-lymphoma#.VPgrFSx5vU4

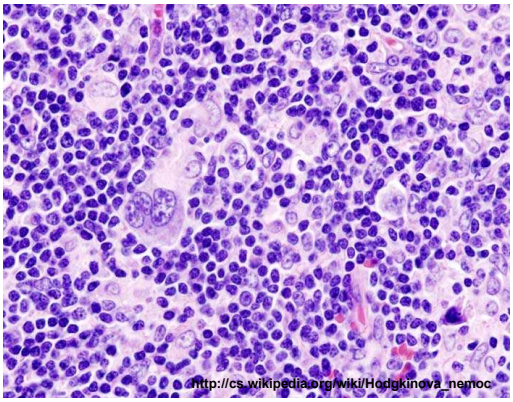
Malignant impact of EBV

Hodgkin lymfoma

High number of patients in long lasting remission.
Higher frequency in younger patients (approx. 20 yrs. of age) and in patients older 50-60 yrs. (median of age at dg. 35 yrs.)

Ratio of malignant and non-malignant cells approx. ~ 1:100

Incidence 2.4/100000 in ♀ and 3.1/100 000 ♂.



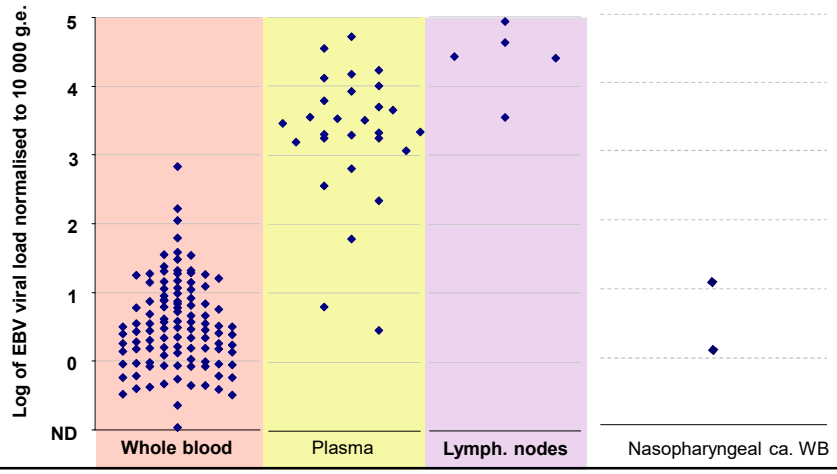
http://cs.wikipedia.org/wiki/Hodgkinova_nemoc

Histologically divided according to no. of **Reed-Sternberg's cells** (cells developed by mutation from B-cells) and according to the cellular frections:

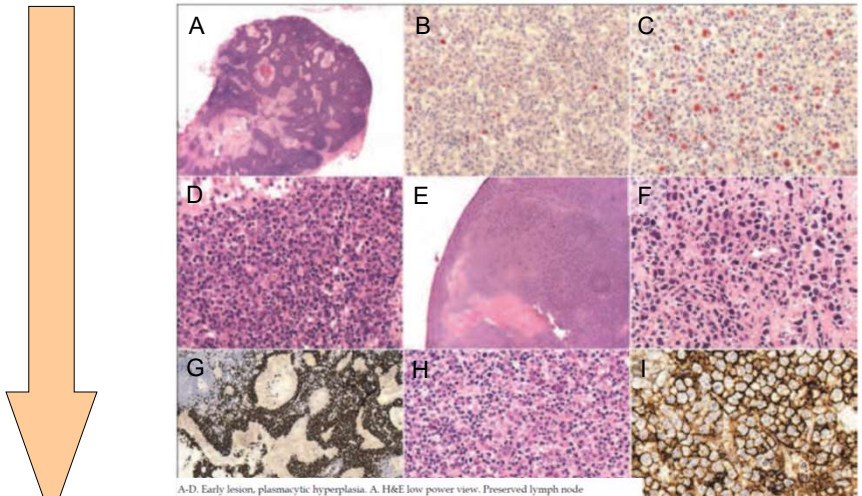
typ I with dominance of lymfocytes (few R-S cells, dominance of lymfocytes; best prognosis) (5 %);
typ II nodular-sclerotic (nodular centres, cells (reticular, lymphocytes, histiocytes) in colagen fibres) (70 %);
typ III mixed (20–25 %);
typ IV classical, few lymphocytes (No. of Sternberg's cells increased; worse prognosis) (1 %).

Patients with Hodgkin L. and NF ca.

- Positive HL – 69 patients (38%)
  - positive 110 whole blood samples (17%) and 30 plasma samples (4.8%)
  - median of positivity in whole blood 3.45 copy (range 0.11 - 721)
  - median plasma positivity 5,400 copies/ml (range 600 – 126,600); after normalisation to 10 000 g.e median 2,500 (range 3 - 52 162)

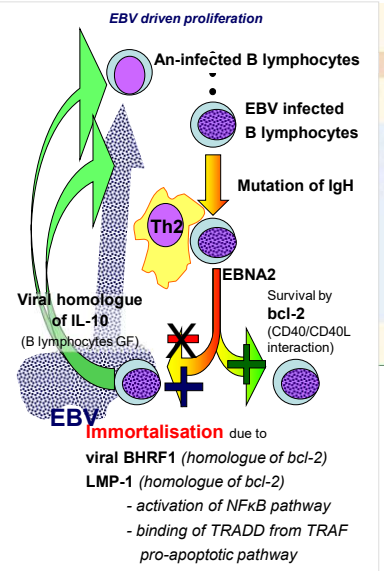


EBV associated posttransplant proliferative disease (EBV-LPD)



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-type 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)

EBV-LPD incidence and risk factors

Risk Factor	Degree of Risk	Study Reference(s)
EBV seronegativity pretransplant	24 × average risk	11–13
Younger age at transplantation	4–8 × adult risk	1,11
Type of immune suppression		
– Tacrolimus	2–5 × risk with cyclosporine	1,16,17
– OKT3 and/or ATG	3–4 × risk without these drugs	1
Type of organ transplant		9
Kidney	1%–3% of all transplant patients	
Liver	1%–3% of all transplant patients	
Hear	1%–6% of all transplant patients	
Heart-lung	2%–6% of all transplant patients	
Lung	4%–10% of all transplant patients	
Small bowel	20% of all transplant patients	
Time from transplant < 1 year	5–10 × risk at > 1 year	1
De novo CMV infection:		
CMV-positive recipient of a CMV-positive organ	4–6 × risk of CMV-negative recipient	21

ATG = anti-thymocyte globulin; CMV = cytomegalovirus; EBV = Epstein-Barr virus; OKT3 = muromonab-CD3 (Orthoclone OKT3); PTLD = post-transplant lymphoproliferative disorder.

In allogeneic HSCT incidence 2-25%.

- Cumulative intensity of immunosupresive treatment
- Use of anti-T lymfocytic antibodies in conditioning and/or posttransplant treatment
- T-cell depleted graft
- Intensive GvHD treatment
- Activation about 60 days after HSCT

EBV-LPD diagnosis

Diagnosis of <u>neoplastic</u> EBV-LPD should fulfill at least 2 of the following criteria:

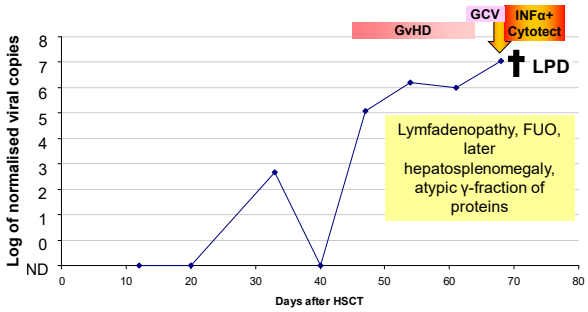
- **Change and/or destroy of the cell tissue culture by lymphoproliferative process**
- **Presence of monoclonal, or oligoclonal proliferation proven with cell and/or viral markers**
- **Evidence of EBV infection in many cells (e.g.. DNA, RNA, protein…**

EBV DNA detection in whole blood is not enough.

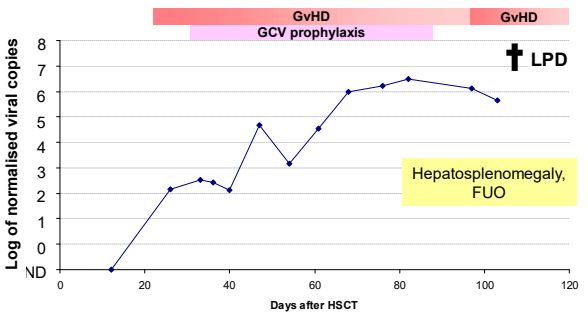
Dle definice EBMT IDWP, 2007

- Clinical symptoms
- Imagine 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

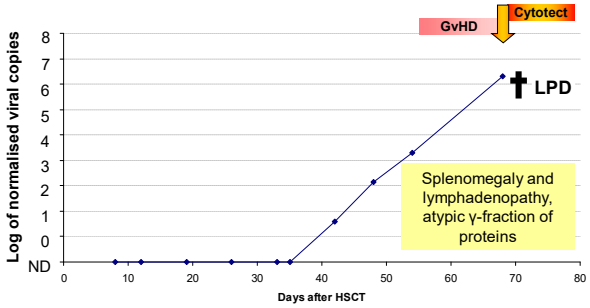


Girl, 5.5 yrs., HSCT for ALL CR2.
Donor: MMUD (8/10)
Graft: BM
Conditioning: TBI (12 Gy)
cyclophosphamide (120 mg/kg)
ATG (16 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade III (D+ 49; GIT)
Deceased on D+74 due to MOF.

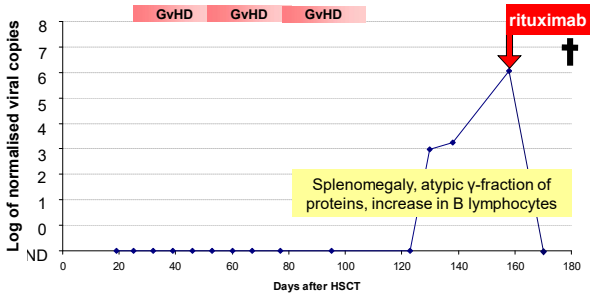


Girl, 4 yrs., HSCT for AML PR2
Donor: MMUD (9/10)
Graft: BM
Conditioning:busulphan(16 mg/kg)
cyclophosphamide (120 mg/kg)
melphalan (140 mg/m²)
ATG (40 mg/kg)
GvHD: grade III (D+ 25; GIT)
Deceased on D+117 due to bleeding and respiratory failure.

Retrospectively tested patients

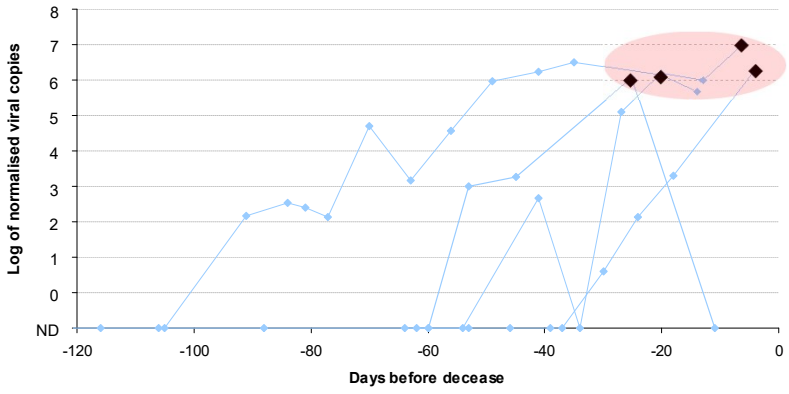


Boy, 5 yrs., HSCT for JMML
Donor: MUD
Graft: BM
Conditioning: busulphan (18 mg/kg)
cyclophosphamide (120 mg/kg)
ATG (16 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade II (D+ 56; GIT)
Deceased on D+72 due to MOF.



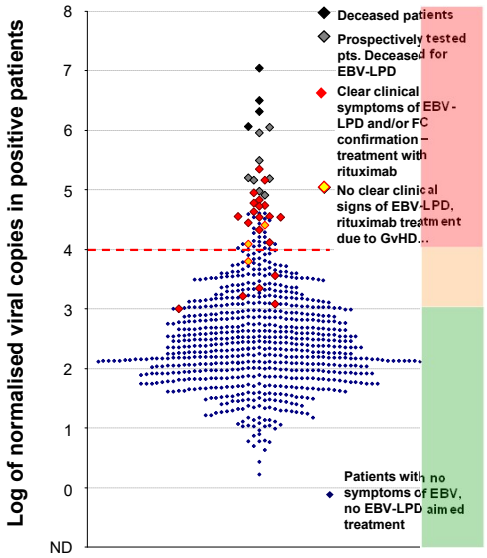
Boy, 11 yrs., HSCT for AML CR2.
Donor: MUD
Graft: BM
Conditioning: busulphan (16 mg/kg)
cyclophosphamide (120 mg/kg)
melphalan (140 mg/m²)
ATG (40 mg/kg)
GvHD: grade III-IV (D+ 23; GIT)
Deceased on D+182 due to MOF.

Retrospectively tested patients



Maximum detected quantity was between
1.16×10^6 and **1.17×10^7 NVCs**

Prospective testing – maximal quantity

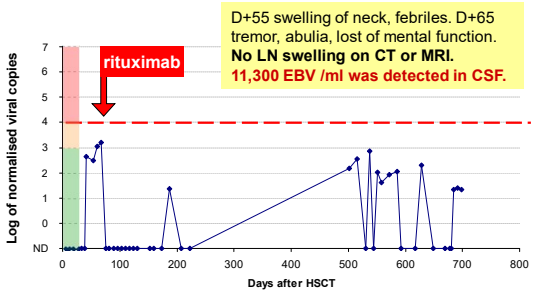


- **Very high risk level reactivation**
  > 10^4 NVCs – ≈ 50 IU/ng
  suspected and/or correlated with signs of EBV-LPD - 102 samples from 26 children (7.1%) and 22 adults (3.2%)
  EBV-LPD was confirmed in 22 (45%)

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

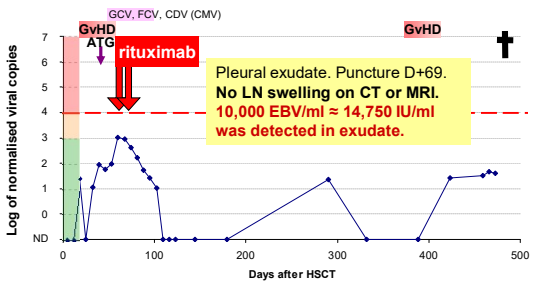
- **Benign level reactivation**
  < 10^3 NVCs ≈ 5 < IU/ng
  low quantities with no clinical signs of EBV were detected in majority of samples from 275 children and 481 adults.

Localised EBV-LPD



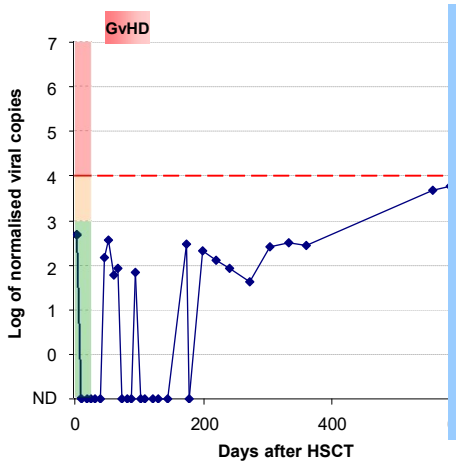
Woman,58 yrs.,HSCT for MDS/AML
Donor: SD (8/10)
Graft: PBSC
Conditioning: idarubicine (21 mg/m²)
fludarabine (25 mg/m²)
TBI (12 Gy) ATG (40 mg/kg)
GvHD prophylaxis: CyA, MMF
GvHD: grade II (D+22)

Outcome: rapid improvement,alive



Woman, 39 yrs., HSCT for AML
Donor: MMUD (9/10)
Graft: PBSC
Conditioning: idarubicine (21 mg/m²)
fludarabine (25 mg/m²)
TBI (12 Gy) ATG (40 mg/kg)
GvHD prophylaxis: CyA+MMF
GvHD: grade III-IV (D+15)
Other: pulmonary proces of unknown origin
Outcome: deceased on D+478 due to MOF

Localised EBV-LPD (NHL)



Boy, 15 yrs., HSCT for ALL in 1. CR
CMV, EBV, HSV seronegative

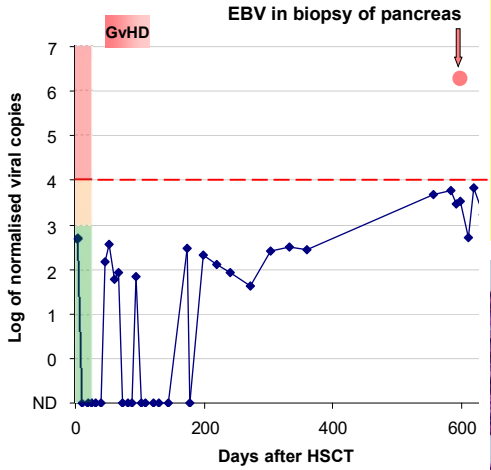
Donor: MSD (brother)
Graft: PBSC
Conditioning: TBI (12 Gy)
etoposid (60 mg/kg)
GvHD prophylaxis: CyA

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)



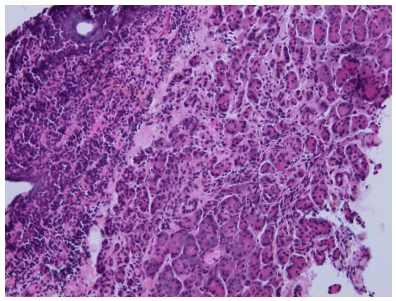
Pancreatopathy of unknown origin, confirmed by CT and US.
MRD negative.

↑CRP, ↑ IgG, no autoantibodies.

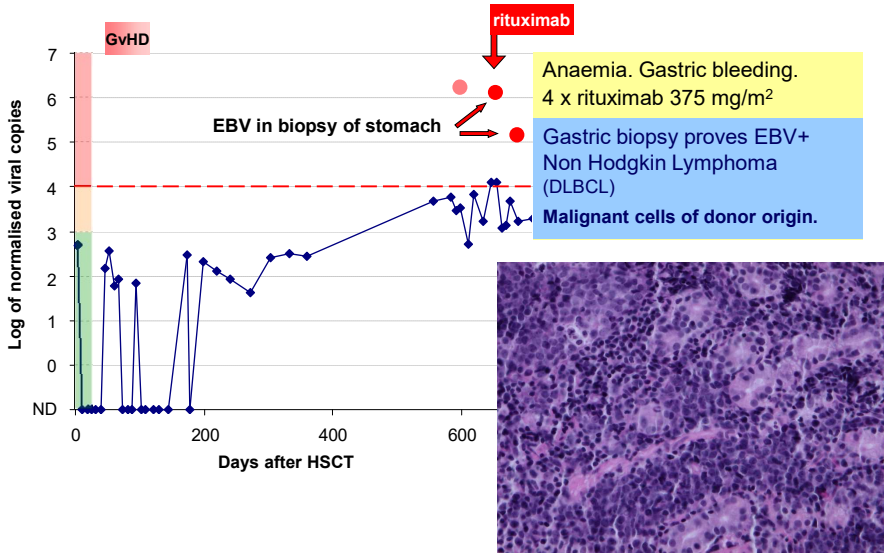
Laparotomy.

All types of lymphocytes detected in the biopsy. No malignant cells.

DR+ lymphocytes detected



Localised EBV-LPD (NHL)



Anaemia. Gastric bleeding.
4 x rituximab 375 mg/m^2

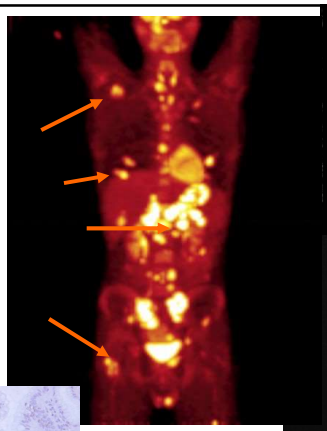
Gastric biopsy proves EBV+ Non Hodgkin Lymphoma (DLBCL)

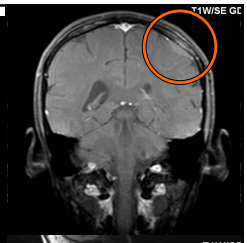
Malignant cells of donor origin.

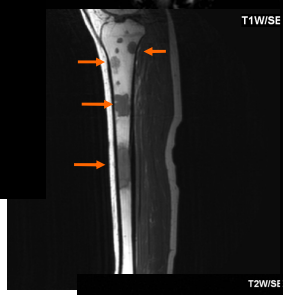
Treatment according to Protocol **BFM NHL 2004**

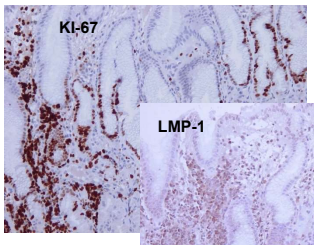
During last chemotherapy sepsis cased by Pseudomonas aeruginosa.

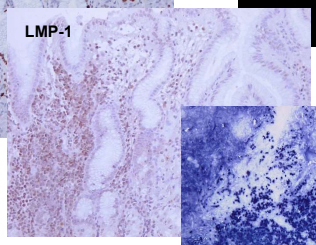
Last PET CT confirmed Remission of NHL.

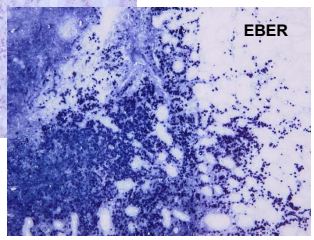


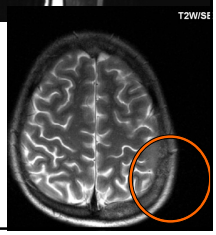




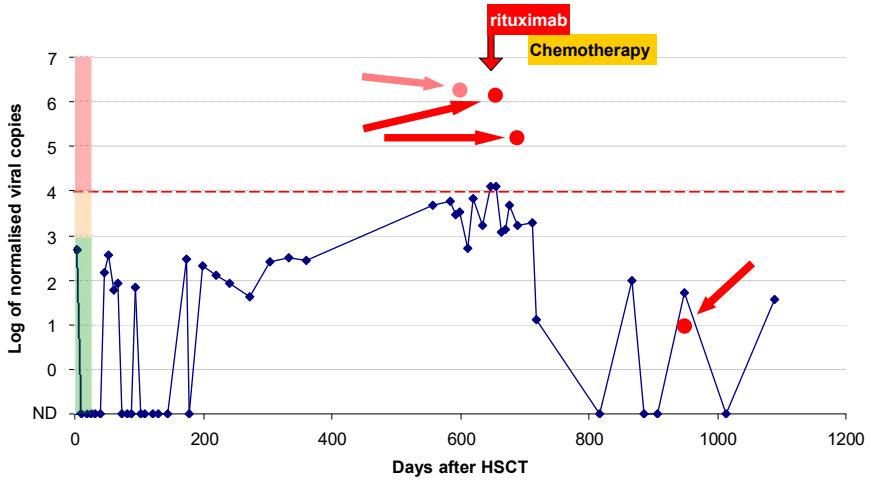






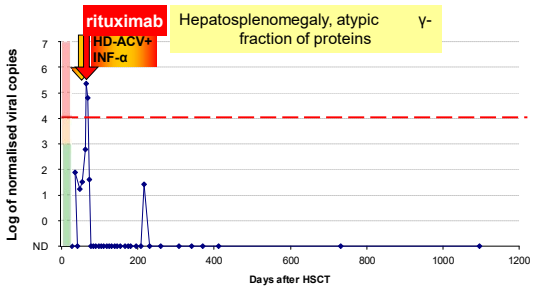


Localised EBV-LPD (NHL)

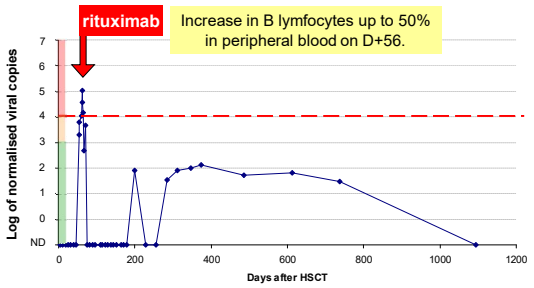


The patient remains in the remission of ALL and NHL.

Generalized EBV-LPD

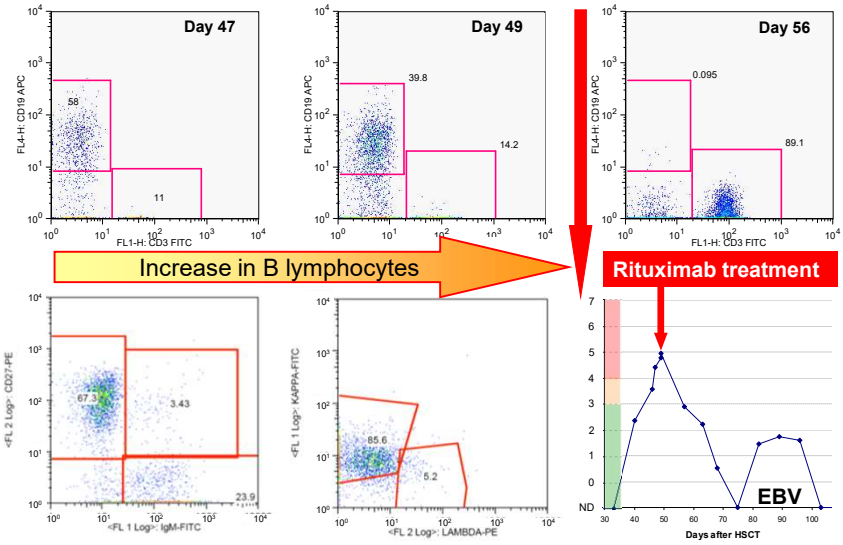


Boy, 3.3 yrs., HSCT for MPS type I
Donor: MMUD (5/6)
Graft: CB
Conditioning: busulphan (24.5 mg/kg)
cyclophosphamide (200 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA, MP
GvHD: grade II (D+49; GIT)
rituximab on D+69
Outcome: alive, no clin. problems



Boy, 4.4 yrs., HSCT for WAS
Donor: MUD
Graft: BM
Conditioning: busulfan (15.3 mg/kg)
cyclophosphamide (200 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA, MTX
GvHD: grade II (D+54; GIT)
rituximab on D+56.
Outcome: alive, no clin. problems

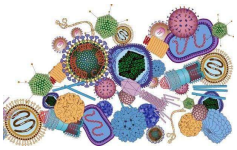
Flow cytometry EBV-LPD confirmation



Proliferation of memory B cells

KAPPA IgL+ cells

Possibilities of influence of viral infection



Immune system

(immunosupresive treatment, chemotherapy, …)

Patogens

Lymfocyte regulated – viruses , mykoses

1. Prevention – vaccination
2. Decrease of immunosupresive therapy
4. Improvement of lymfocyte function
6. Improvement/adding specific lymfocytes

3. Decrease of viral proliferation by virostatic therapy
5. Destruction of permissive cells

1. Prevention - Vaccination

Acyclovir

TBE

Influenza

Rotaviruses

Human papilomaviruses

Hepatitis A

Travel and special vaccin

Lyssa

Yellow fever

…

2. Decrease of the immunosupression treatment intensity

Autoimmune disease

Transplant patients

Iatrogenic immunosupression

STEROIDS

> 2 mg/kg leads to lymfopenia

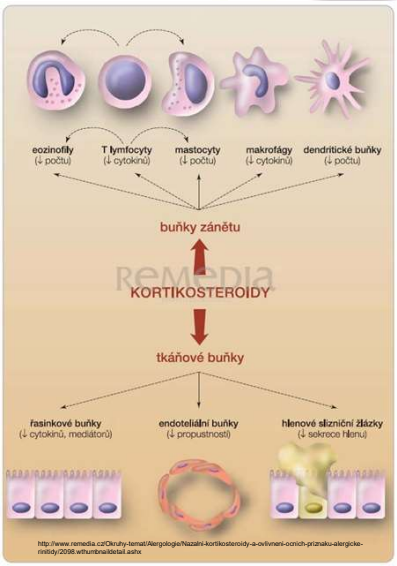
„BIOLOGIC TREATMENT "

infliximab (anti TNF-α)

basiliximab (anti CD25 – α řetězec IL-2R)

Campath (anti CD-52)

Antithymocytární globulin (ATG) …



3. Decrease of viral proliferation

Aiming proliferation improtant viral genes

DNA/RNA polymerase

herpesviruses, AdV

Reverse transkriptase

Protease

Neuraminidase

HCV, HBV, HIV

herpesviruses

Antibodies against permissive cells

anti CD20 - rituximab

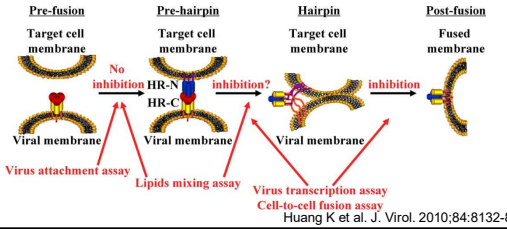
Neutralising antibodies

Profylactic prevention

motavizumab

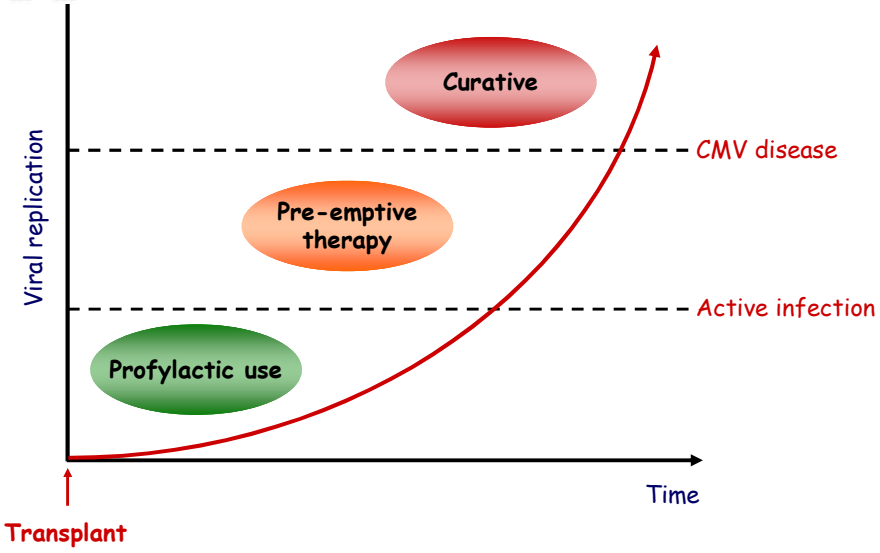
palivizumab (Synagis)

Humanisided neutralizating antibody against F- protein of RSV



Huang K et al. J. Virol. 2010;84:8132-8140

3. Decrease of viral proliferation

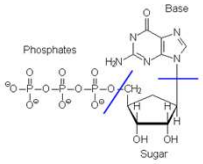


In HSCT recipient: pre-emptive and curative therapy

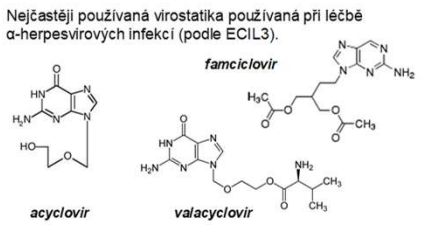
Virostatic drugs impact

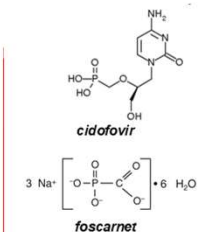
Virostatics

usually cellular nucleotides analogues blocking (more or less specifically) viral polymerase (acyklovir, ganciklovir, cidofovir…), or polymerase directly blocking drugs without similarity to nucleosides (e.g. foscarnet) or viral protein blocking drugs (neuraminidase inhibitors..)



Guanidine trifosfát (GTP)

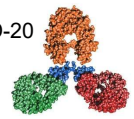




Antibodies with virostatic effect

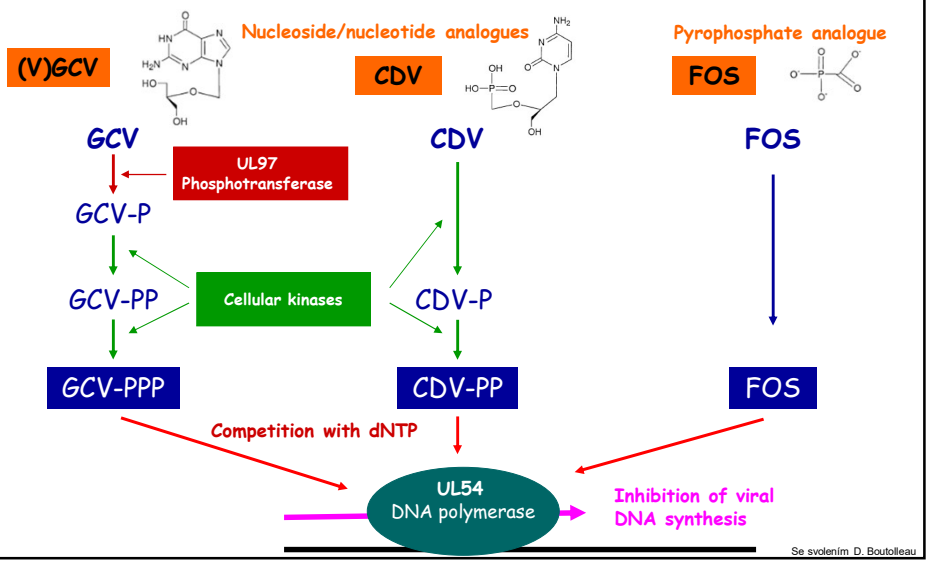
Neutralising antibodies against certain proteins important in pathogenesis of viral disease (F protein in RSV) or aimed against target cells (anti-CD20 in EBV).

Anti CD-20



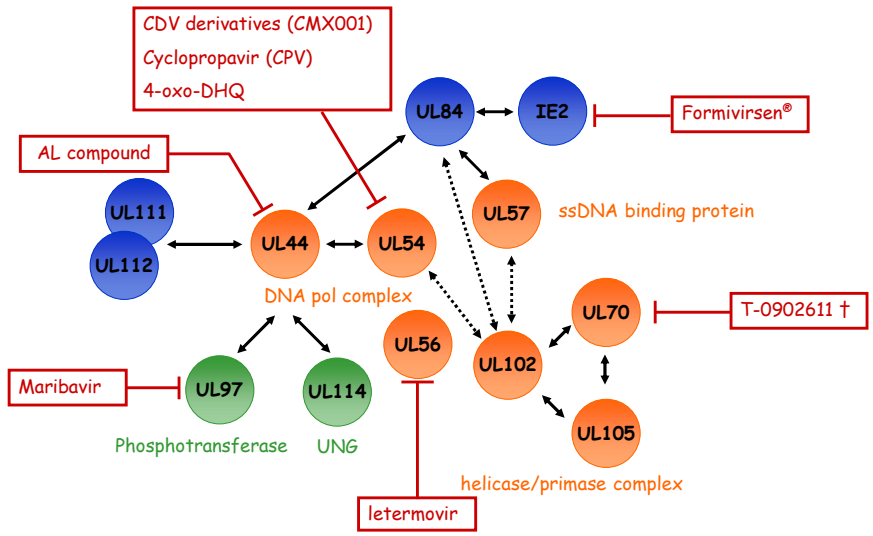
3. Decrease of viral proliferation

➔ Inhibitors of CMV DNA polymerase UL54



Se svolením D. Boutolleau

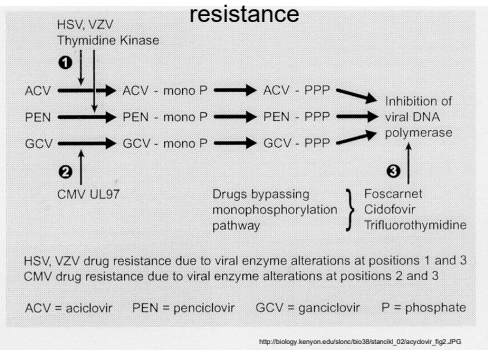
CMV replication complex

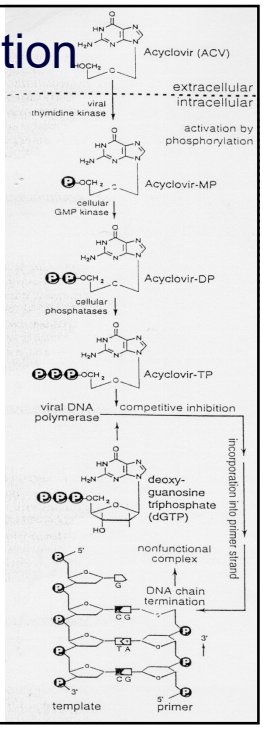


Se svolením D. Boutolleau

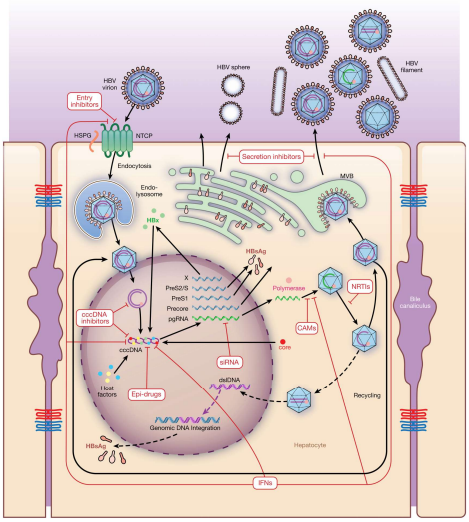
3. Decrease of viral proliferation

Inhibition of herpesviruses and the possible resistance



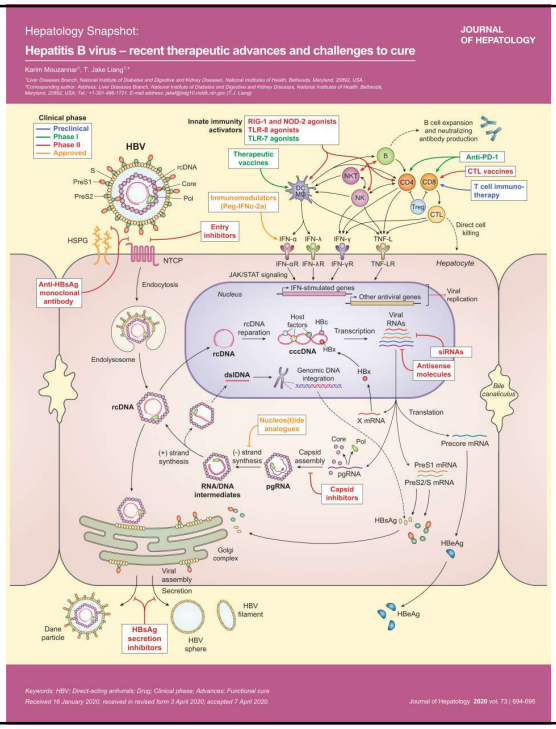


3. Direct acting antivirals (DAA) and future in HBV

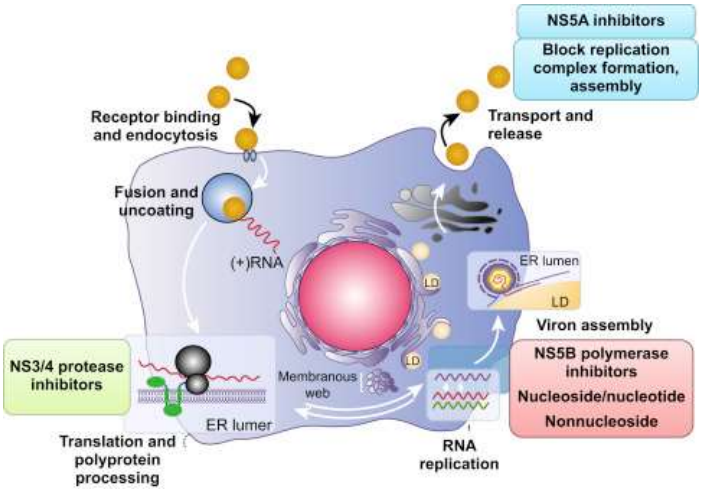


Gastroenterology 2019156311-324DOI: (10.1053/j.gastro.2018.07.057)

3. Direct acting antivirals (DAA) and future in HBV



3. Direct acting antivirals (DAA) in HCV



3. Direct acting antivirals (DAA) in HCV

PROTEASE Inhibitors (PI)
Block active site viral enzyme
-PREVIR

LOW BR
1st Gen.1st wave (GT1):
TELAPREVIR *
BOCEPREVIR *
1st Gen.2nd wave (all GTs except 3)
SIMEPREVIR *
ASUNAPREVIR
PARITAPREVIR/ritonavir *
VANIPREVIR (MK-7009)
DANOPREVIR

HIGHER BR
2nd Generation. Pangenotyping less effective for GT3:
MK-5172 (Grazoprevir-GRZ)
ACH-2684

NS5A Inhibitors (NS5AI)
Block Replication complex, particle assembly & release
-ASVIR

LOW BR
1st Gen. (GT1 and GT4. Other genotypes variable)
DACLATASVIR *
LEDIPASVIR *
OMBITASVIR (ABT-267) *

Slighlty HIGHER BR
2nd Gen. (Pangenotyping)
MK-8742 (Elbavir-EBR)
GS-5816
ACH3102
SAMATASVIR (IDX719)

ORAL IFN-free treatment (+/- Ribavirin)
HARVONI: LEDIPASVIR+SOFOSBUVIR

NS5B Nucleos(t)ide Inhibitors (Nucs or NI)
Block active site.
Inhibits RNA elongation
-BUVIR

HIGH BR
Pangenotyping (less SVR GT3)
SOFOSBUVIR *
MERACITABINE
ACH-3422
IDX21437
IDX21459

NS5B Non-Nucleos(t)ide Inhibitors (Non-Nucs or NNI)
Allosteric site.
Change tridimensional structure
-BUVIR

LOW BR
Narrow genotyping coverage
DASABUVIR (ABT-333) *
DELEOBUVIR
BMS-791325 (Beclabuvir)
PPI-383
GS-9669
TMC647055

ORAL IFN-free treatment (+/- Ribavirin)
VIEKIRAX: OMBITASVIR-PARITAPREVIR-ritonavir +
EXVIERA: DASABUVIR

BR= barrier to resistance. * Approved

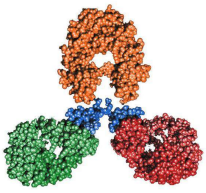
Resistance of Hepatitis C Virus to Inhibitors: Complexity and Clinical Implications. 2015 Viruses 7(11):5746-5766

3. Decrease of viral proliferation

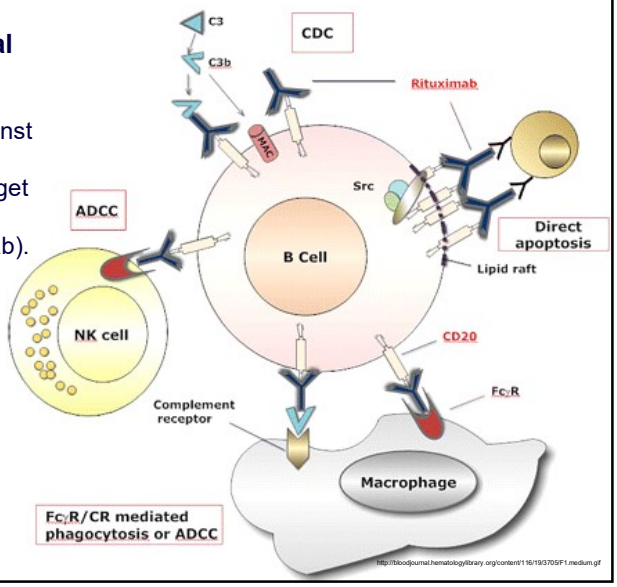
Antibodies

Antibodies with antiviral effect

Neutralising antibodies against proteins important in viral pathogenesis or against target cells of virus
(anti-CD20 u EBV - rituximab).



http://www.curaxys.com/images/img1B.jpg



http://bloodjournal.hematologylibrary.org/content/116/19/3705/F1.medium.gif

Dosing of most frequently used virostatic drugs

- **acyclovir** (HSV, VZV)
  - ***Prophylactical dosing*** – 500 mg/m^2/dose in infusion for 60 minut twice daily with maximum 750 mg/dose
  - ***Therapeutical dosing*** – for 7–10 days
    250 mg/m^2/dose in infusion for 60 minutes á 8 hours with maximum of 500 mg/dose (resp. 10-15 mg/kg/dose)
- **ganciclovir** (CMV, HHV-6, HHV-7)
  - ***Therapeutical dosing*** – at least 3 weeks
    2 weeks 5 mg/kg/dose in infusion for 60 min á 12 hours, 2 týdny; subsequently
    5 mg/kg/dose in infusion for 60 min/ day
- **foscarnet** (CMV, HHV-6, HHV-7, HSV, VZV)
  - ***Therapeutical dosing*** – for 3 weeks
    60 mg/kg/dose in infusion for 60 min (or i.v.) á 12 hours, 1- 2 weeks; subsequently 90 mg/kg/dose in infusion for 60 min (or i.v.) á 24 hours
- **cidofovir** (CMV, HHV-6, HHV-7, HSV, VZV, adenoviruses, BKV, …)
  - In case of CMV disease 5 mg/kg/dose in infusion (1/1 fysiological solution) 1x week
- **oseltamivir** (Influenza)
  - ***Prophylactical dosing*** - 30-60 mg in children younger 12 yrs. according to the weight (>15 kg - 30 mg, 15 to 23 kg - 45 mg, 23 to 40 kg – 60 mg), in patients older 13 yrs. and heavier 40 kg then 75 mg for at least 10 dní.
  - ***Therapeutical dosing*** – at least 10 days in children and adults; dvojnásobek prophylactic dosing – in adults 75 mg 2x day, in very severe cases 150 mg 2x day.

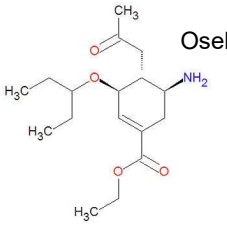
Adverse effects of the virostatic drugs

- **Acyclovir/valaciclovir**
  - ***AE usually reversible***, usually in patients with hepatopathy.
  - rarely haematopoietic and lymphatic system disorders (anaemia, leucopenia, thrombocytopenia), hepatitis, nefrotoxicity.
- **Ganciclovir/valganciclovir**
  - ***myelosupressive effects*** (neutropenia (25–40 %), thrombocytopenia (9-20 %
  - nauzea, vomiting and diarrhea, increase of the liver enzymes: confusion and seizures; renal insufficiency (rarely in patients after heart tx.); enormously rare exanthema or eosinophilia
- **Foscarnet**
  - ***Nephrotoxicity-*** rarely acute renal failure (uremia and polyuria), potencially metabolic acidosis and diabetes insipidus
  - Increase of the liver enzymes, LDH, ALP and amylasis; often nauzea, vomitting nad diarrhea, rash (exanthema), tremor, muscle weakness and increase in body temperature, thrombocytopenia, hypokalemia, hypomagnezemia, hypo- or hyperfosfataemia, ***hypocalcemia*** (shortly after infusion or tonic-clonic seizures) – increased risk in CNS disorder or ciprofloxacine administration
  - Headache, tiredness, paresthesia, tremor, ataxia. Neuropathy, hypestazia, confusion, depression, psychosis, agressive reactions, psychosis, agressive reactions; changes in ECG, hyper- hypotension, rarely even chamber arythmias
  - Often Phlebitis (thrombophlebitis) in addministration of concentrated solutions (> 12 mg/ml) to peripheral vein.

Adverse effects of the virostatic drugs

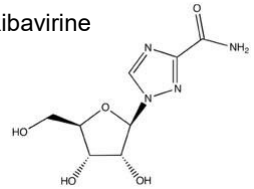
- **Cidofovir**
  - ***nephrotoxicity*** – proteinuria, creatinine increase; acute and even with delay; - good hydration, together with probenecid
  - potencially to ***chronic renal failure*** with dialysis
  - other more common neutropenia, headache, nauzea, vomitting, alopecia, rash, weakness and fever. Described also occular toxicity.
- **Oseltamivir**
  - most frequent AE are nausea, vomitting and belly pain
- **Ribavirine**
  - ***Haematopoietic disorders, depression, teratogenic effect (inhalation)*** from that reason there must not be exponed men or women about the conception. In case of higher cumulative dose risk of teratogenicity lasts for months; nausea, pain in belly….

Oseltamivir



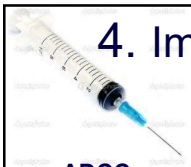
http://en.citizendium.org/images/thumb/6/68/Oseltamivir_structure.jpg/350px-Oseltamivir_structure.jpg

Ribavirine



4. Improvement of the lymfocyte function

Antibodies

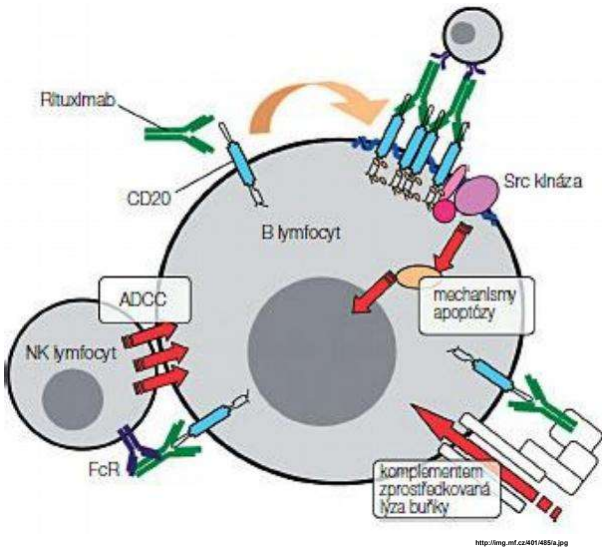


Acyclovir

ADCC

– antibody dependent cellular cytotoxicity

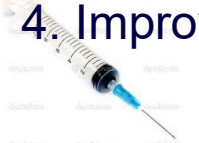
Both „non-specific “ IVIG, and hyperimunne globulins (e.g. Cytotect®) might be used.



http://img.mf.cz/401/485/a.jpg

4. Improvement of the lymfocyte function

Interferon α



Používá se zejména při léčbě hepatitidy B.

Na trhu několik přípravků lišících se typem interferonu I – α2a

Např. (rekombinantní Roferon A), α2b (rekombinantní - INTRON A), případně pegylované interferony tj. s polyetylenglykolem (Pegasys^{TM}, PEG-INTRON)

Dávka: obvykle 2,5 - 5,0 milionů IU/m^2, resp. až 10 milionů IU/m^2 u dětí s.c., 3× týdně po dobu 4–6 měsíců.

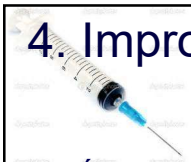
Dávkování může být v případě nežádoucích účinků upraveno.

Není-li po 3–4 měsících léčby zlepšení, je třeba uvažovat o přerušení terapie.

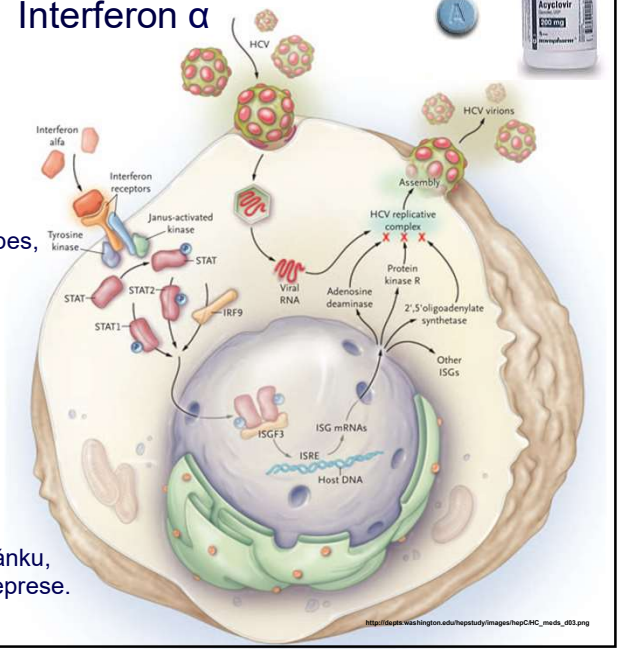
Pacientům nad 18 let je v současnosti doporučen pegylovaný interferon- α2a v dávce 180 μg/týden v jedné dávce s.c.; délka léčby dle odpovědí na léčbu - při dobré odpovědi trvá 48 týdnů.

4. Improvement of the lymfocyte function

Interferon α

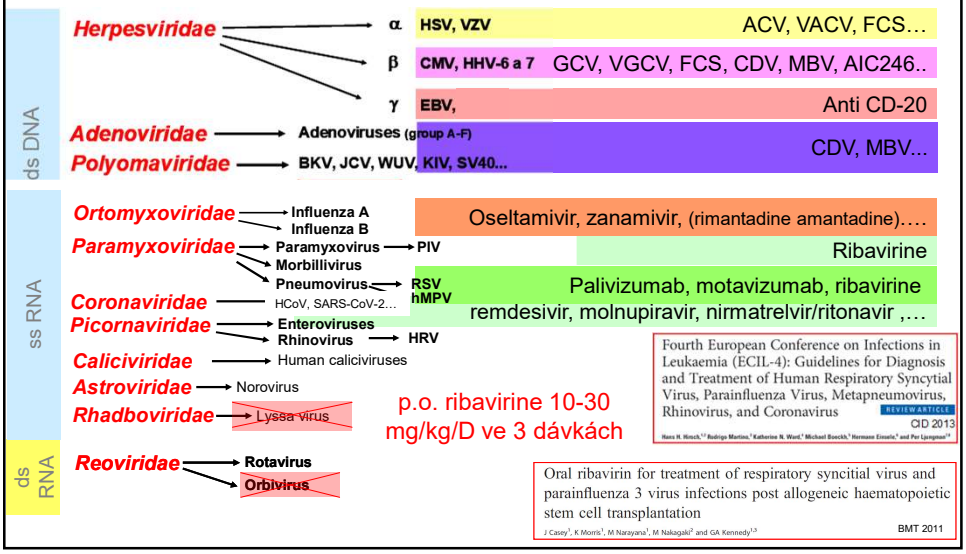


NÚ:
„flu-like“: únava, zimnice, bolest svalů nebo kloubů, bolest hlavy, pocení nebo horečka.
Vzácněji pneumonie a herpes, anémie, trombocytopenie, Leukopenie, autoimunitní stavy, sarkoidóza, poruchy štítné žlázy, zažívání, hypo- a hypertenze, proteinurie a poruchy renálních funkcí, glykémie a homeostázy.
Případně účinky na CNS např. poruchy citlivosti, spánku, nervozita, stavy úzkosti, deprese.



Therapeutical possibilities of virostatics and specific antibodies

More or less specific for certain viral groups:



Fourth European Conference on Infections in Leukaemia (ECIL-4): Guidelines for Diagnosis and Treatment of Human Respiratory Syncytial Virus, Parainfluenza Virus, Metapneumovirus, Rhinovirus, and Coronavirus
REVIEW ARTICLE
CID 2013

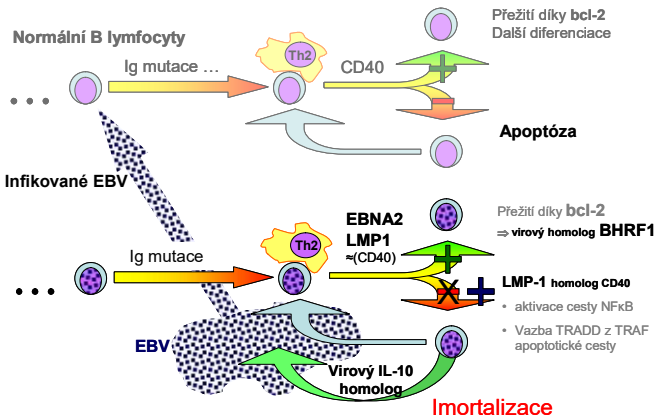
Oral ribavirin for treatment of respiratory syncitial virus and parainfluenza 3 virus infections post allogeneic haematopoietic stem cell transplantation
J Casey[1], K Morris[1], M Narayana[1], M Nakagaki[2] and GA Kennedy[1,3]
BMT 2011

Acyclovir

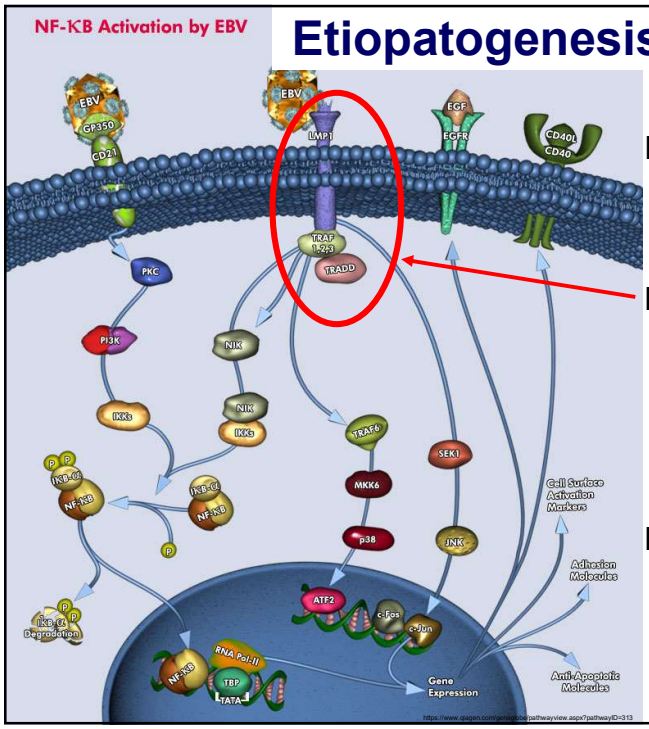
5. Destruction of permissive cells

Used in EBV associated malignant disease (HL, NHL), or post-transplant EBV-LPD.

Anti CD-20 – rituximab (MabThera)



Etiopatogenesis of EBV-LPD



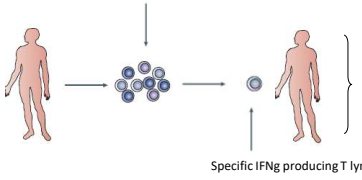
EBER
- – ↓kaspase 3, PARP
- -↑ bcl-2 - ↓PKR fosf.

LMP1
- – homologue CD40
- - binds TRADD from TRAF apoptotic pathway
- –NFkB activation, AP1, STAT1/3

BHRF1
- – viral homologue bcl-2

6. Present possibilities of specific cell production and its limitations

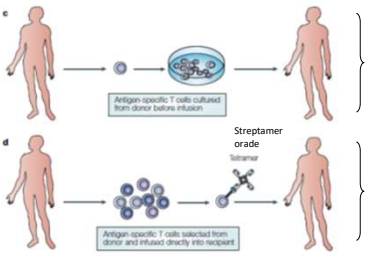
Acyclovir



Non-specific T cells, technically and financially difficult
ATMP (advanced therapeutic medicinal product)

Feuchtinger et al BJH 2006
Feuchtinger et al Blood 2010

Unrelated donors: preparation about 8-9 weeks from first demand to product



Literatura:

4-12 weeks of production,
ATMP (advanced therapeutic medicinal product)

Leen et al Nature Medicine 2006
Leen et al Blood 2009

So far - for prophylactic use only!

HLA-string, technically and financially difficult

Unrelated donors: preparation about 8-9 weeks from first demand to product

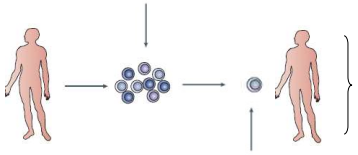
Cobbold et al J. Exp. Med. 2005
Schmitt et al Transfusion 2011

Moss P and Rickinson A Nature Reviews 2005 (5)

Acyclovir

6. Present possibilities of specific cell production and its limitations

Viral antigen stimulation



Specific IFNg producing T lymfocyte selection

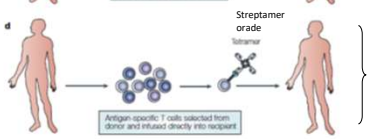
Non-specific T cells, technically and financially difficult
ATMP (advanced therapeutic medicinal product)

Feuchtinger et al BJH 2006
Feuchtinger et al Blood 2010

Unrelated donors: preparation about 8-9 weeks from first demand to product

Promissed results, however so far not useful for wide clinical practice. Price approx. 8-14 000 Eur

So far - for prophylactic use only!



HLA-string, technically and financially difficult

Unrelated donors: preparation about 8-9 weeks from first demand to product

Cobbold et al J. Exp. Med. 2005
Schmitt et al Transfusion 2011

Moss P and Rickinson A Nature Reviews 2005 (5)

However – for success of the therapy is still crucial …

… reconstitution of immunity!


Thank you for you attention
Petr.Hubacek@Lfmotol.cuni.cz