# General virology and detection techniques

FN MOTOL

## What is virus?

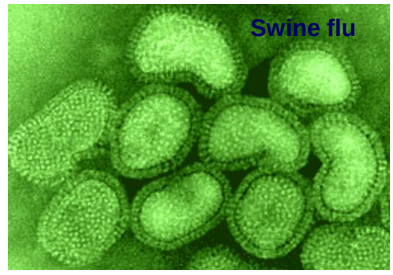
**It is a submicroscopical pathogen containing the nucleic acid and proteins, which infects and reproducts in host cells.**

**Proliferation and multiplication of the virus is possible only in infected cells.**

Viruses do not have translation system (ribosomes and transfer RNA) necessary for proteosyntesis. That is the reason why proliferation is possible in host cells only (bacterias, animals and plants).

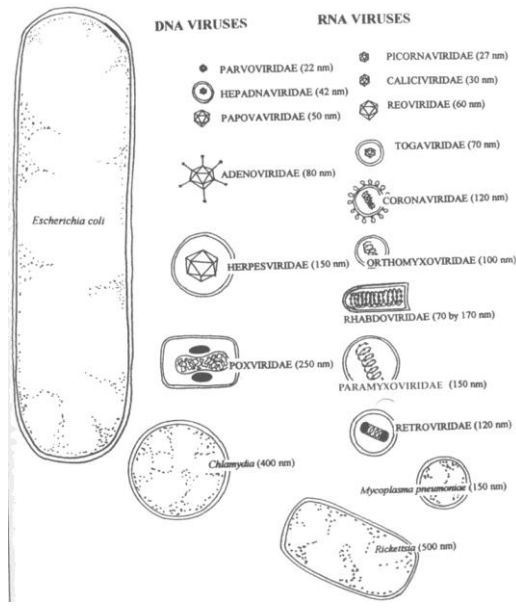
**Some viruses (poxviruses, herpesviruses or rhabdoviruses..) contains enzymes important for viral reproduction inside the virions.**

**Virion is complete fully matured viral particule able to infect the cell.**




**Life is fight.**

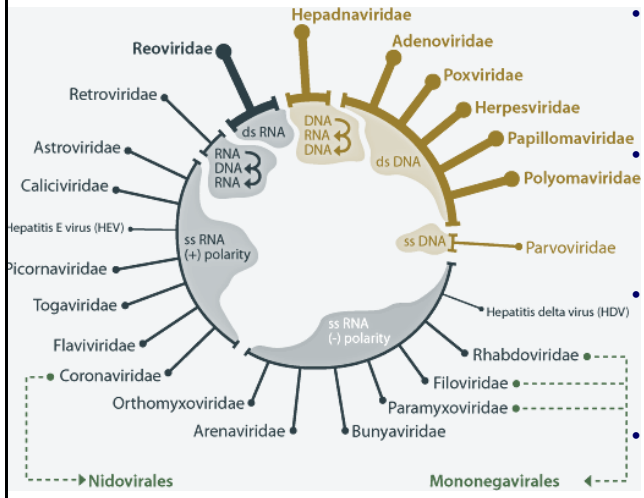
# How they looks like?




http://www.epubbud.com/read.php?g=2RBLFKRP&tocp=5

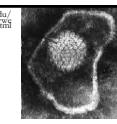
- Different capsid symetry(22 nm-250nm)
- May be enveloped or non-enveloped
- For infection are important molecules on the viral surface which determines the cell receptors for virus binding and so specificity of viral infection for different cell types.

# How they looks like?



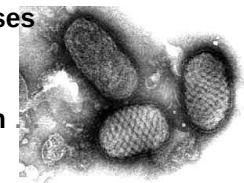

- Coding nucleic acid can be both ss or ds and RNA and DNA
- Size of the genome is approx. between 3 kB and ≈ 200 kB
- Genome may be segmented or non-segmented
- Linear or circular

# DNA viruses



ds DNA

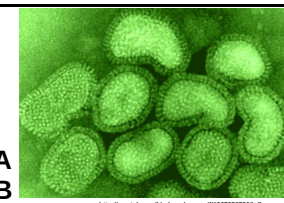
- ***Herpesviridae*** → α HSV 1 and 2, VZV; β CMV, HHV-6 a 7; γ EBV, HHV8
- ***Adenoviridae*** → Adenoviruses (group A-F)
- ***Polyomaviridae*** → BKV, JCV, WUV, KIV, SV40...
- ***Papillomaviridae*** → Papillomaviruses
- ***Poxviridae*** → Variolla virus, vaccinia, molluscum contagiosum
- ***Hepadna*** → HBV



ss DNA

- ***Parvoviridae*** → Parvovirus B19; Dependovirus

# RNA viruses



ss RNA

- ***Ortomyxoviridae*** → Influenza A; Influenza B
- ***Paramyxoviridae*** → Paramyxovirus → PIV 1-4; Morbillivirus; Pneumovirus → RSV, hMPV
- ***Coronaviridae*** → HCV
- ***Picornaviridae*** → Enteroviruses; Rhinovirus → HRV
- ***Caliciviridae*** → Human caliciviruses → Norovirus, Sapovirus
- ***Astroviridae*** → Astrovirus
- ***Rhadboviridae*** → Lyssa virus

ds RNA

- ***Reoviridae*** → Rotavirus; Orbivirus

# Does it make sense to know it?

# Many viruses around

**Enveloped / non-enveloped** – diffrent physical and chemical stability
**RNA / DNA** - differennce in stability and contagiousness

## DNA

ds DNA

- *Herpesviridae* → α HSV, VZV; β CMV, HHV-6A a B a HHV-7; γ EBV, HHV-8
- *Adenoviridae* → Adenoviruy (skupina A-G)
- *Polyomaviridae* → BKV, JCV, WUV, KIV, SV40...
- *Papillomaviridae* → Papillomaviry
- *Poxviridae* → Variolla virus, vaccinia, molluscum contagiosum

ss DNA

- *Hepadna* → HBV
- *Parvoviridae* → Parvovirus B19, HBoV; Dependoviry
- *Anelloviridae* → TTV, TTMDV, TTMV

## RNA

ss RNA

- *Ortomyxoviridae* → Influenza A; Influenza B
- *Paramyxoviridae* → Paramyxovirus → PIV 1-4; Morbillivirus; Pneumovirus → RSV, hMPV
- *Coronaviridae* → Lidské coronavíry: OC43, NL63, 229E, HKU1, MERS
- *Picornaviridae* → Enteroviruses; Rhinovirus → HRV
- *Caliciviridae* → Norovirus, Sapovirus
- *Astroviridae* → Astrovirus
- *Rhadboviridae* → Lyssa virus

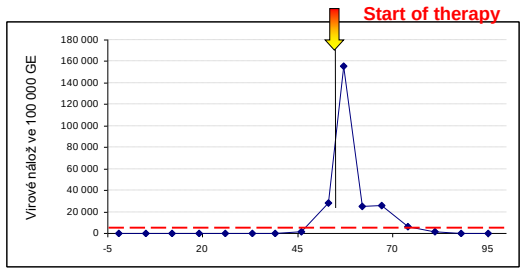
ds RNA

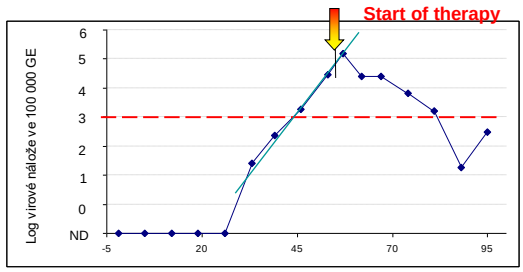
- *Reoviridae* → Rotavirus; Orbivirus
- *Retroviridae* → HIV, HTLV

***Bigger, ussually more genes.***
***Often – chronic and latent infections.***
***Immune systém manipulation.***
***Changes in cell cycle regulations genes – anti-apoptotic.***

***Smaller, less genes.***
***Rapid and acute disease.***
***Through TRL, IFN- bigger systemic impact of the infection.***
***Higher mutation rate.***

# Exponential virus proliferation




**In vivo CMV doubling time**

**48-72 hours**

| Day | Diff. in days | CMV NVCs | Vypočítaná nálož do dalšího vyšetření při čase 48 hodin |
|---|---|---|---|
| 19 | 7 | 0 | |
| 26 | 7 | 0 | |
| 33 | 7 | 260 | |
| 39 | 6 | 2 300 | 2 880 |
| 46 | 7 | 18 700 | 27 500 |
| 53 | 7 | 281 700 | 224 400 |

**Patient with AML dg. after allo-HSCT**

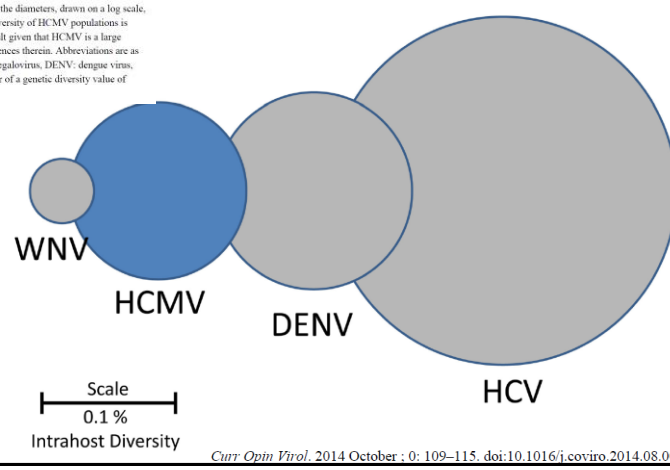
# Rapid changes in CMV genome

*Whole genome sequencing – NGS.*

**Human Cytomegalovirus Intrahost Evolution – A New Avenue for Understanding and Controlling Herpesvirus Infections**

Nicholas Renzette[1], Laura Gibson[2], Jeffrey D. Jensen[3,4,5], and Timothy F. Kowalik[1,6,*]

**Figure 1. HCMV Intrahost genetic diversity as compared to RNA viruses**
Viral intrahost diversities are represented as circles with the diameters, drawn on a log scale, representing reported values of diversity. The genetic diversity of HCMV populations is comparable to those of RNA viruses, an unexpected result given that HCMV is a large dsDNA virus. Values were obtained from [16] and references therein. Abbreviations are as follows: WNV: West Nile Virus, HCMV: human cytomegalovirus, DENV: dengue virus, HCV: hepatitis C virus. Scale bar represents the diameter of a genetic diversity value of 0.1%.

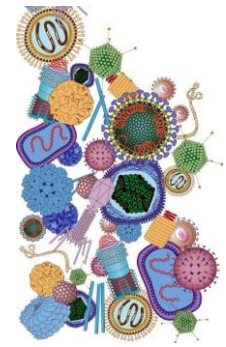



Re-infections in10%

Predominant sequence in about 90% of viral population

*Curr Opin Virol*. 2014 October ; 0: 109–115. doi:10.1016/j.coviro.2014.08.001.

# Balance in the (immunocompromised) patient



**Immune system**

**(immunocompromised treatment, chemotherapy, …)**

**Pathogens**

**Lymfocyte regulated – viruses, mycoses**

# Balance in the immunocompromised patient

**Immune system**

**(immunocompromised treatment, chemotherapy, …)**

**Pathogens**

**Lymfocyte regulated – viruses, mycoses**

***In patient with steroids and immunosupressive therapy, symptoms do not have to develop completely, or might be modified including the rapid and severe course of infection. Vaccination may fail.***

**Always higher risk of symptomatic end-organ disease.**

# Detection methods in virology

- Microscopic **Direct detection**
- Cultivation
- Detection of the antigen
- Detection of the nucleic acid

- Detection of the antibodies
- (Signs of disease)

**Indirect detection**

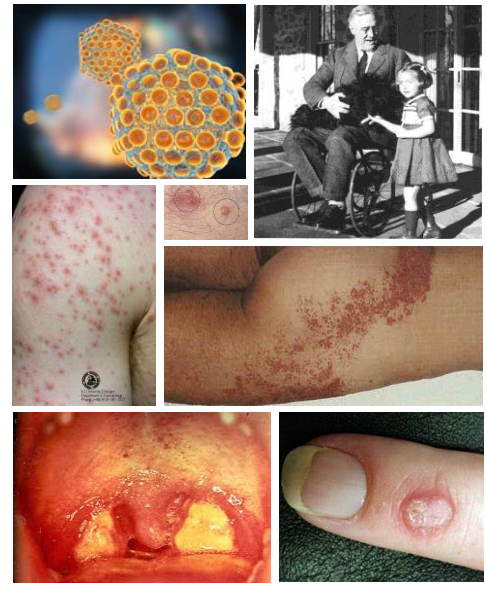


# Signs of the disease

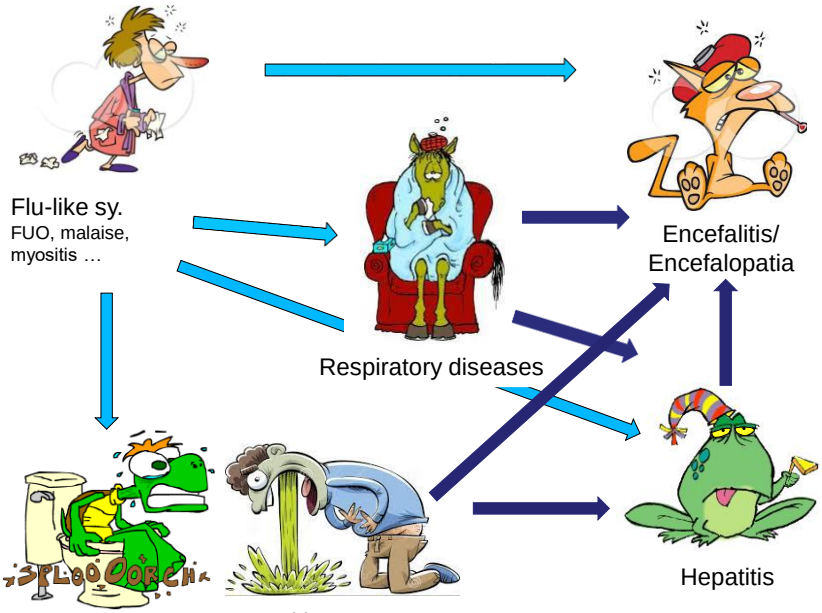
Clinical signs of disease leading to suspition of viral infection (poliomyelitis) were described first 3 700 BC in Egypt.

Typical signs are e.g. in:

- varicella
- zoster
- fully deleloped IM
- papillomaviral infection (wart)
- also in HHV-8 and other viral infections



# Clinical consequences



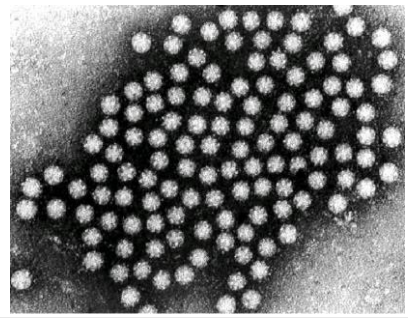

MAJOR ARTICLE

# Astrovirus VA1/HMO-C: An Increasingly Recognized Neurotropic Pathogen in Immunocompromised Patients

**Julianne R. Brown,[1,2] Sofia Morfopoulou,[3] Jonathan Hubb,[4] Warren A. Emmett,[3] Winnie Ip,[5] Divya Shah,[2] Tony Brooks,[6] Simon M. L. Paine,[7,9] Glenn Anderson,[7] Alex Virasami,[2] C. Y. William Tong,[4] Duncan A. Clark,[4] Vincent Plagnol,[3] Thomas S. Jacques,[7,9] Waseem Qasim,[5] Mike Hubank,[6] and Judith Breuer[1,8]**

[1]Virology Department, Great Ormond Street Hospital for Children NHS Foundation Trust, [2]NIHR Biomedical Research Centre, Great Ormond Street Hospital for Children NHS Foundation Trust and University College London, [3]UCL Genetics Institute, University College London, [4]Virology Department, Barts Health NHS Trust, [5]Molecular and Cellular Immunology, [6]Molecular Haematology and Cancer Biology Unit, Institute of Child Health, University College London, [7]Department of Histopathology, Great Ormond Street Hospital for Children NHS Foundation Trust, [8]Department of Infection and Immunity, and [9]Birth Defects Research Centre, Institute of Child Health, University College London, United Kingdom





http://www.oardc.ohio-state.edu/tsailab/pictures/astro%20virus%204x4.jpg

# Microscopic techniques

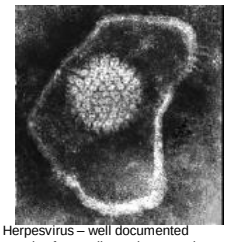
- Elektronmicroscopic detection of virus
  - In liquid materials after virus concentration
  - In the tissues
  - Immunoelectron microscopic detection after signing of virus with specific antibody
- Immunohistochemical detection of virus in the cells
  - Methods for histology testing of biopsies
  - Cytological technique



# Electronmicroscopic evidence

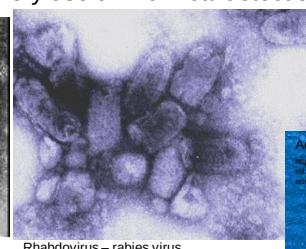
First photography of virus was published in 1939. Further development of electronmicroscopy established this technique in rutine diagnostic of viral infections. Different morphology and size of viral particules makes this technique still very useful in clinical detection.

http://sanidadanimal.info/cursos/curso11/galerias/paginas/picornavirus.htm



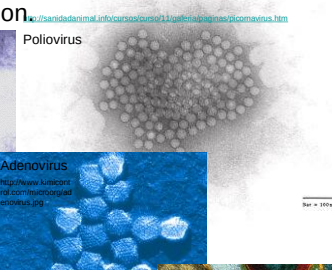
Herpesvirus – well documented envelop from cell membrane and capsid.

Zdroj:



Rhabdovirus – rabies virus

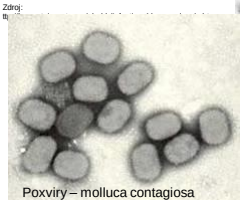
http://www.stanford.edu/group/virus/rhabdo/2005/Rabies%20Virus.htm



Poliovirus

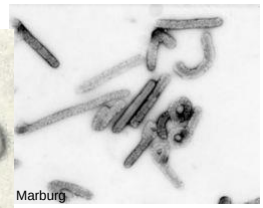
Adenovirus

http://www.luriconsul.com/microorganismos/adenovirus.jpg -



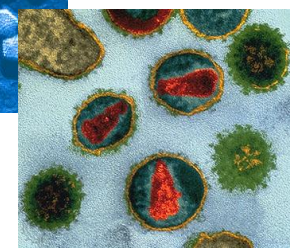
Poxviry – molluca contagiosa

www.equeme.com



Marburg

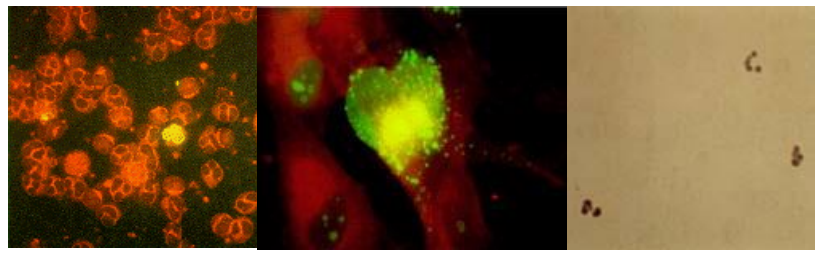
http://content.answers.com/main/content/wp/en-commons/thumb/9/91/250px-Marburg_virus.jpg



http://kirstyne.files.wordpress.com/2007/09/hiv.jpg

# Immunohistochemical detection of viral proteins

**At the present time, detection of viral proteins is based on using of monoclonal antibodies fluorescent microscopy.**



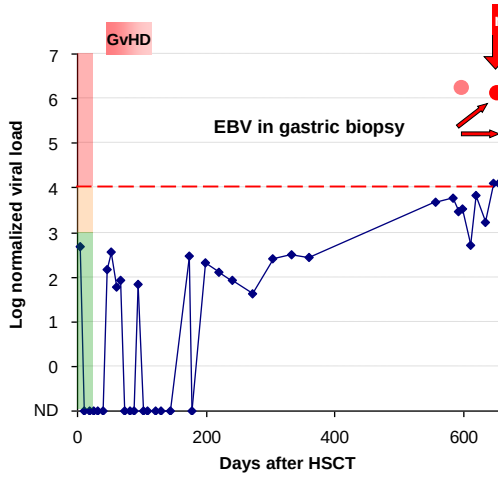
**Detection of pp65 CMV antigen using fluorescent microscopy.** Zdroj: ttp://home.teleport.com/~bobh/InfectiousMononucleosis.htmhttp://www.argene.com/pictures_gallery/zoom_images_ang/CMV_Antigenemia_perox.php



# Using of microscopical techniques

- Histochemical detection
  - Especially during pathological testing
- Electron microscopy
  - For particular types of samples and viruses
  - Lower sensitivity comparing to the cultivation and PCR
- Optical microscopy
  - Might be useful supplemental technique
    - Signs of inflammation without bacterias suggests viral ethiology

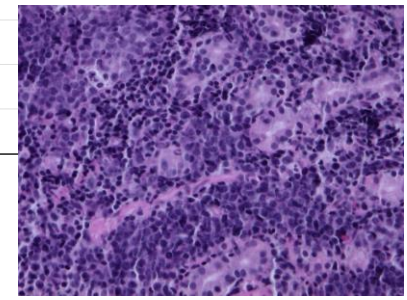
# Localised EBV-LPD (NHL)




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

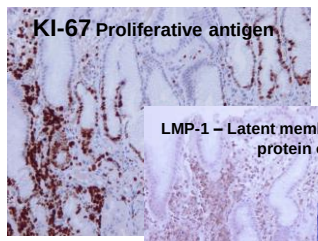
Gastric biopsy proves EBV+ Non Hodgkin Lymphoma (DLBCL)

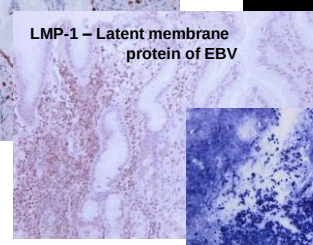
**Malignant cells of donor origin.**

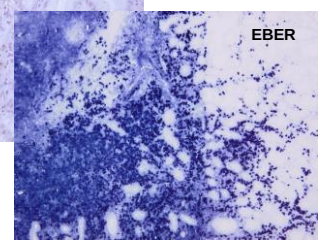


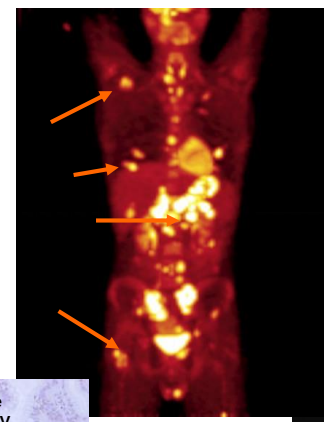
Treatment according to the **BFM NHL 2004** protocol.

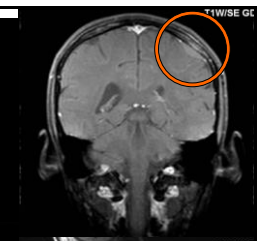
During last block of chemotherapy Pseudomonas aeruginosa sepsis.

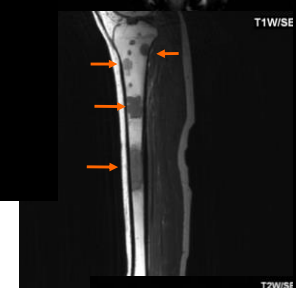


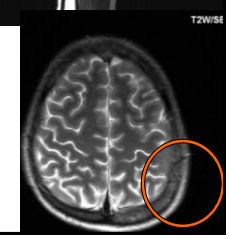





## Methods of cultivation

- Cultivation on cell (tissue) cultures
  - Classic with cytopathic effect
  - Rapid with immunochemical visualiazation of the virus
- Cultivation on chicken embryos
- Test on the animal



## Tissue cultivation

**Pros**

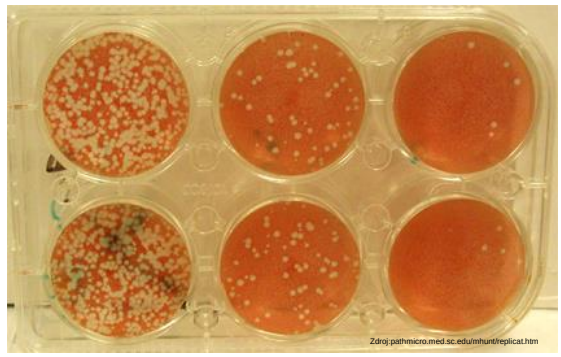
- Prooving a „living“ virus
- Ability to do additional tests
- Detection wider spectrum of viruses

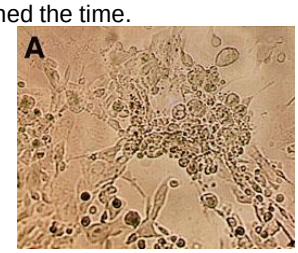
**Cons**

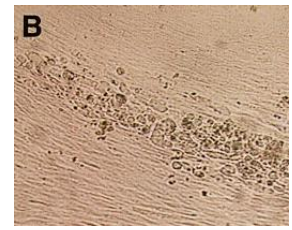
- Sensitive to transport conditions
- Some viruses are badly cultivated in vitro (longer time to detection)
- Difficulties in work with tissue monolayers (contamination with bacterias and mycoses)

# Viral cultivation

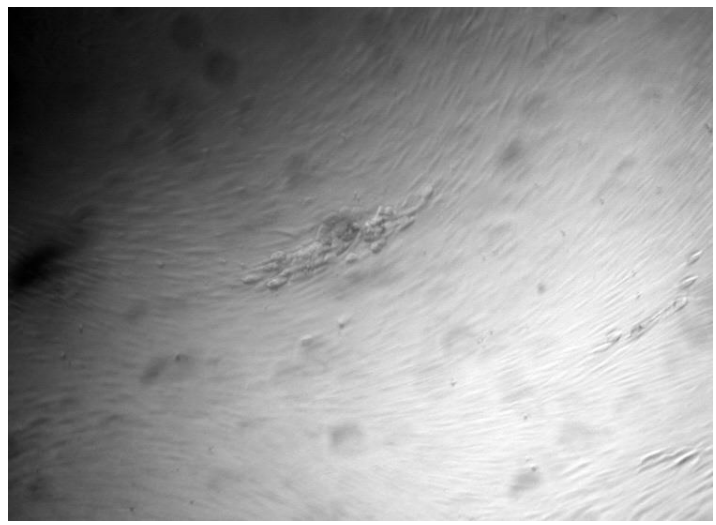
Additional possibility for viral detection is cultivation on tissue monolayers. J. Enders used it for the first time for poliovirus in 1949. Plaque forming assay was first used in 1952 by R. Dulbecco. Subsequently, plaque forming units (PFU) were established. Shell vial cultivation shortened the time.



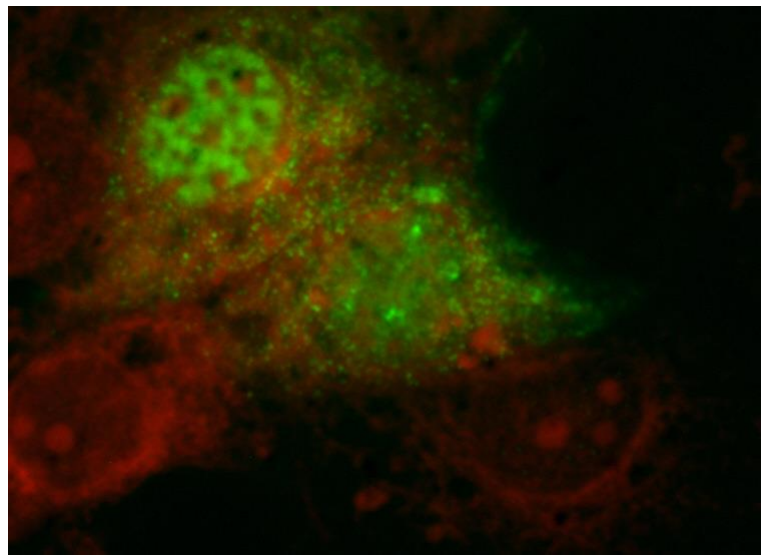




# Cytopatic effect - CMV



# Influenza A virus on tissue monolayers

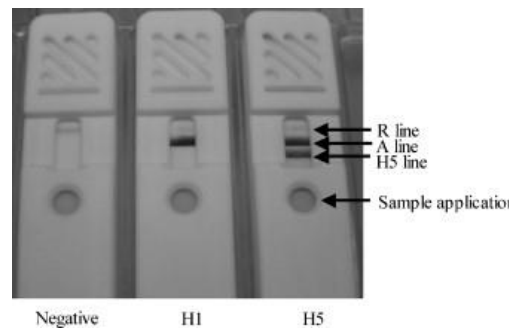
**- monoclonal antibody stained with FITC**



Methods of the viral detection - DIRECT

# Viral antigen detection

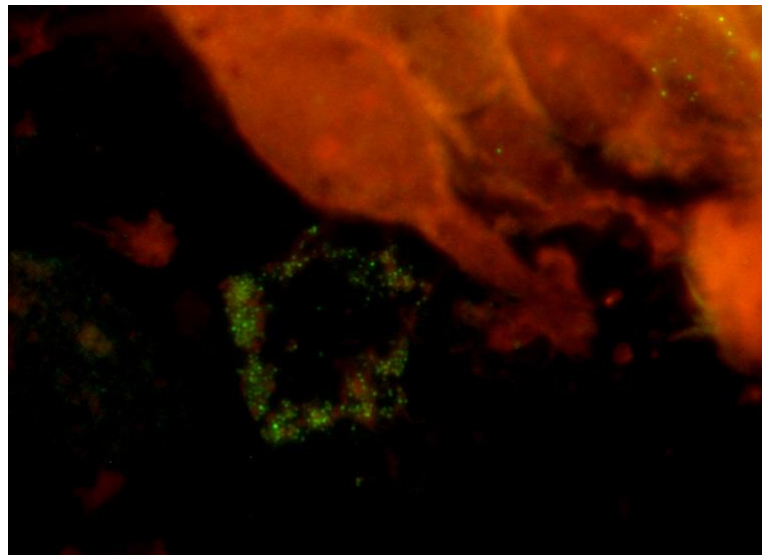
- Detection of single or only couple of pathogens
- Detection of presence/absence; sometimes possibility of (semi-) quantification.
- Based on antigen-antibody reaction




**Sensitivity approx. about 30-40% compared to PCR (real ≈20%).**

**Price approx. 4-6 €**

## Antigen detection of Adenovirus in the lung tissue





## Using of antigen detection

- In case of infection with defined clinical picture and only few possible pathogens (e.g. respiratory tract inf.)
- Infection necessary to be monitored in defined group of patients (e.g. CMV in the immunoxompromissed host).
- Infection, in which is antigen present regularly in huge amounts (hepatitis B)

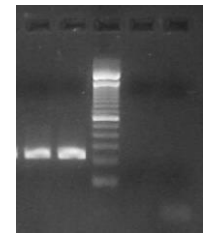
# Nucleic acid detection

- Detection of one or couple agens only
  - Amplification (PCR, NASBA) - sensitive
  - Without amplification (gene probes) – less sensitive
- Rapid and perspective



# PCR reactions

- Qualitative
  - Basic diagnostics
  - Detection of presence/absence of single agens only
- Multiplex
  - Detection of more pathogens in a single reaction
  - Important is detection of product
- Quantitative
  - Competitive
  - Real - time PCR



# Using of PCR

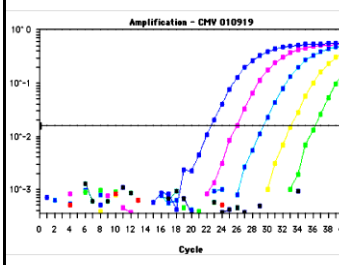
- **Pros**
- High sensitivity
- rapid
- Highly specific
- Possibility of quantification

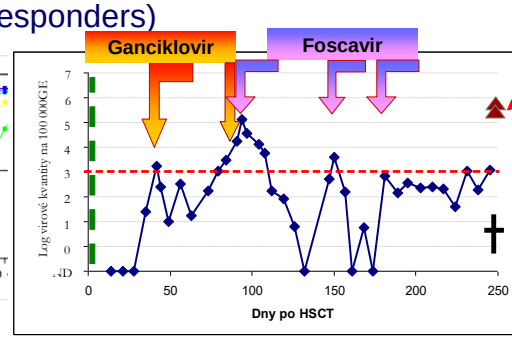
- **Cons**
- Sensitive for manupulation
- Detection of vial and non-vial agens
- Risk of inhibition and false positivity



# Using of Real-time PCR

- Quantification of microbial agens to find diagnosis and prognosis
- Monitoring of patients in immunosupression (quick start of the treatment)
- Monitoring of virostatic treatment (detection of resistence in pacient - nonresponders)



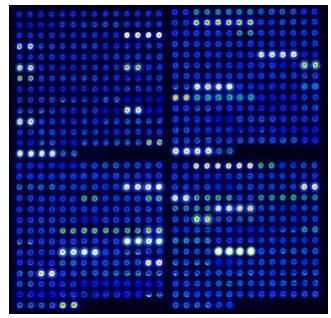
# Sequencing and detection of agent according to the database

- Detection of nucleotid sequence according to the database
- Less useful in virology
- Quality of database matters
- At the present time more supplementary

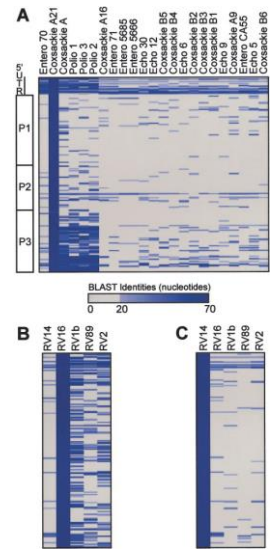


# Detection of nucleic acids using CHIP technique

**Since 2000, there are first papers describing possibility of viral detection by CHIP technique.**



**This approach was used also for discovery of two new human polyomaviruses WU and KI in 2007 which were isolated from respiratory tract.**



## Comparing of techniques – sensitivity

- PCR and cultivation amplified samples quantity of the agens - sensitive
  - PCR depends on type of techniques (primers, multiplex…)
- Cultivation os easily influence by experience and type of agens (growth factors)
- Detection of antigens in sampled quantity only – less sensitive
- Microscopy is more or less for orientation only

## Comparing of techniques – specificity

- Cultivation has minimum of false positive reactions
- PCR – depends on quality of detection and primers – detects all not only viable virus
- Antigen detection has lower specificity

## Comparing of techniques – necessary time

- Antigen detection – results normally within 30 minutes
- PCR – result can be generated within couple of hours
- Cultivation – takes usually days to weeks

## Comparing of techniques – possibility of wide detection

- PCR and antigen detection detects only particullar pathogens (with exception of sequencing)
- Cultivation can detect more viruses
- Electron microscopy detect widest spectra of viruses (limited by staff experience)

**Detection of antibodies reflects only reaction of part of the immune system against infection.**



## Detection of antibodies

- Can detect immunoglobuline in different classes (IgG, IgM, IgA)
- At the beginning of the infection, there is no specific antibody production
- Not suitable for monitoring of the treatment

## Main usage of the antibody detection

- Viral infections with huge systemic response (influenza, rubella, hepatitis)
- More severe bacterial infections (pertusse, syfilis)
- Systemic infections with single cell parasites (toxoplasmosis)



## Limited or small impact of antibody detection

- Infections with intracellular bacterias (Mycobacterium tuberculosis)
- Local infections (uncomplicated salmonelosis, tonsilitis, urinary tract infections)
- Reactivation of persistent infections (herpes)

# Classical methods of antibody detection

- Complement fixation
  - Good specificity, reasonable sensitivity, cheap.
  - Application: especially for respiratory tract infection
- Haemagglutinin inhibition
  - specific, reasonable sensitivity, cheap.
- Agglutination and precipitation
  - specific, less sensitive, cheap.

**Major disadvantage is necessity of pair serum testing.**



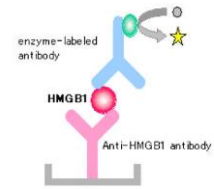
# Immunochemical methods

EIA (enzyme immunoanalysis),

IF (immunofluorescence),

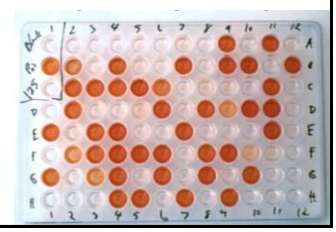
RIA (radio-immunoanalysis),

ELISA (enzyme-linked immunosorbent assay)




Advantage: very good reproducibility of resul, discrimination of immunoglobulin classes, high sensitivity

Disadvantage: more expensive, sometime unspecific results



## Why can antibody detection fail?

- Significant part of infection is destroyed by unspecific immunity
  (no activation of specific immunity)
- Detection has limited sensitivity, method is inappropriate or blood sample has been drawn too early
- Infection was caused by another than tested pathogen.

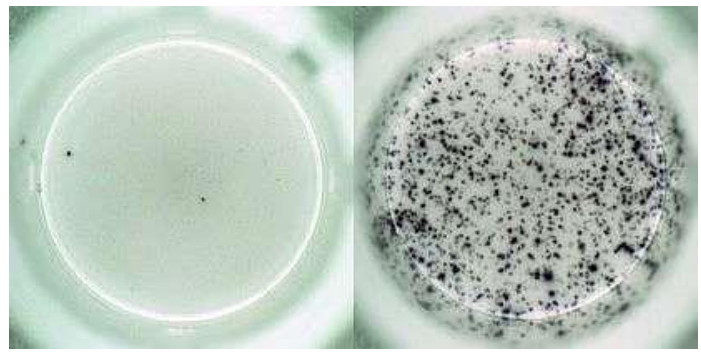


## Is antigen detection really easy?

- Technically is antigen detection easy
- Difficult is interpretation of result
  - There is no „normal range“
  - Sensitivity of methods depend on type of used antigen – no standardisation
  - Immune system of every person react in a unique way.

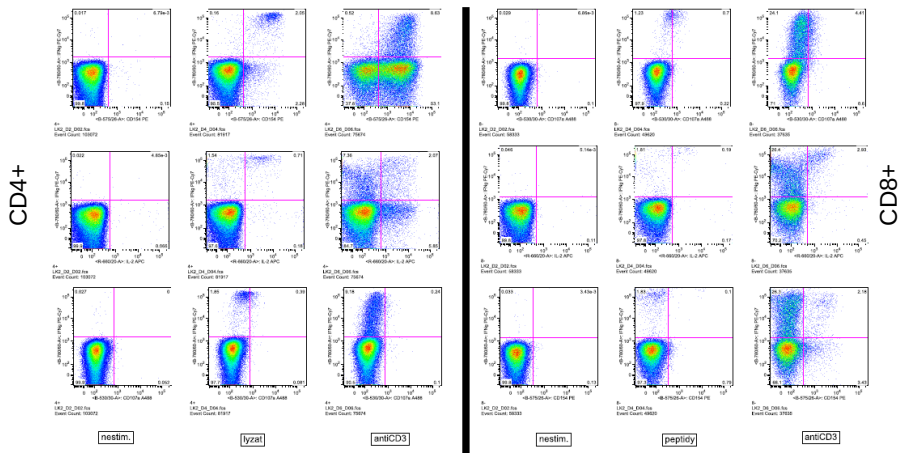
# Detection of specific lymphocytes

Further step in detection of viral infection consequence using molecular biology. Detection of lymphocytes producing IFN-γ after antigen stimulation – by ELISPOT assay or flow cytometry.





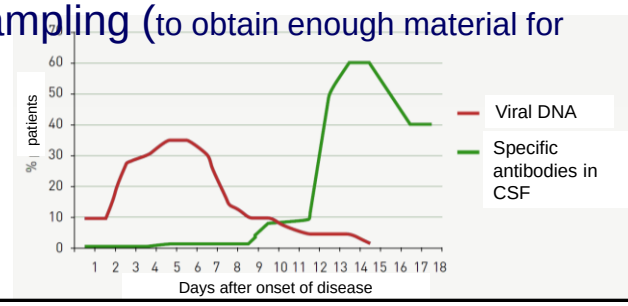
# Detection of specific lymphocytes



## Sampling of material for cultivation

- In an acute phase of infection
  - Smaller viability in latter phases
- Sampling from the place with highest pathogen concentration
  - Important is the knowlegde about pathogenesis of infection
- Vigorous sampling (to obtain enough material for testing)

PCR and antibody response against herpesvirus infection in CSF




## Sampling for direct detection

- Antigen detection (and Nucleic Acid) without amplification)
  - Very important is sampling of vigorous volume of material in acute phase
- Nucleic acid detection with amplification
  - Can be used also in situation when number and viability of pathogens decrease (but there are also some limitations!)
  - There is necessary to use special clean sampling and transport sets (due to risk of DNA and especially RNA destruction)

## Transport

- Cultivation
  - transport medium according to the suggestion of the lab
  - Maintaining and transport at fridge temperature
  - Important is lenght of transport (up to 24 hours)
- Antigen detection
  - It is important to avoid sample destruction
- Nucleic acid detection
  - It is important to avoid sample destruction (destruction of NA or adding of inhibitors into the samples)

## What is important for communication with the lab

- To know what I need

  (Ask a proper question – differential diagnostics)
- To know what and how quickly can be tested
- To know pathogenesis of infection and test the samples according to the phase of infection
- To be able to communicate with people from lab

## What should good lab do

- Standart result in a reasonable time
- Express testing in severe clinical situations
- Consultation of diagnostical possibilities
- Interpretation of the result and advise with further steps in diagnostics and therapy
- Inform clinical staff about diagnostical impact of the result (sensitivity, specificity, negative and positive predictive value)

Before sampling a sample, you have to know what will be impact of the result for management in case of
positivity or negativity
If there will be no difference in both cases – test is senseless.

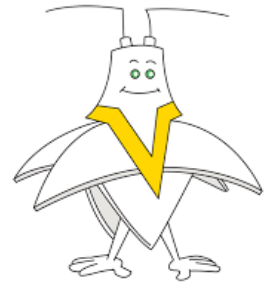
**2. Are the signs of cervical carcinoma from the diagnostical point of view more diagnostics**

**Direct**

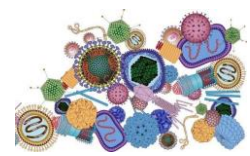
**Indirect**

# How long does it take to produce detectable specific antibodies?

A) 0.5 day
B) 3 days
C) approx. a week
D) approx. 3 weeks
E) approx. 6 weeks



# 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


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**

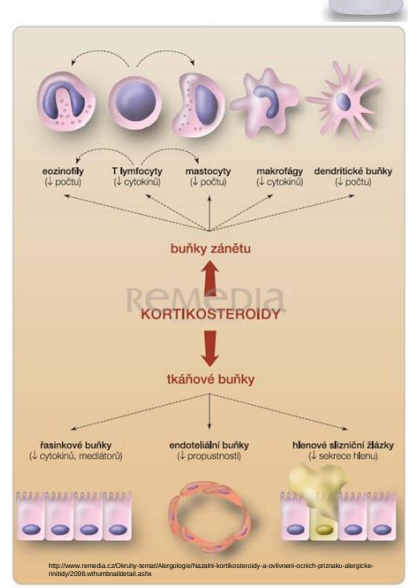
**„BIOLOGIC TREATMENT "**

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

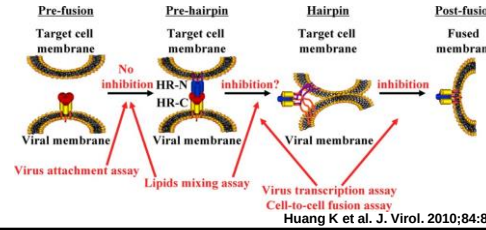
**Neutralising antibodies**

**Profylactic prevention**

motavizumab

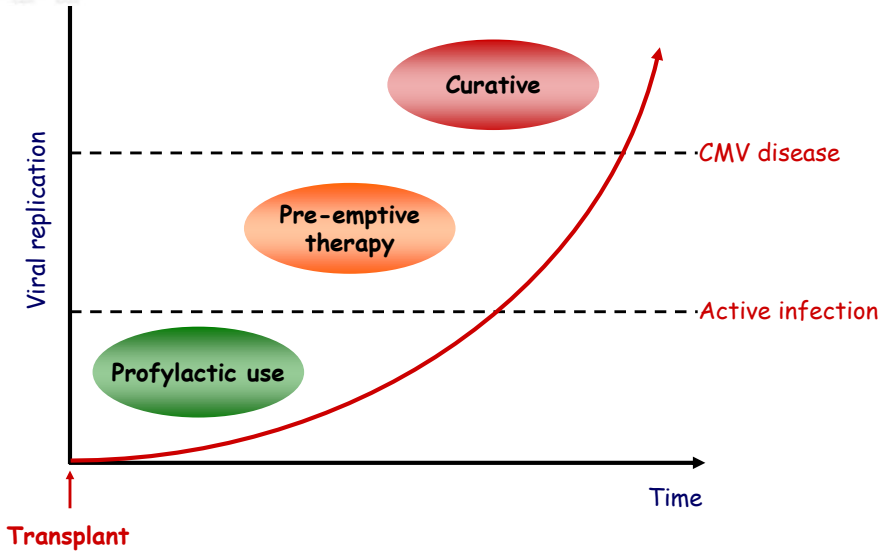
palivizumab (Synagis)

Humanisided neutralizating antibody against F- protein of RSV




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

# 3. Decrease of viral proliferation

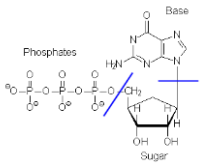




In HSCT recipient: pre-emptive and curative therapy

# Virostatic drugs impact

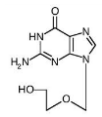
## Virostatics

**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..)**

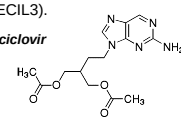



*Guanidine trifosfát (GTP)*

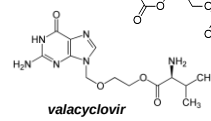
Nejčastěji používaná virostatika používaná při léčbě α-herpesvirových infekcí (podle ECIL3).



*acyclovir*

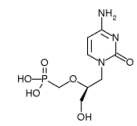


*famciclovir*

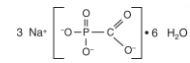


*valacyclovir*

*Léky první volby.*



*cidofovir*



*foscarnet*

*Léky používané při HSV rezistentní k léčbě.*

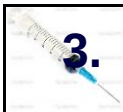
## Antibodies with virostatic effect

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

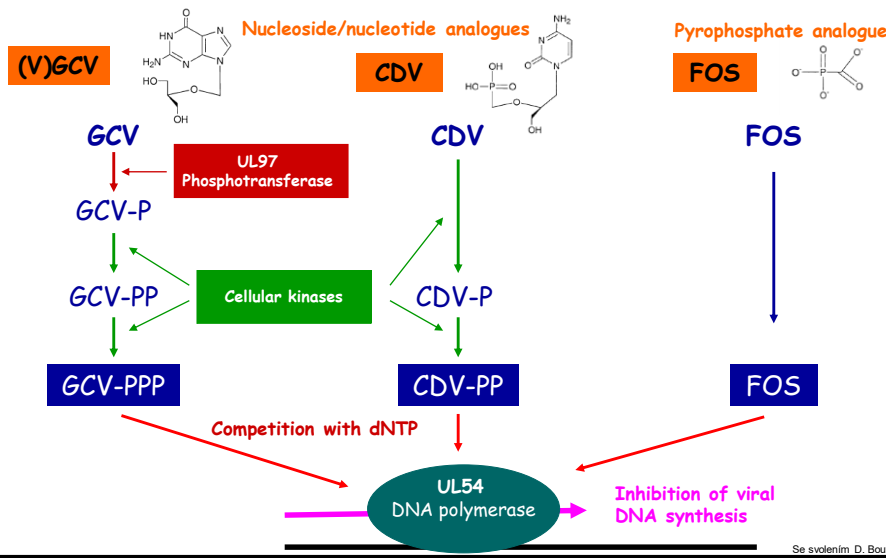
**Anti CD-20**

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

# 3. Decrease of viral proliferation

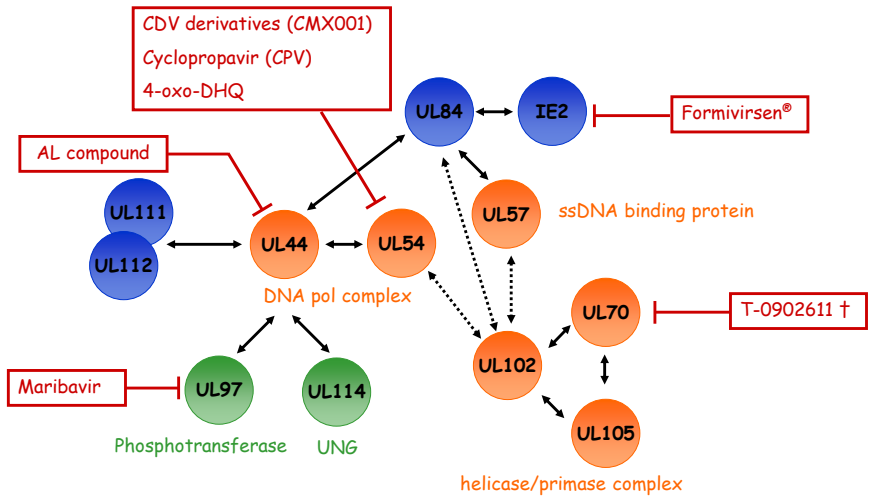



➔ Inhibitors of CMV DNA polymerase UL54




Se svolením D. Boutolleau

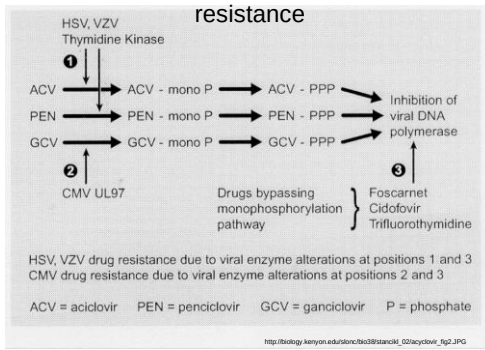
## CMV replication complex

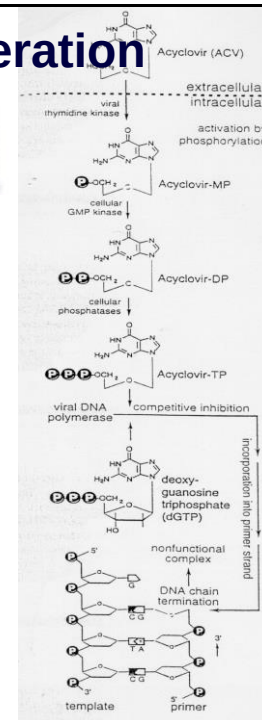



Se svolením D. Boutolleau

## 3. Decrease of viral proliferation


Inhibition of herpesviruses and the possible resistance



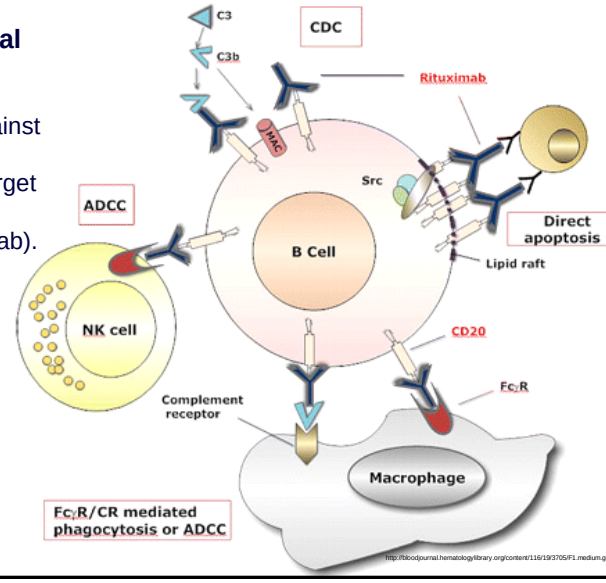
# 3. Decrease of viral proliferation

## 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.

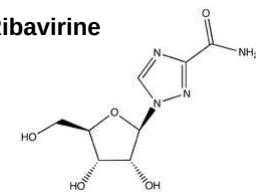
# Adverse effects of the virostatic drugs

- **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

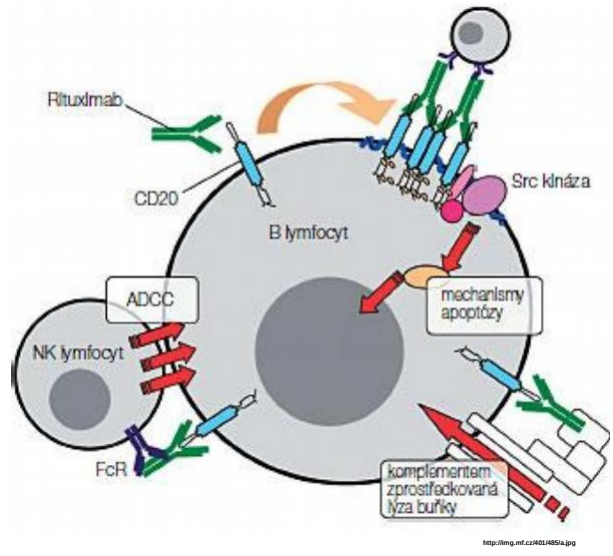
## Antibodies




**ADCC**

**– antibody dependent cellular cytotoxicity**

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




# Therapeutical possibilities of virostatics and specific antibodies

More or less specific for certain viral groups:

**ds DNA**

- ***Herpesviridae*** → α **HSV, VZV** — ACV, VACV, FCS…
- ***Herpesviridae*** → β **CMV, HHV-6 a 7** — GCV, VGCV, FCS, CDV, MBV, AIC246..
- ***Herpesviridae*** → γ **EBV,** — Anti CD-20
- ***Adenoviridae*** → **Adenoviruses (group A-F)**
- ***Polyomaviridae*** → **BKV, JCV, WUV, KIV, SV40...** — CDV, MBV...

**ss RNA**

- ***Ortomyxoviridae*** → **Influenza A**, **Influenza B** — Oseltamivir, zanamivir, (rimantadine amantadine)….
- ***Paramyxoviridae*** → **Paramyxovirus** → **PIV** — Ribavirine
- ***Paramyxoviridae*** → **Morbillivirus**
- ***Paramyxoviridae*** → **Pneumovirus** → **RSV**, **hMPV** — Palivizumab, motavizumab, ribavirine
- ***Coronaviridae*** → HCV — Ribavirine, interferon, boceprevir,…
- ***Picornaviridae*** → **Enteroviruses**
- ***Picornaviridae*** → **Rhinovirus** → **HRV**
- ***Caliciviridae*** → Human caliciviruses
- ***Astroviridae*** → Norovirus
- ***Rhadboviridae*** → ~~Lyssa virus~~

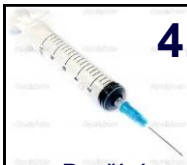
**p.o. ribavirine 10-30 mg/kg/D ve 3 dávkách**

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

**ds RNA**

- ***Reoviridae*** → **Rotavirus**
- ***Reoviridae*** → ~~Orbivirus~~

Oral ribavirin for treatment of respiratory syncitial virus and parainfluenza 3 virus infections post allogeneic haematopoietic stem cell transplantation
BMT 2011



# 4. Zlepšení funkce lymfocytů

## Interferon α


Používá se zejména při léčbě hepatitidy B a C.

Na trhu několik přípravků lišících se typem interferonu I – α2a

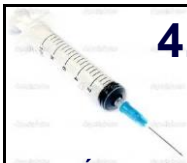
Např. (rekombinantní Roferon A), α2b (rekombinantní - INTRON A), případně pegylované interferony tj. s polyetylenglykolem (Pegasys™, PEG-INTRON)

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.

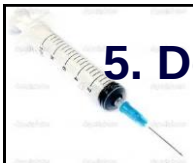


# 4. Zlepšení funkce lymfocytů

## Interferon α

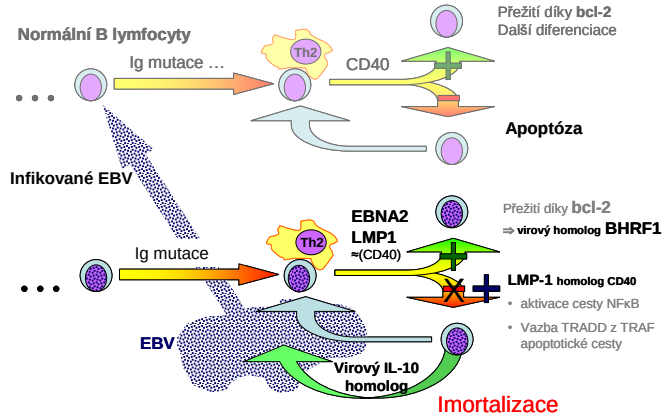

**NÚ:**
„flu-like“: únava, zimnice, bolest svalů nebo kloubů, bolest hlavy, pocení nebo horečka.
Vzácněji pneumonie a herpes, anémie, trombocytopenie, Leukopenie, autoimunitní stavy, sarkoidóza, poruchy štítné žlázy, zažívání, hypo- a hypertenze, proteinurie a poruchy renálních funkcí, glykémie a homeostázy.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

# 5. Destruction of permissive cells

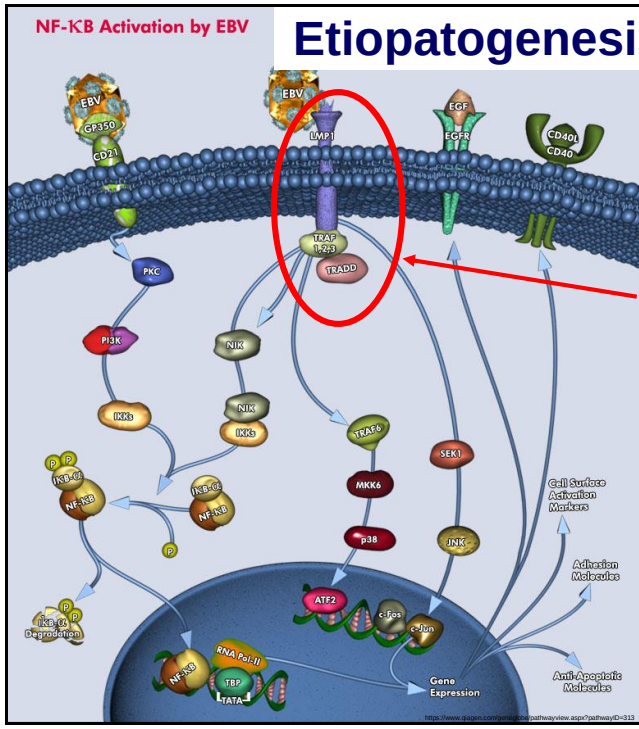



Used in EBV associated malignant disease (HL, NHL), or post-transplant EBV-LPD.

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**

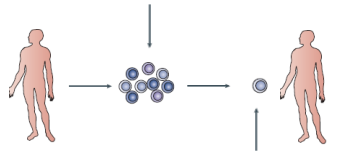
**BHRF1**

– viral homologue bcl-2

Acyclovir

# 6. Present possibilities of specific cell production and its limitations

Viral antigen stimulation



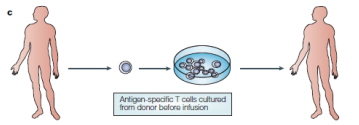
Specific IFNg producing T lymfocyte selection

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

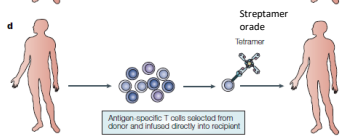
Literatura:




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

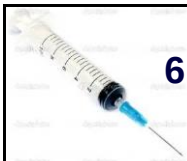
Unrelated donors: preparation about 8-9 weeks from first demand to product

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

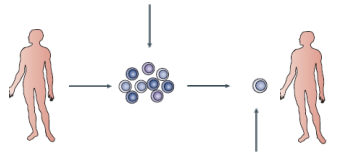
Moss P and Rickinson A Nature Reviews 2005 (5)

Acyclovir

# 6. Present possibilities of specific cell production and its limitations



Viral antigen stimulation



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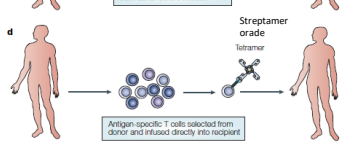
Unrelated donors: preparation about 8-9 weeks from first demand to product

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


So far - for prophylactic use only!

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Thank you for you attention
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