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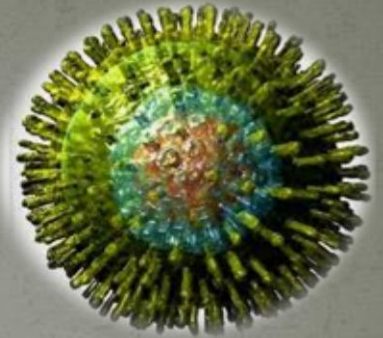
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Herpesviruses Infection and Diseases of Nervous System

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Content of presentation



1. Overview on the influence of human herpes viruses on nervous system



2. Report of the results from our studies on association of HHV-6,7 and demyelinating diseases of CNS and PNS

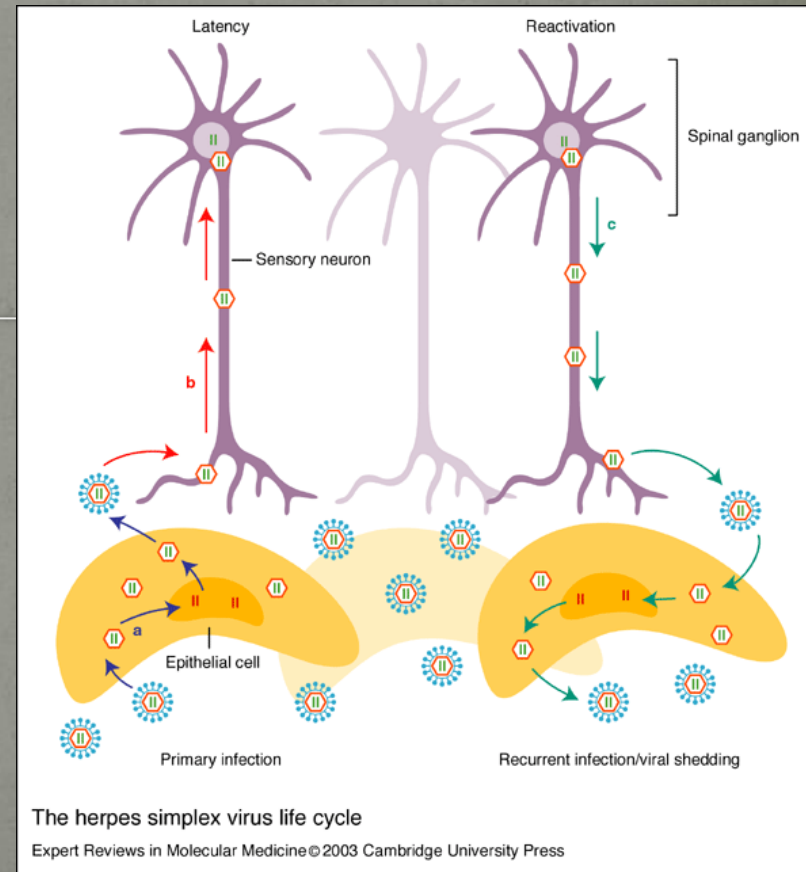
The first 3 types or *Alphaherpesvirinae* subfamily are acknowledged as typical **neurotropic and neuroinvasive** viruses

Name	Sub-family	Target cell type	Latency	Transmission
Herpes simplex -1 (HSV-1)	Alphaherpesvirinae	Mucoepithelia	Neuron	Close contact
Herpes simplex -2 (HSV-2)	Alphaherpesvirinae	Mucoepithelia	Neuron	Close contact, usually sexual
Varicella Zoster virus (VZV)	Alphaherpesvirinae	Mucoepithelia	Neuron	Contact or respiratory route

HSV-1, HSV-2, VZV persist latent in the **cell bodies of sensory nerves**

(Ryan, Ray, 2004)

- HSV-1 – typically tends to reside in the **trigeminal nerve ganglia**
- HSV-2 - in the **sacral ganglia**
- VZV - in the **trigeminal ganglia** and higher **spinal ganglia (cervical, thoracic)**
- Activation of the virus in a nerve cell →
 1. virus is transported via the nerve' **axon to the skin** - replication occur → **cold sores or herpes zoster**
 2. **dissemination to the central nervous system** – to brain or spinal cord → **encephalitis, meningitis**



The cell tropism of *Betaherpesvirinae* subfamily is linked and proven in the peripheral blood mononuclear cells (PBMC)

Name	Sub-family	Cell tropism	Transmission
Cytomegalovirus (CMV)	Betaherpesvirinae	Epithelia, PBMC	Contact
Human Herpes virus-6 (HHV-6)	Betaherpesvirinae	PBMC	Contact, transmission
Human Herpes virus-7 (HHV-7)	Betaherpesvirinae	PBMC	Contact

Data on **neurotropism** of these viruses :

detected in **cerebrospinal fluid, normal and encephalitic brain**

(Luppi et al., 1994; Chan et al, 1999; 2001; Cuomo et al, 2001)

Different neurotropism of HHV-6A, HHV-6B (Hall et al, 1998)

HHV-7 can be found in brain tissue at lower frequency than HHV-6

(Chan et al, 1999).

Cell tropism of Epstein-Barr Virus (EBV) from *Gammaherpesvirinae* subfamily is related to B lymphocytes

Name	Sub-family	Cell tropism	Transmission
Epstein – Barr virus (EBV)	Gamma-herpesvirinae	B lymphocytes epithelia	Body fluids

Several different neurological manifestations initiated by HHV

- Shingles or *Herpes zoster*
- Postherpetic neuralgia and other peripheral nerve damage
- Acute neuroinfections
- Immune-mediated demyelinating inflammatory diseases of the CNS and PNS
- Neurodegenerative diseases and paroxysmal states

Skin lesions caused by herpes viruses → typical initial manifestations or latent virus reactivation

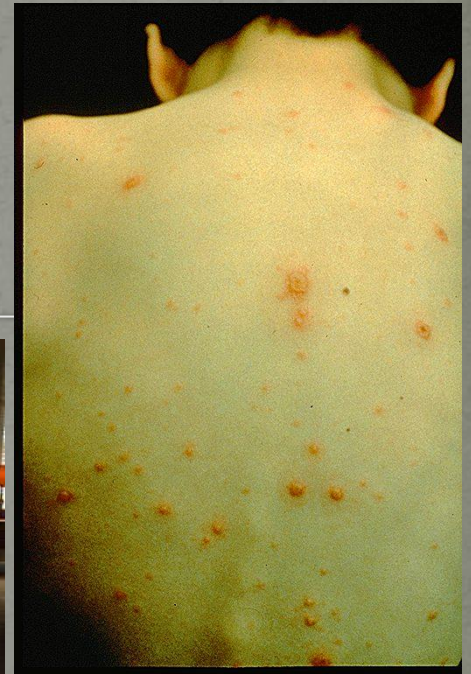
- Cold sores and other herpetic vesicles →

HSV-1, HSV-2



- Chicken pox and Herpes zoster →

VZV



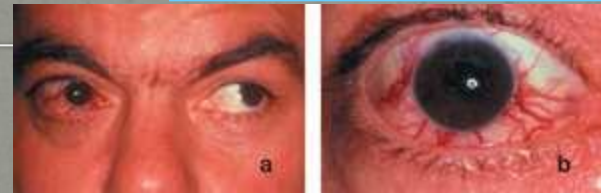
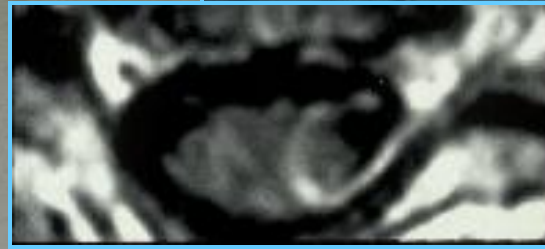
- *Exanthema subitum* →

HHV-6, HHV-7



Herpes zoster

- Reactivation of VZV replication in sensor neural ganglia
 - also HSV-1,2 - sometimes
- Symptoms and signs :
 - Severe **radicular pain** – in area corresponding involved nerve dermatome
 - A few days later **herpetic vesicles** occur
- Reactivation can affect :
 - **Eye** – uveitis, conjunctivitis, ophthalmoplegia or **Tolosa-Hunt syndrome** → via trigeminal nerve
 - **Bell's or facial palsy** and **Ramsey-Hunt syndrome** → via VII cranial nerve and *ganglion geniculi*
- **Postherpetic neuralgia**
 - Chronic burning or itching pain in affected area
 - Increased sensitivity to touch and pain (allodynia, hyperesthesia).
 - The pain may last well after the rash has healed (even months or years)



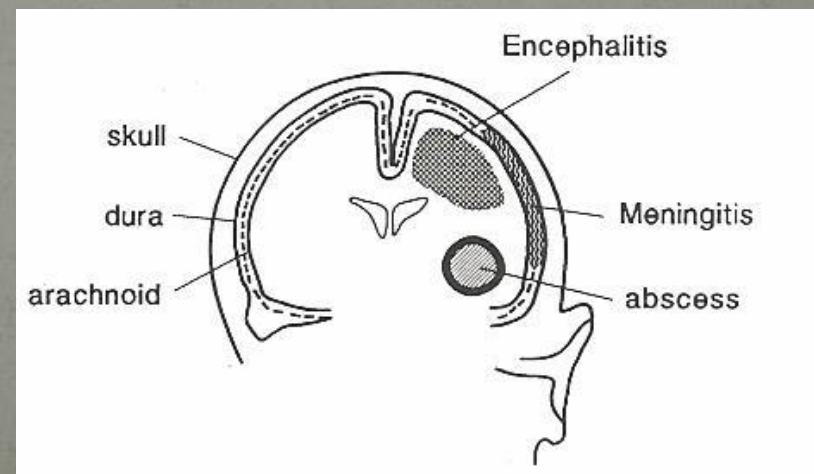
Neuroinfections and herpes viruses

- Neuroinfections – group of the devastating infectious diseases affecting the nervous system in children and adults worldwide

(Steiner et al, 2010)
- The burden of disease is high as survivors may have severe neurological sequelae

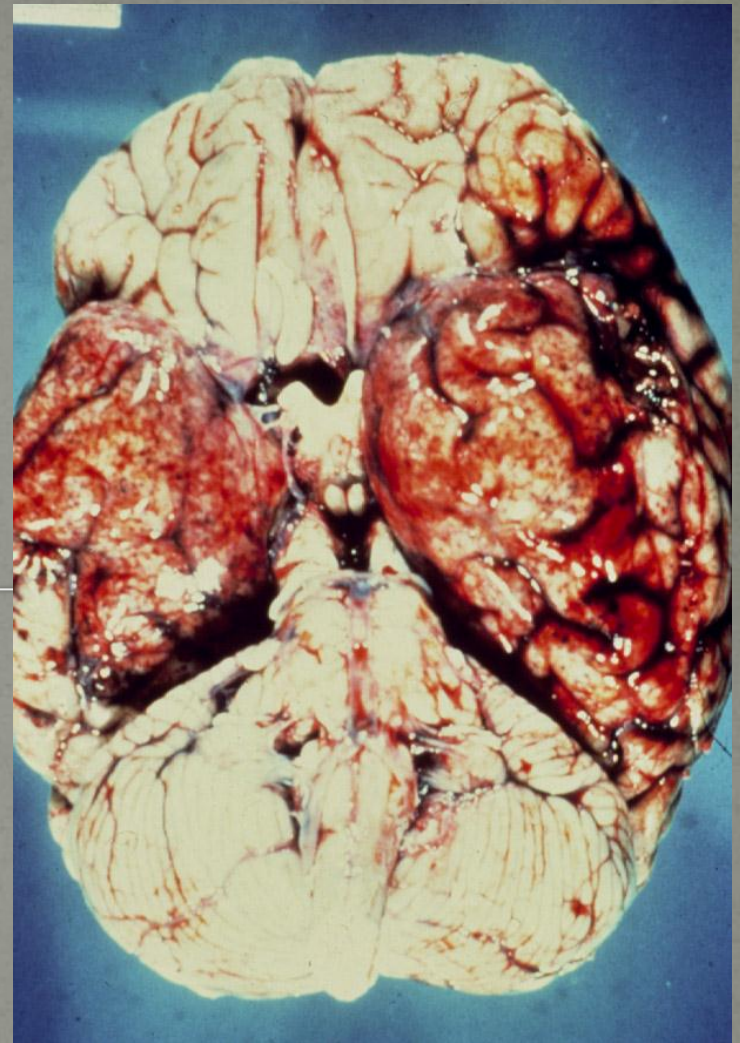
Management of neuroinfections depend on categorisation into :

- The causative infectious agent
 - **Viral**
 - Bacterial
 - Protozoal, fungal parasital
- The mode of pathogenesis or route of penetration
 - **Haematogenous spread**
 - Direct implantation
 - Secondary extension from local purulent foci
 - **from PNS – viruses from peripheral neural ganglia**
- Geographical distribution of infectious vectors
 - (eg TBE, tbc, malaria etc)
- The localisation of inflammation :
 - **Meningitis** - primarily involving of meninges ,
 - **Encephalitis** – primarily confined to the parenchyma
 - **Myelitis** – inflammation in the spinal cord



Encephalitis

- is defined by the **presence of an inflammatory process of the brain parenchyma**
- in association with **clinical evidence** of brain pathology :
 - cognitive dysfunction,
 - behavioural changes,
 - focal neurological abnormalities
 - seizures
- accompanied by
 - a febrile disease,
 - headache
 - and altered level of consciousness.

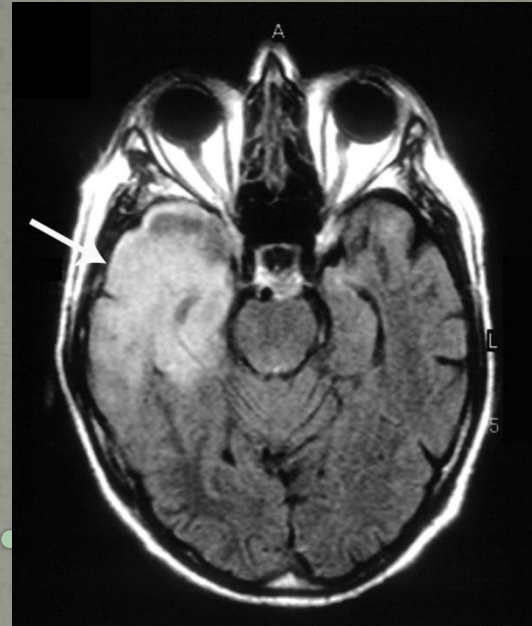


Herpes simplex encephalitis

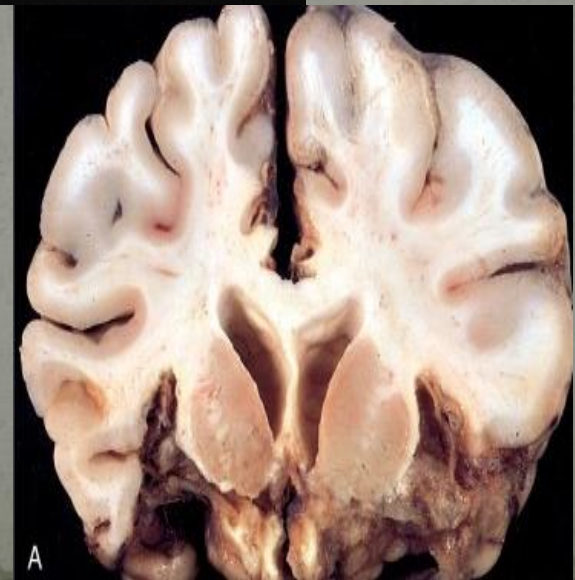
- Herpes simplex encephalitis is the most common cause of sporadic and lethal encephalitis
 - occurring in about 1 person per 250,000-500,000 population per year
 - Before the availability of acyclovir the mortality rate of herpes simplex encephalitis in untreated patients was ~70%.

Herpes simplex encephalitis

- The HSV preferentially involves the temporal lobe and orbital surfaces of the frontal lobes

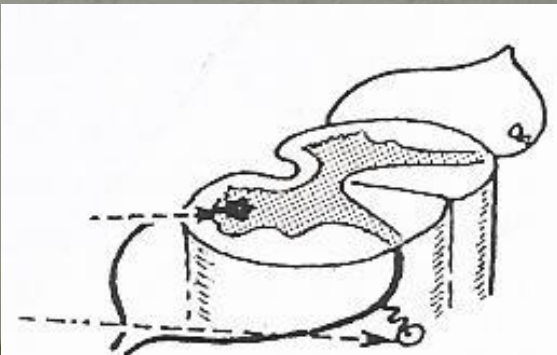
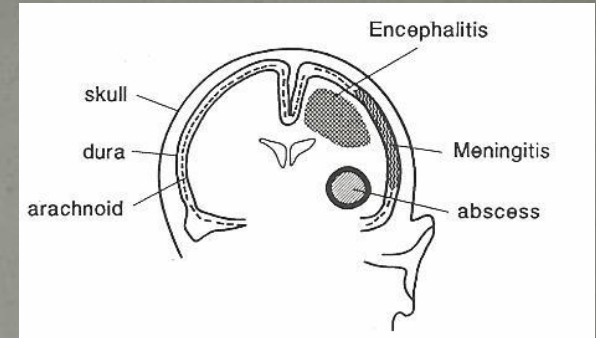


- Herpes viruses cause a hemorrhagic necrosis and inflammatory infiltrates



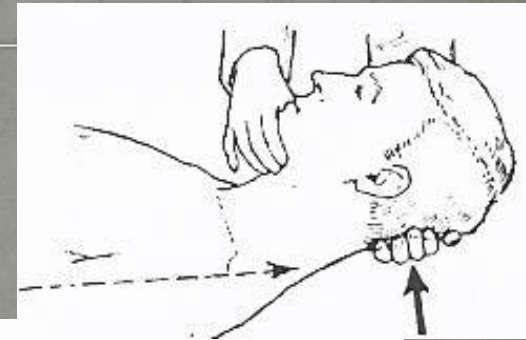
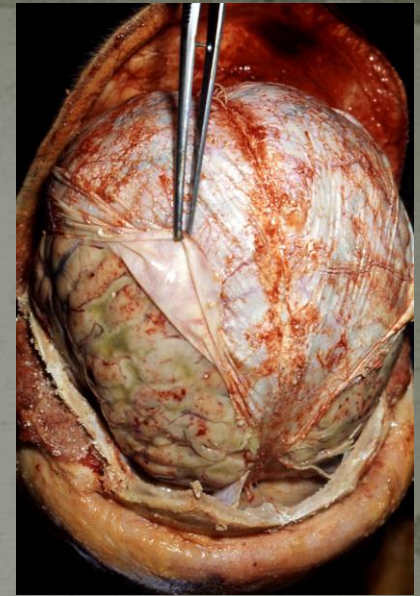
Herpes viral neuroinfections

- Commonly approved causes of infectious viral encephalitis are :
 - HSV-1 HSV-2 (rarer), VZV, EBV, CMV**
- Also **HHV-6** can be responsible for :
 - Encephalitis
(Steiner et al, 2010; Gilden et al., 2000)
 - Febrile convulsions in complicated **exantema subitum** and encephalitis in infants and immunocompromised
(Hall et al., 1994; Asano et al., 1992; Dewhurst S, 2004, Mori et al., 2010).
- HHV-7** has not been shown to cause a specific disease but is associated with febrile convulsions and encephalitis
(Chan et al, 2002)
- CNS complications of **VZV, CMV** and **HSV** include other localizations as well :
myelitis/myeloradiculitis, arteriitis, ventriculitis, meningitis
(Steiner et al, 2010; Gilden, et al., 2000)



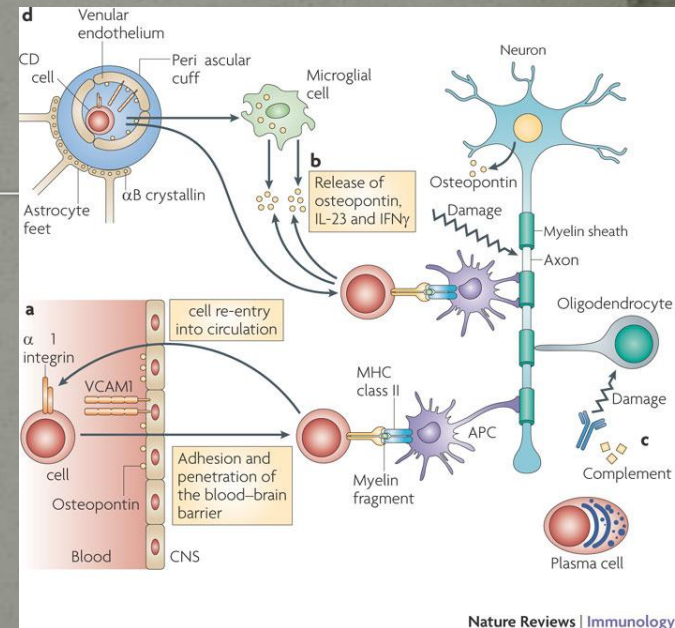
Herpes viral meningitis

- Primary encephalitic processes by HHV mainly **secondarily** involve the meninges
- Viral **meningoencephalitis** has denoted viral infection process of both the brain/spinal cord and the meninges.
- **Isolated aseptic meningitis** is uncommon manifestation of human herpes viral infection
 - however most commonly because of **HSV-2**
- Inflammation of meninges results in symptoms of meningeal irritation :
 - headache,
 - photophobia,
 - nuchal rigidity,
 - nausea
 - usually mild CSF pleocytosis.



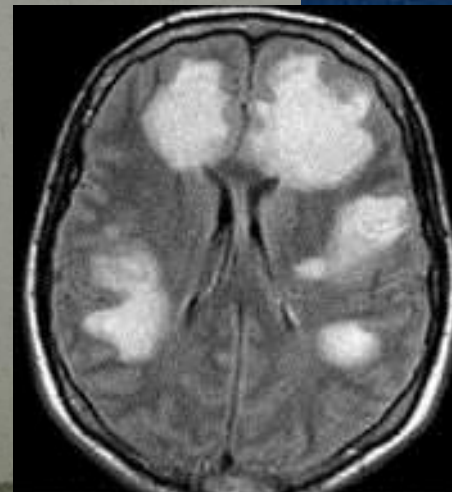
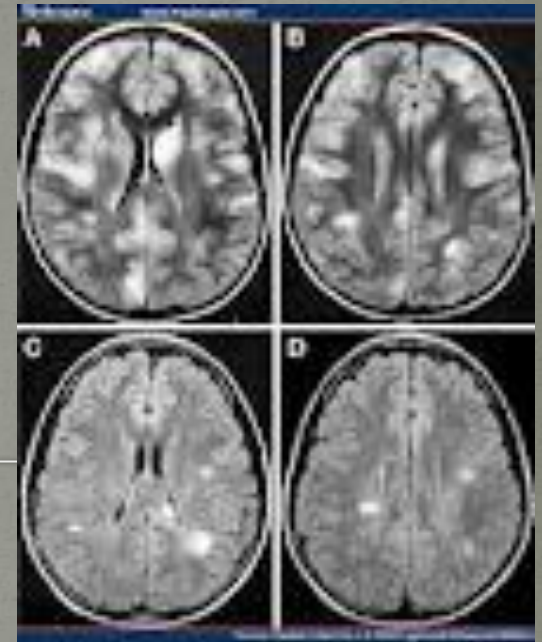
HHV and immune-mediated inflammatory diseases of the nervous system

- Lot of data have been accumulated on
 - the immunomodulating properties of herpes viruses
- The most studied immune-mediated diseases of the nervous system are :
 - multiple sclerosis (MS)
 - acute disseminated encephalomyelitis (ADEM)
 - postinfectious polyradiculoneuritis or Guillain-Barre syndrome (GBS)



Acute disseminated encephalomyelitis (ADEM)

- ADEM is the immune-mediated condition with multiple small demyelinated inflammatory foci around small veins of the white matter
(Budka, 1997)
- ADEM is an autoimmune disease with evidence of cell-mediated immunity to the myelin basic protein.
- Unlike multiple sclerosis ADEM is monophasic disorder with rapidly progressive course, on the other hand sometimes it is hard to distinguish it from acute viral encephalitis.



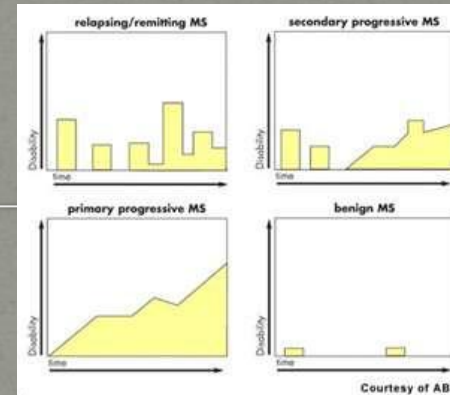
Multiple sclerosis (MS)

- CNS disorder marked by decreased nerve function due to inflammation of the protective myelin and manifests with
 - motor weakness – pareses,
 - vertigo and disbalance,
 - optic and oculomotor symptoms,
 - sensory disturbances,
 - behavioural and cognitive signs etc



- Symptoms and signs progress and course of MS may be :

- RR : relapsing – remitting
- SP : secondary progressive
- PP : primary progressive



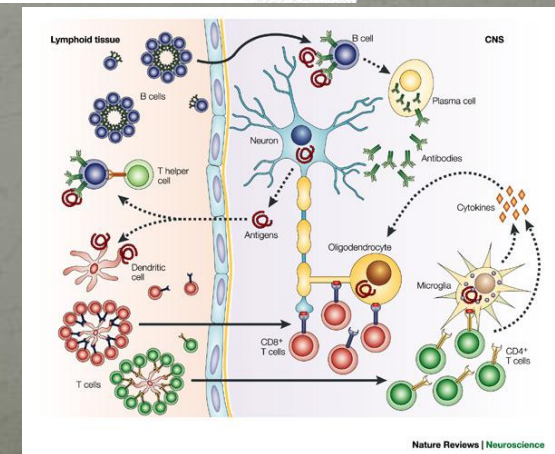
- It is generally accepted that MS is a systemic T-cell-mediated autoimmune disease

(Martino,Hartung, 1999)

- with a T-helper type 1 (Th1) profile of cytokine production

(Carrieri et al., 1992);

- However, the precise etiology of MS remains unknown.



Multiple sclerosis (MS)

- **Viruses** have long been proposed to be either initiating factors for MS or directly pathogenic in the development of MS

(Cermelli, Jacobson, 2000).

- Recently much attention has been paid to the relationship between **HHV-6** and MS:

- HHV-6 antigens are expressed in the nuclei of oligodendrocytes in inflammatory lesions of brain tissue

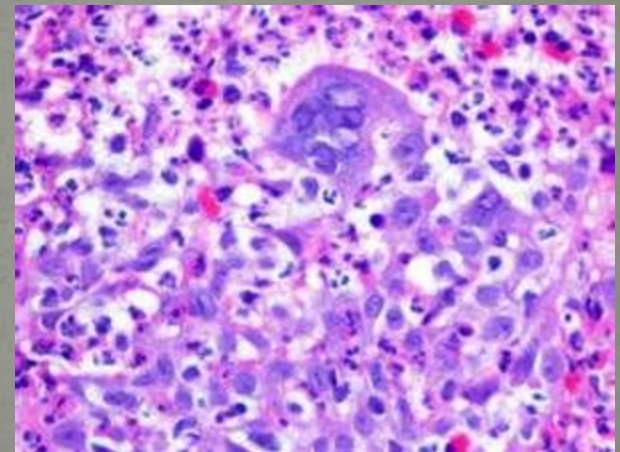
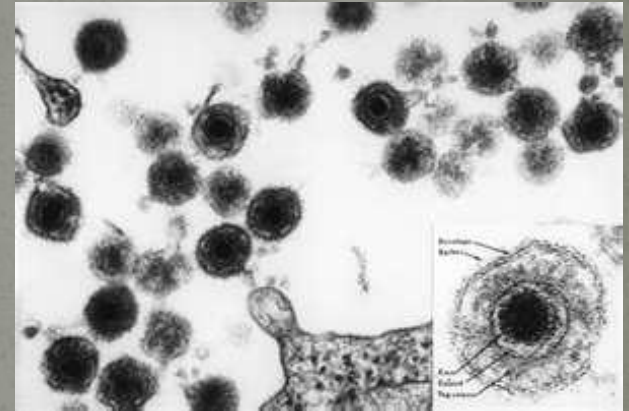
(Challoner PB, Smith KT, Parker JD, et al., 1995)

- IgM and increased IgG specific anti-HHV-6 antibody titres are detected in plasma/serum

(Soldan SS, et al., 1997; Ablashi DV, et al., 1998; Ablashi DV, et al., 2000) and cerebrospinal fluid (Ablashi DV, et al., 1998)

- **HHV-7** is similar to HHV-6 in its genetic content, which includes the possible association with MS.

(Nicoletti, 1996; Martino, Hartung, 1999)



Results of our study : Association of Herpesviruses with multiple sclerosis

(Nora Z., PhD, 2008)

- We studied 67 (48 females and 19 males) patients with MS diagnosis confirmed
 - clinically according to the criteria of McDonald (2001)
 - by MRI and laboratory tests
- The mean duration of disease was 6 years
- MS forms :
 - 36 patients had remitting/relapsing (RR) MS type,
 - 26 pts – secondary progressive (SP) MS type
 - 5 pts – primary progressive (PP) MS type
- Activation of HHV-6 and HHV-7 infection was detected in correlation with exacerbation of MS that was approved by acute lesions detected in patients by MRI
 - RR/MS patients were examined: 27 - during exacerbation, 3 - during exacerbation and remission and 6 – in remission only.
 - 19 from 26 SP/MS patients : 19 during exacerbation, 3 - during exacerbation and remission and 4 – in remission only

The prevalence of latent/persistent **HHV-6 and HHV-7** infection and frequency of active infection in studied MS patients

- **Active infection** (DNA in blood plasma) was detected :
 - **HHV-6** : in 26,7% MS patients (12/45)
 - not in any from control group patients with other neurological diseases (OND) and blood donors
 - **HHV-7** : in 29,4% MS patients (15/51)
 - statistically significant difference between patients with OND ($p=0.00036$) and blood donors ($p=0.00049$)
- **Latent infection** (DNA in peripheral blood leucocytes - PBL) was present in :
 - **HHV-6** : in 66.2% (45/67)
 - **HHV-7** : in 75% (51/67)
- In order to confirm PCR results, patients' blood and plasma was checked for **HHV-6 and HHV-7 specific IgG and IgM antibody** presence
 - Patients with SP/MS and PP/MS had similar results.
 - Totally in 26.7% MS patients **HHV-6** specific and
 - in 29.4% - **HHV-7** specific IgM antibodies were present.

Part of MS patients were examined during periods of **exacerbation and remission.**

- The **viremia and virus specific IgM antibodies** were found only during disease exacerbation confirmed by the presence of Gd-enhancing lesions on MRI
- Overall we found **active HHV-6** infection
 - during exacerbation of RR/MS in 23.1% of all cases,
 - during exacerbation of SP/MS – in 31.3% of patients
- **Active HHV-7 infection**
 - during exacerbation of RR/MS – in 28.0%,
 - during exacerbation of SP/MS – in 28.6%
- Thus we conclude that **HHV-6 and HHV-7 activation** does not depend on MS course type.



Increased cytokine level expression were found in MS patients (not in OND control group patients) :

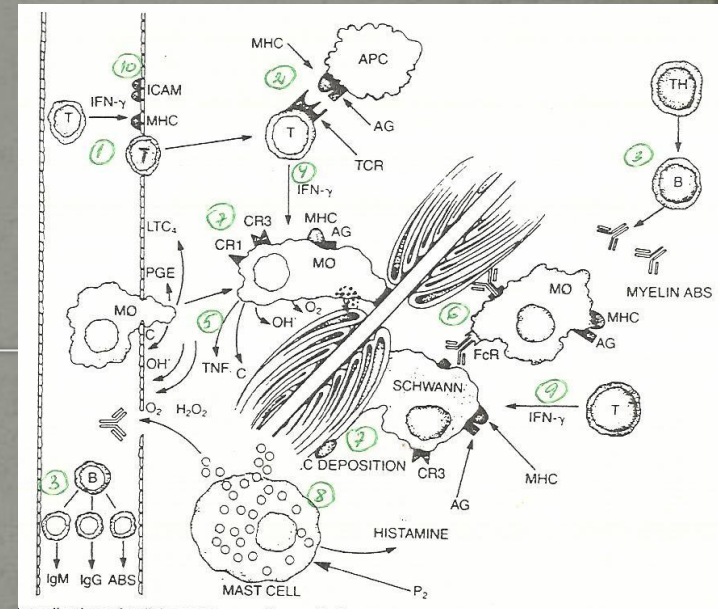
- **RR/MS patients group** - a correlation between the concentration of IL-12 and TNF- α in plasma and disease exacerbation was shown
 - a higher IL-12 level in RR/MS patients is found only in disease exacerbation periods (22.8 ± 16.3 pg/ml)
 - also TNF- α concentration in the patients' plasma is considerable higher during flare-ups than during periods of remission ($p=0.001$).
- **Patients with SP/MS** show a higher plasma concentration of IL-12 and TNF- α in both relative remission and during flare-ups, however :
 - during exacerbation the IL-12 concentration is 1.8 times (17.7 ± 12.9 pg/ml and 9.7 ± 6.9 pg/ml) greater,
 - the TNF- α concentration - 3 times greater ($p=0.001$).
- **Increased proinflammatory cytokines IL-12 and TNF- α levels during exacerbation phase were detected in MS patients with active HHV-6 and/or HHV-7 infection.**
 - **Correlation between HHV-6 and HHV-7 reactivation, elevated concentrations of IL-12 and TNF- α in plasma and RR/SM and SP/MS exacerbation** indicates these viruses' involvement in disease pathogenesis – by possible changes in functioning of immune cells.

Autoimmune inflammatory demyelinating neuropathy - Guillain-Barre syndrome

- Pathology in GBS is attributed :
 - to sensitized T cells mediated autoimmune response directed to peripheral nerve sheaths and myelin involving macrophages, cytokines and autoantibodies produced by B lymphocytes.
 - often preceded by a bacterial or viral infection - *Campylobacter jejuni*, *Mycoplasma pneumonia* or **CMV, EBV**.
 - GBS association with **HHV-6** has been mentioned, too
 - **HHV-7** association with diseases is not reported widely.

(Merelli et al, 1992)

- Symptoms of this disorder include
 - varying degrees of weakness or tingling sensations in the legs, spreading to the arms and upper body.
 - In severe cases the patient is almost totally paralyzed and the disorder is life threatening – potentially interfering with breathing and autonomic regulation.

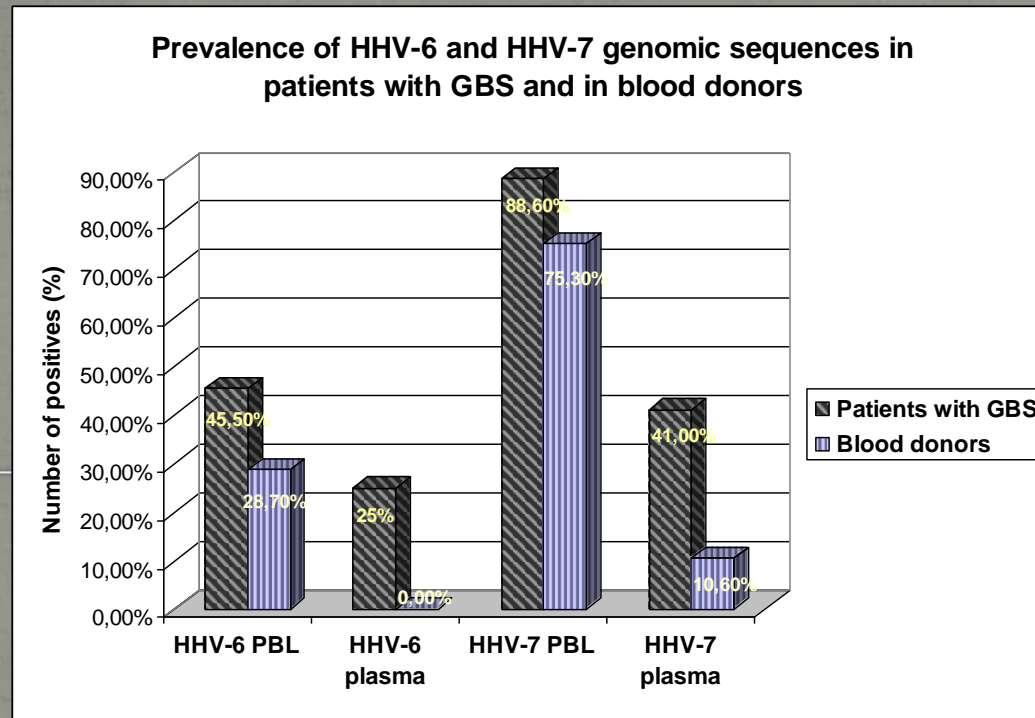


Study of **HHV-6 and HHV-7** association with acute demyelinating polyneuropathy

- We studied 44 patients (20 females, 24 males)
- A clinical diagnosis of GBS was confirmed according to the criteria of Asbury A.K. and Cornblath D.R. (1990.)
- Blood samples were collected during a period of 2 -8 weeks after appearance of symptoms - in acute period of disease.

HHV-6 active infection was detected in 25.0% pts ($p=0.04$).

HHV-7 active infection – in 41% pts with GBS ($p=0.000077$)



The results of our study show that HHV-6 and HHV-7 are related to GBS. Active HHV-7 infection is even more frequent than active HHV-6 infection. All cases approved by IgM specific viral antibodies and elevated IgG titer. The relationship of HHV-6 with GBS is discussed in some publications, but data are controversial. There was not known literature on the possible association of HHV-7 with GBS before.

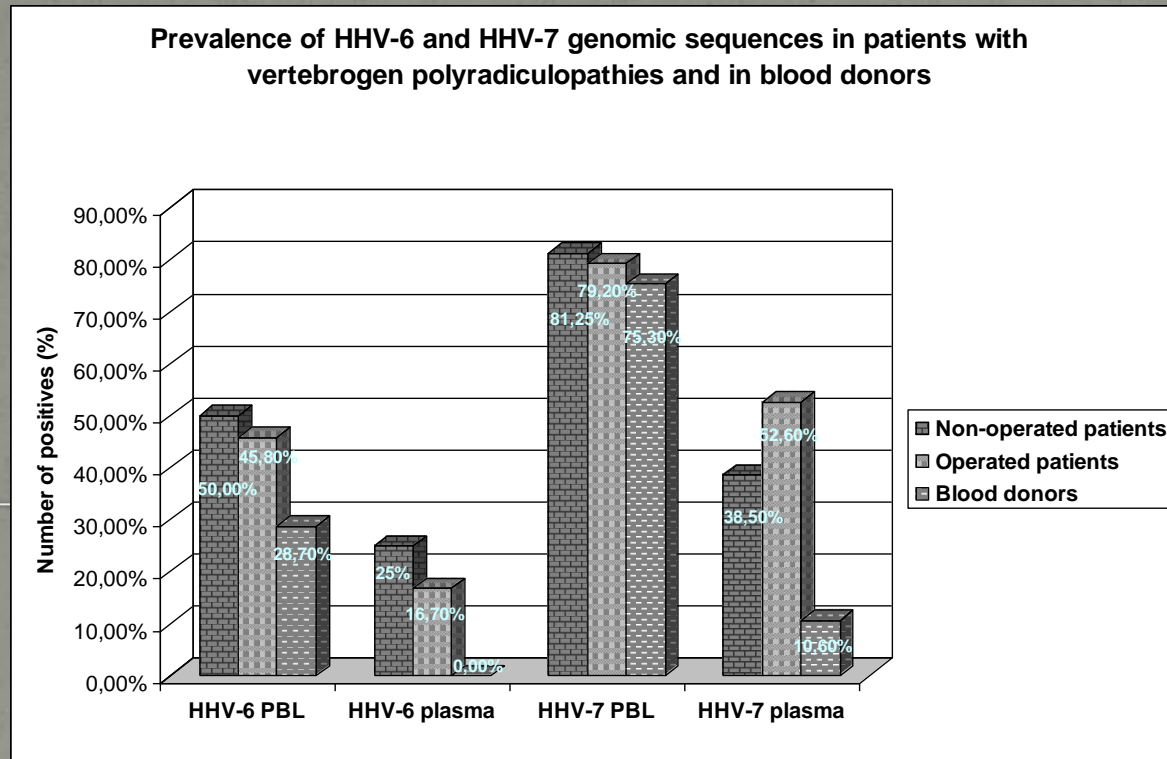
We did original study on the relationship between HHV-6, HHV-7 infection and cytokine expression in GBS

- GBS patients **without latent/persistent HHV-6 and/or HHV-7 infection** do not have a higher IL-12, TNF- α , IL-1 β and IL-6 concentration in the plasma
- In the case of **active HHV-6 infection**
 - IL-6 and TNF- α levels are slightly higher in the plasma
- In the case of **active HHV-7 infection**
 - IL-1 β and IL-6 levels are significantly higher in comparison with latent/persistent HHV-7 infection
($p=0.025$ and $p=0.0001$, respectively)
 - a higher TNF- α and IL-12 concentration was not statistically significant
- In the case of **simultaneous active infection with both HHV-6 and HHV-7 viruses**
 - the IL-12, IL-1 β and IL-6 levels are significantly higher,
($p=0,01$; $p=0.001$; $p=0.0001$)
 - no difference in the TNF- α level

Relationships between HHV-6 and HHV-7 and vertebrogen radiculopathies (VR)

- There were no published data before on relationships between HHV-6 and HHV-7 and mechanical damage of the nervous system with back/radicular pain
-
- This group is of great clinical interest due to the significantly large proportion of patients with back pains in general practice.
 - Frequent is also failed back surgery syndrome – recurrent pain after invasive treatment of pain.

Latent/persistent HHV-6 and HHV-7 was observed in all studied groups without significant difference



Active HHV-6 and HHV-7 infection were found significantly more frequent in patients with back problems : HHV-6 in 25.0% of non-operated patients; HHV-7 in 38.5% non-operated ($p=0.0161$) and in 52.6% operated patients with VR ($p=0.00008$).

All samples with active viral infection had both IgM specific viral antibodies and elevated IgG titer

- **Activation of HHV-6 and HHV-7**

correlated with clinical exacerbation of VR in both groups of patients (operated and non-operated) :

- Clinical escalation of pain
- Radiological signs of inflammation - Gadolinium-enhancement on MRI what means active local inflammation

(Nora, Logina, Murovska etc, 2006)



Conclusion from our study :

- Our study confirmed the possible relationship of HHV-6 and HHV-7 to autoimmune inflammatory demyelinating and non-demyelinating CNS and PNS diseases,
- as well as the relationship between virus activation and the clinical activity of the diseases.
- This is especially important because there have been no strong evidence on the role of HHV-7 in infectious demyelinating polyneuropathies and vertebrogen radiculopathies.
- Testing for the HHV-6 variants was performed on all patient groups studied. Patients with MS and patients with PNS diseases had HHV-6B variant in PBL.

More and more reports have been issued not only about the infectious and immunomodulating properties of herpes viruses but also about the regulating **influence on the host cell genome**

- During latent infection in the nerve cells, **HSVs** express Latency Associated Transcript (LAT) RNA

- LAT is known to regulate the host cell genome and interferes with natural cell death mechanisms

(Ryan, Ray, 2004).

- In the presence of a certain gene variation (APOE- ϵ 4), a possible link between **HSV-1 and Alzheimer disease** was reported

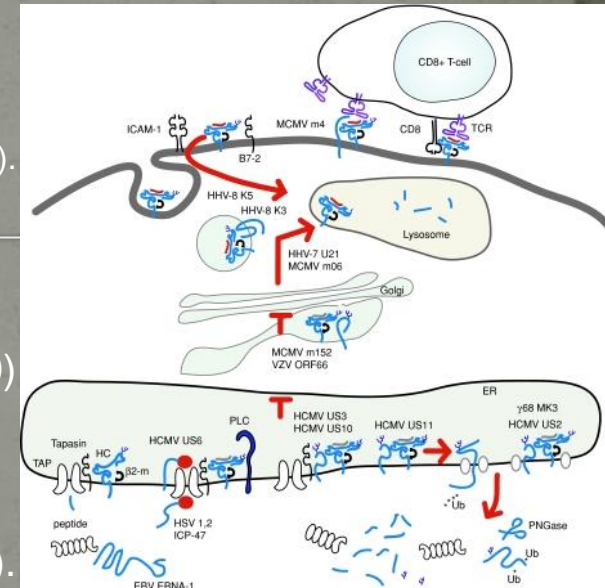
(Middleton et al., 1980)

- The virus interacts with the components and receptors of lipoproteins, which may lead to the development of Alzheimer's disease

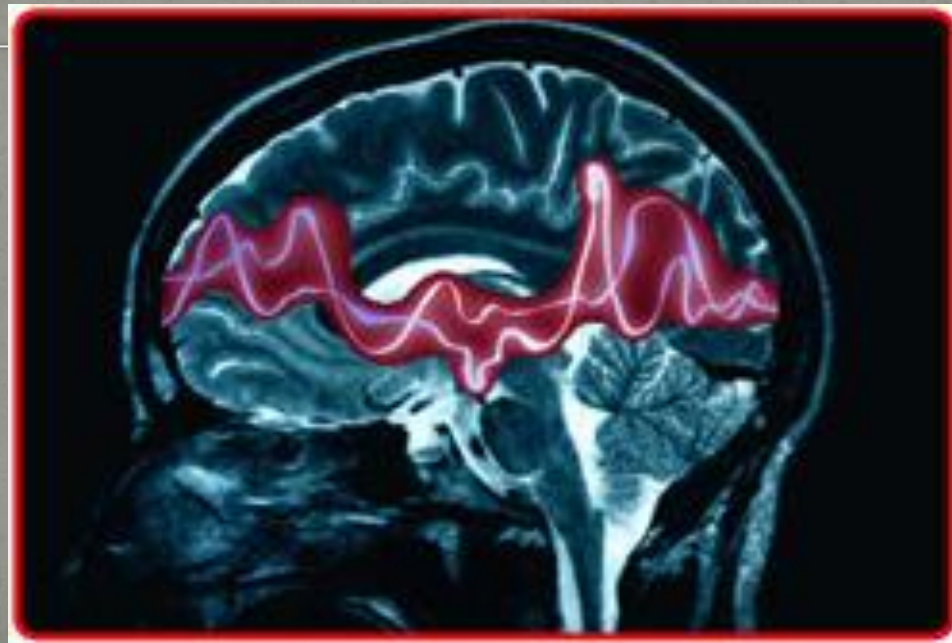
(Dobson, Itzhaki, 1999; Pyles RB, 2001).

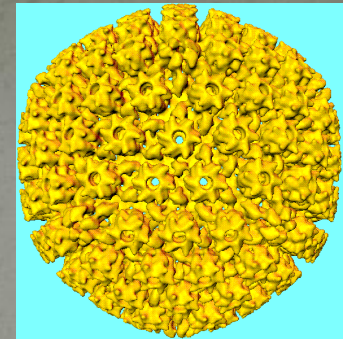
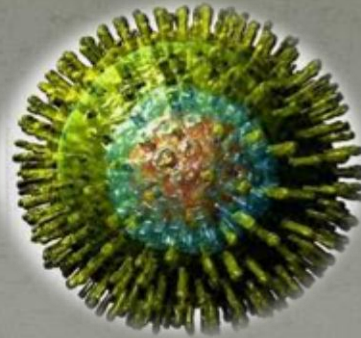
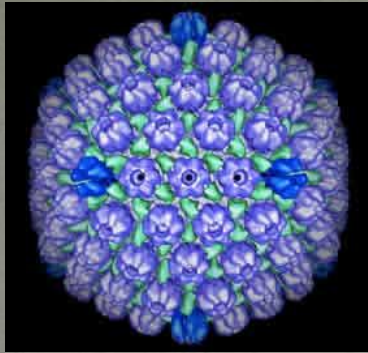
- Without the presence of the gene allele, HSV-1 does not appear to cause any neurological damage or increase the risk of Alzheimer's.

- The ideas exist and have been worked out that **HHV-7** also might be linked to the development of neurodegenerative diseases.



- Several studies stated association with **HHV-6** and potential pathogenetic mechanisms of mesial temporal lobe epilepsy
(*Fotheringham J et al, 2007; Niehusmann P et al, 2010*).





- Human herpes viruses are the hidden neighbours for majority of people lifelong and worldwide, however, for some of them they might turn into dangerous tools and can cause serious diseases of the nervous system.