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Assessment & Implications of Viruses in Debilitating Fatigue in CFS & MS Patients

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Viruses Implicated In CFS

Human herpesviruses
- EBV (HHV-4)
- CMV (HHV-5)
- HHV-6
- HHV-7

Stealth Virus (Simian)

Enteroviruses
- Polio
- Coxsackie A & B
- Echovirus

Foamy virus, also known as spuma virus

Parvo virus
- B-19

Hepatitis-C virus

JHKV

Rubella virus

Ross River virus (RRV)

Inoue-Melnick virus

Borna virus

Viruses Implicated In Multiple Sclerosis

Corona virus
Toga virus
Rubella virus
Tick borne encephalitis
Herpes simplex-1 (HSV-1)
Varicella zoster virus (HHV-3)
EBV (HHV-4)
HHV-6
Rabies virus
Scrapie agent
SMON (subacute myelo-optico-neuropathy)

Paramyxo virus
Measles virus
Mumps virus
Para-influenza virus-1
Simian virus-5
HTLV-1
MSRV (MS associated retrovirus)
HRES-1
Retrovirus-like particles
Bone marrow agent

Reasons to suspect viruses as a cause of CFS & MS

CFS:

- CFS often starts with a flu-like episode
- CFS symptoms wax and wane
- Antiviral pathways are activated
- CFS symptoms are similar to many viral conditions including:
  - EBV mononucleoisis
  - polio post-viral fatigue
- Geographic outbreaks have been reported
- Gene expression profiling found genetic variants that reduce antiviral defenses
- Antiviral treatments have been effective in in small studies
  - Ampligen, isoprinosine, beta interferon, cidofovir

MS:

- Antiviral pathways are activated
- Geographic outbreaks have been reported
- All demyelinating disorders with known etiology have been caused by viruses
- Antiviral treatments have been effective in MS
  - beta interferon
- MS symptoms wax and wane
- MS symptoms worsen with viral infections

Enterovirus, Rubella, Parvovirus & EBV in CFS

Enterovirus in CFS

Two papers by Douche-Aourik (2003) and Lane (2003), demonstrated enterovirus (Coxsackie virus IB) DNA in skeletal muscle of patients with CFS (20%) and fibromyalgia (13%) by PCR. Douche-Aourik also detected VP-1 protein in inflammatory muscle disease. The authors found no enteroviral sequences in healthy donor specimens.

Rubella in CFS

Allen et al (1988) showed IgG to rubella were positively correlated with the intensity of symptoms in CFS patients compared to controls.

Parvovirus in CFS

Kerr (2002) studied 51 patients with acute B19 infections and found that 15% developed chronic fatigue (10% fulfilled the CDC criteria for CFS diagnosis). Sixty percent developed fibromyalgia.

EBV in CFS

Elevated EBV VCA IgM antibodies and EA antibodies have been elevated in in several studies. (Ikuta, Lerner, 2004)

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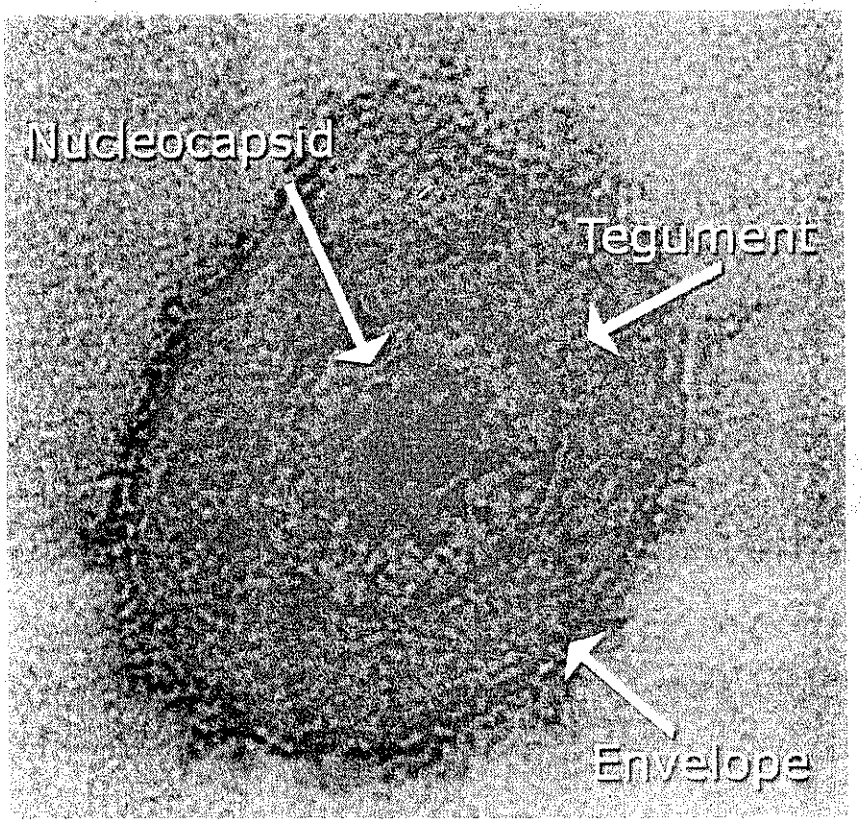
HHV-6

HHV-6 Overview

- Discovered in 1986 by Ablashi and Salahuddin at NCI in patients with lymphoma
- Beta herpesvirus
- Over 90% of adults seropositive
- Predominantly CD4 lymphotropic
- Cell membrane target receptor: CD46
- Two very distinct variants with 90% nucleotide sequence homology

Structure of HHV-6



HHV-6: General Characteristics Related to the Disease Process

•HHV-6 has two variants, A and B. Each variant is associated with a subset of illnesses.

•HHV-6A infection comes later in life and is strongly linked to the pathogenesis of CFS and MS. It also plays a role in HIV, causing more immunosuppression in AIDS patients.

•HHV-6B is the causative agent of *Exanthem subitum*, is strongly linked to infections in transplant patients and is also associated with epilepsy.

•HHV-6A is more lytic, and readily infects a variety of neural and other cells such as astrocytes , and is also more neurotropic, leading to cognitive disorders in CFS and MS patients.

•Both A & B strains cause encephalitis, facial paralysis, chronic myelitis and transverse myelitis.

•HHV-6 is an enveloped, double-stranded DNA virus with an icosahedral capsid. The genome contains 70 proteins. Some of these proteins (early antigen and immediate early antigen) can be used to detect active infections.

HHV-6: General Characteristics

(continued)

HHV-6A can lead to enhanced production of EBV, HSV and HHV-8.

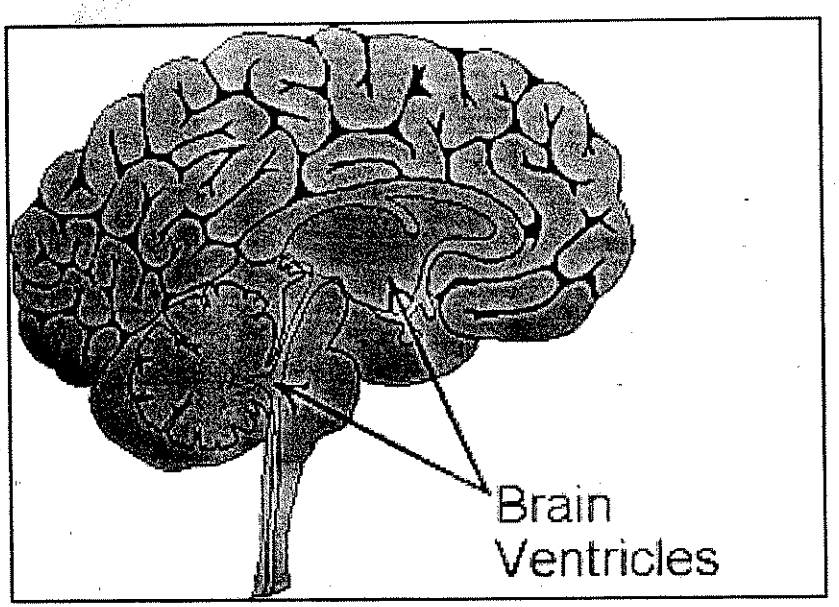
- Both strains establish latency in monocytes, macrophages and target CD4 cells for infection.
- Since HHV-6, like other human herpesviruses, is ubiquitous, its role in the pathogenesis is established when the virus is in the active state of infection.
- Some anti-viral agents that inhibit CMV or EBV infection do not block HHV-6 infection, especially of the A variant.

HHV-6 infection contributes to immune suppression by:

- Disturbing key immune activation pathways and cytokine networks.
- Depleting CD4 T lymphocytes via direct infection and induction of apoptosis (Lusso)
- Upregulating TNF alpha, TNF- gamma, IL-1beta and IL-10.(Flamand, Dockerell, Li)
- Downregulating complement activity through the CD46 receptor
- Suppressing the ability of macrophages to produce IL-12 upon stimulation with interferon gamma. (Lusso 2004)

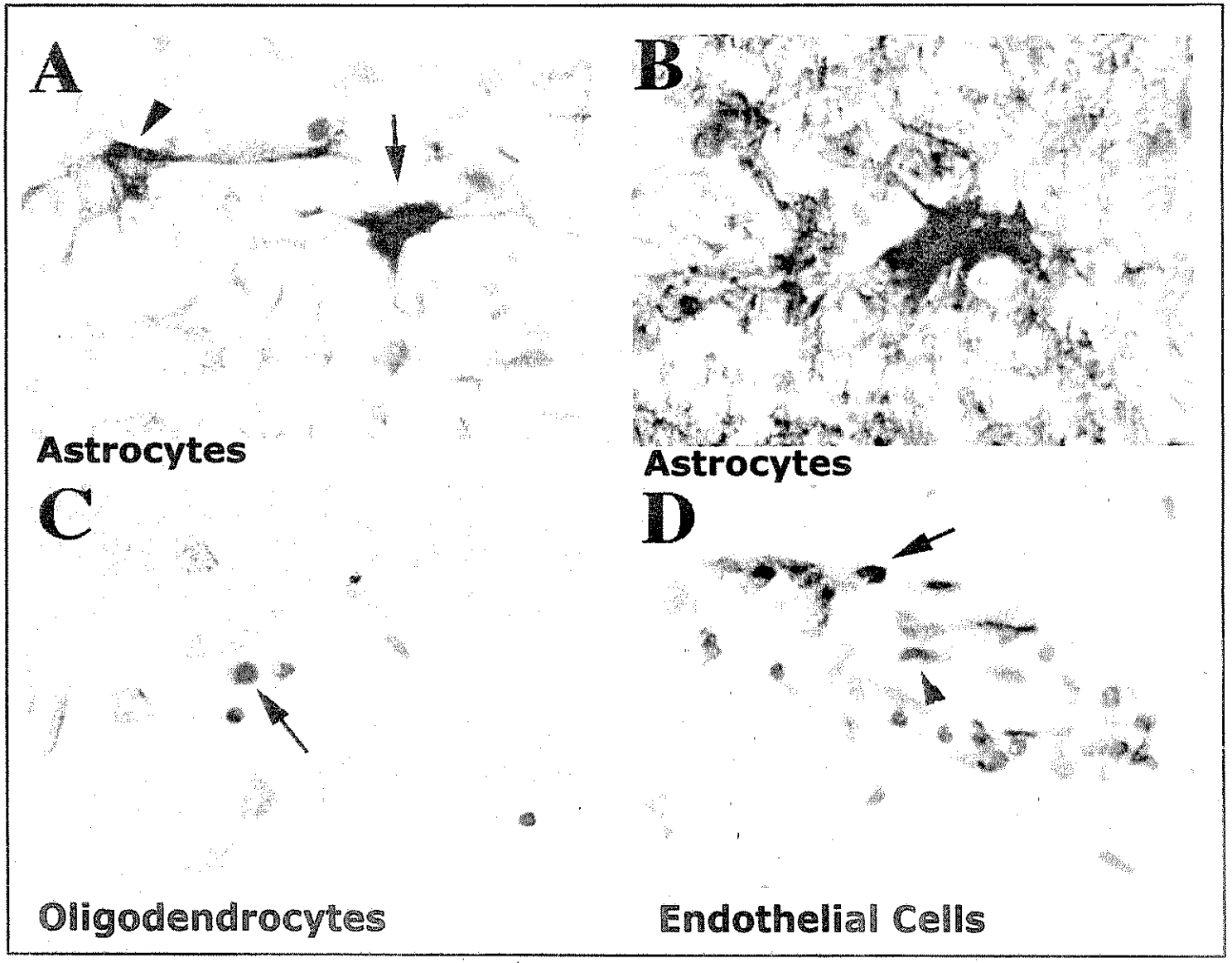
Evidence of CNS Abnormalities In CFS are Similar to Those In MS:

- Reduced grey matter volume in bilateral prefrontal cortex
- Abnormal uptake of of acetyl-L-carnitine in the prefrontal cortex
- Enlarged ventricle volumes
- Increased small punctuate lesions on MRI in MS and a subset of CFS
- Fatigue
  - MS: >85%
  - CFS: 100%
- Reduced information processing speed
- Memory and cognitive problems



HHV-6 virus in MS plaques

Arrows indicate HHV-6 A antigen in red stain.



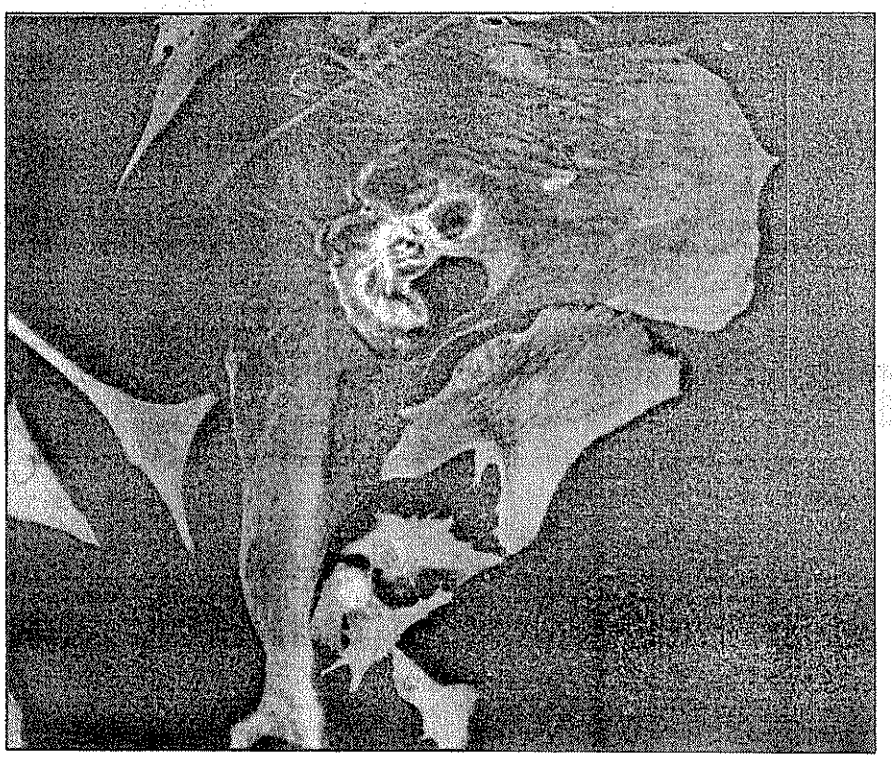
Photos courtesy of S. Jacobson, NINDS, NIH

Myelin Basic Protein Was Recently Found to Cross-react with HHV-6

- Viral infections are thought to play an important role in the pathogenesis of multiple sclerosis (MS) potentially through molecular mimicry.

- An **identical sequence** was found in both myelin basic protein and human herpesvirus-6

- Tejada-Simon et al found that greater than 50% of T cells recognizing this MBP sequence cross-reacted with a synthetic peptide corresponding to the identical HHV-6 U24 sequence in MS patients. (Ann Neurol. 2003 Feb)

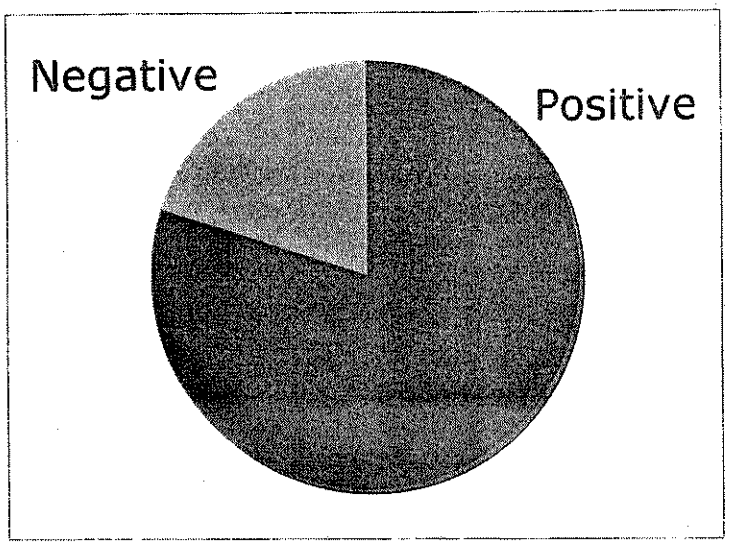


Human astrocyte infected with HHV-6A virus.

Photo courtesy of S. Jacobson, NINDS, NIH

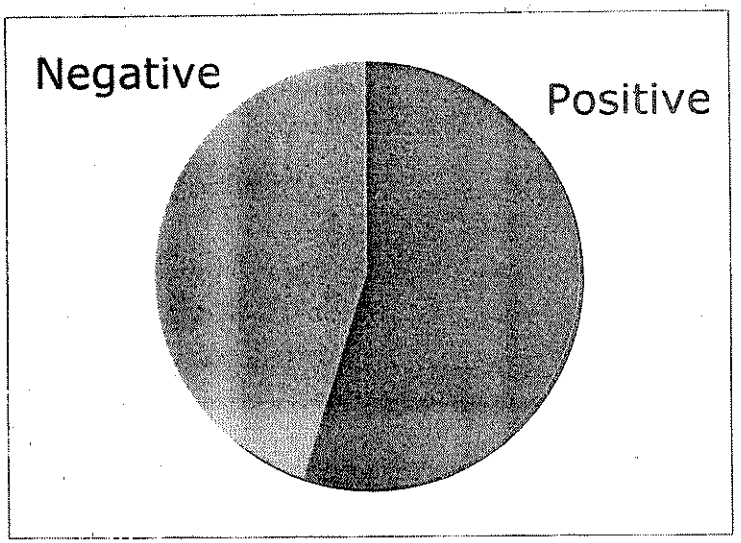
Analysis of Studies of HHV-6 in MS

Differentiated between active and latent virus



N = 36
Positive = 32 (81%)
Negative = 8 (19%)

Did <u>not</u> differentiate between active and latent virus

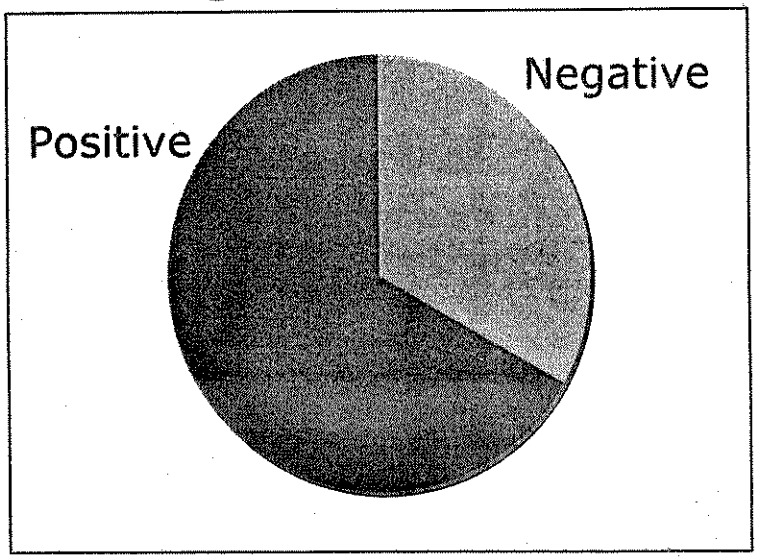


N = 20
Positive = 11 (55%)
Negative = 9 (45%)

Serologic Assays are More Sensitive than PCR Assays in Looking for HHV-6 in MS

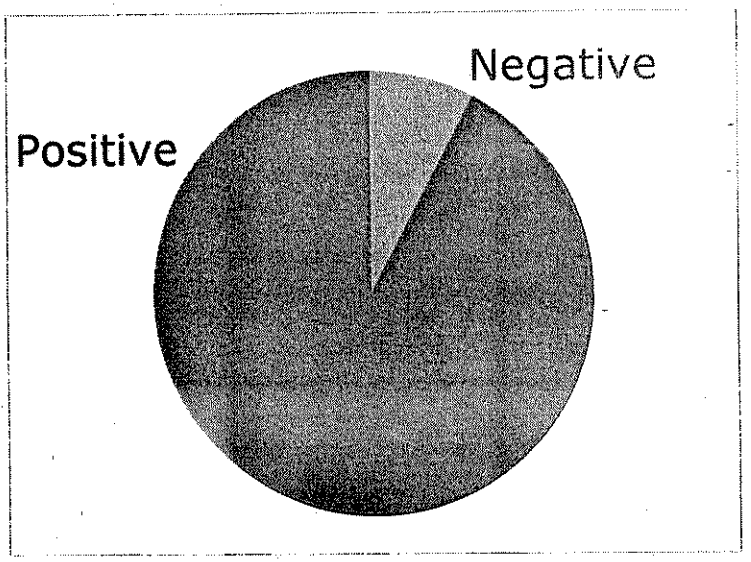
This is similar to what others have found with HHV-8.

Serological Based Studies



N= 13
Positive= 12 (92%)
Negative = 1 (8%)

PCR Based Studies



N=27
Positive= 18 (66%)
Negative = 9 (33%)

Source: NINDS, NIH

HHV-6

Tests That Differentiate Between Active and Latent Infections

Tests that can differentiate:

IgM antibodies to early antigen
PCR on serum, plasma or acellular CSF
In situ PCR (quantitative)

Tests that can differentiate poorly:

IgM antibodies to late antigen
Lymphoproliferative response (high cutoff)
IgG titres (4X elevated)

Tests that can <u>not</u> differentiate:

PCR on whole blood
PCR on cellular CSF
PCR on tissue
IgG titres with a low cutoff

Best Tests for Detecting *Active* HHV-6A

IgM antibodies against HHV-6A early antigen	Very sensitive and specific.
IgM antibodies against early antigen (A and B)	Very sensitive but not specific (picks up B variant reactivations)
Lymphoproliferative assays (high cutoff)	Sensitive and specific.
Nested PCR DNA on serum, plasma or CSF	Specific but insensitive, especially for A variant.
PCR DNA on serum, plasma or CSF	Very insensitive to HHV-6A.
Viral isolation	HHV-6A is extremely difficult virus to isolate from PBMCs.

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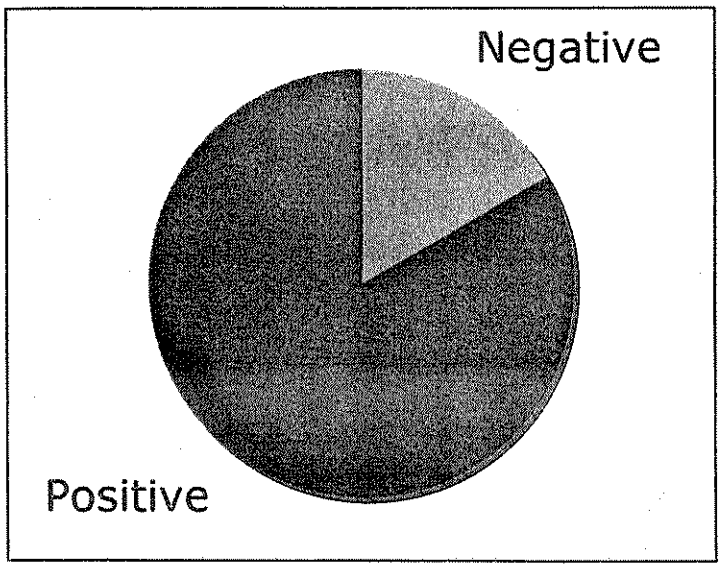
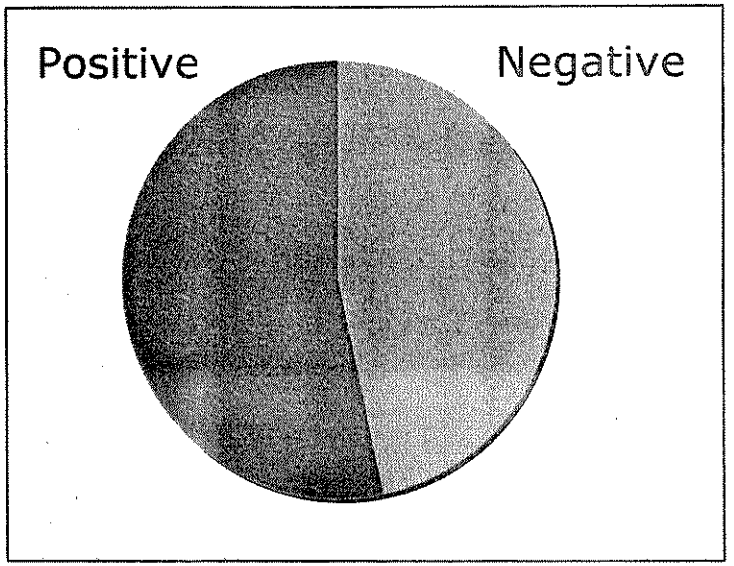
Analysis of Studies of HHV-6 in CFS

Differentiated between active and latent virus



N= 12
Positive = 10 (83%)
Negative = 2 (17%)

Did not differentiate between active and latent virus



N = 15
Positive = 8 (53%)
Negative = 7 (47%)

Studies of HHV-6 in CFS Using Assays That Differentiate Between Active and Latent Virus

Author	Year	Patients Positive	Controls Positive	Testing Method	Result	Size of Study
Nicolson	2003	31%	9%	PCR on serum or plasma	Positive	200 CFS, 100 controls
Koelle	2002	0%	0%	PCR on serum or plasma	Negative	22 CFS, 22 controls (twin pairs)
Ablashi	2000	54%	8%	IgM Early Antigen antibodies	Positive	35 CFS, 25 controls
Reeves	2000	0%	0%	Viral Isolation	Negative	26 CFS, 52 controls
Patnaik	1995	77%	12%	IgM Early Antigen antibodies	Positive	119 CFS, 165 controls
Secchiero	1995	2.90%	0%	PCR on serum or plasma	Positive*	39 CFS, 37 controls
Buchwald	1992	70%	20%	Primary cell culture	Positive	113 CFS, 40 controls
Josephs	1991	43%	0%	Short term culture	Positive	7 CFS, 2 controls

* not statistically significant

HHV-6

Studies of HHV-6 in CFS & MS Using IgM Early Antigen Antibody Assays

Author	Year	%Pos	%Neg	Result	Study Size	
Ablashi	2000	54%	8%	Positive	35 CFS, 25 controls	CFS
Ablashi	2000	57%	16%	Positive	35 CFS, 25 controls	MS
Soldan	1997	73%	18%	Positive	50 MS, 14 Controls	MS
Tourtellotte	1997	71%	18%	Positive	Unpublished data from UCLA	MS
Patnaik	1995	77%	12%	Positive	119 CFS patients, 165 controls	CFS

Note: these studies were done with an assay that picked up both HHV-6 A and HHV-6B. The B variant reactivates in healthy adults in response to illness.

Conclusions

- None of the viruses described here are a single causative agent for CFS. Both molecular and immunological studies suggest that HHV-6 plays a major role in the pathogenesis of disease in a subset of CFS.

- More studies are needed on the association of CFS and HHV-6 using assays that differentiate between active and latent virus.

- Studies are needed on the association of CFS and myelin basic protein antibodies.

- Studies are needed on antiviral treatments for CFS patients with chronic HHV-6A infections.

- An IgM early antigen assay must be made available to researchers and to commercial laboratories for clinicians.

This study was supported by the HHV-6 Foundation

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Studies on CFS & HHV-6 Sorted by Type of Assay

Assays that differentiated between active and latent virus: 83% positive

Author	Year	CFS +	Controls +	Method used	Result	Size of study
Nicolson	2003	31%	9%	PCR on serum or plasma	**Positive**	200 CFS, 100 controls
Koelle	2002	0%	0%	PCR on serum or plasma	Negative	22 CFS, 22 controls (twins)
Ablashi	2000	54%	8%	IgM Early Antigen antibodies	**Positive**	35 CFS , 25 controls
Ablashi	2000	+++	+	Lymphocyte response	**Positive**	10 CFS. 6 controls
Ablashi	2000	57%	16%	IgM Early Antigen antibodies	**Positive**	35 CFS, 25 controls
Reeves	2000	0%	0%	Viral isolation	Negative	26 CFS , 52 controls
Zorzenon	1996	73%	0%	CPE/IFA Positive	**Positive**	52 CFS, 51 controls
Wagner	1996	39%	-	Viral isolation	**Positive**	107 CFS
Patnaik	1995	77%	12%	IgM Early Antigen antibodies	**Positive**	119 CFS , 165 controls
Secchiero	1995	3%	0%	PCR on serum or plasma	**Positive***	39 patients, 37 controls
Buchwald	1992	70%	20%	Primary cell culture	**Positive**	113 CFS, 40 controls
Josephs	1991	43%	0%	Short term culture	**Positive**	7 CFS, 2 controls

Assays that did not differentiate active vs. latent virus: 53% positive

Author	Year	CFS +	Controls +	Method used	Result	Size of study
Koelle	2002	36%	27%	PCR on PBMCs	Negative	22 CFS, 22 controls (twins)
Ablashi	2000	++	+	IgG Antibody titres	**Positive**	21 MS, 35 CFS
Enbom	2000	25%	29%	PCR on PBMCs	Negative	8 CFS, 7 controls
Reeves	2000	8%	28%	PCR on PBMCs	Negative	26 CFS, 52 controls
Wallace	1999	35%	27%	PCR on PBMCs	Negative	74 CFS, 71 controls
Wallace	1999	+	+	IgG Antibody titres	Negative	58 CFS ,51 controls
Wagner	1996	72%	-	IgG and IFA above 1:40	**Positive**	107 CFS
Zorzenon	1996	62%	12%	PCR DNA on PBMC's	**Positive**	52 CFS, 51 controls
Di Luca	1995	22%	4%	PCR on PBMCs	**Positive**	36 CFS, 24 controls
Sairenji	1995	++	+	Total IgG Antibody titres	**Positive**	20 CFS, 3 controls
Wilborn	1994	++	+	IgG Antibody titres	**Positive**	21 MS, 16 controls
Yalcin	1994	53%	0%	PCR on PBMCs	**Positive**	13 CFS, 13 controls
Levine	1992	+	+	Total IgG Antibody titres	Negative	31 CFS, 105 controls
Nishikai	1992	+	+	Total IgG Antibody titres	Negative	51 CFS
Gupta	1991	++	+	Total IgG Antibody titres	**Positive**	20 CFS, 20 controls

*not statistically significant

Studies on the association of HHV-6 & MS using assays that differentiate between active and latent virus: 81% positive

Lead Author	Year	MS +	Controls +	Technique used	Result	Size of Study
Alvarez-Lafuente	2004	16%	0%	messenger RNA	Positive	154 MS (RR), 49 controls
Rotola	2004	22%	9%	CSF DNA (cell free)	Positive	32 MS
Al-Shammari	2003	0%	0%	Nested PCR on serum/ plasma	Negative	24 patients, 20 controls
Chapenko	2003	50%	0%	PCR DNA on serum or plasma	Positive	26 MS, 150 controls
Chapenko	2003	57%	0%	PCR DNA on serum or plasma	Positive	11 MS (RR),150 controls
Goodman	2003	100%	0%	in situ PCR	Positive	9 MS CNS biopsies
Villoslada	2003	++	+	IgM antibodies	Positive	49 MS , 50 controls
Berti	2002	23%	0%	Nested PCR on serum or plasma	Positive	167 MS , 70 controls
Caselli	2002	87%	44%	Early antigen IgG antibodies	Positive	54 MS , 82 controls
Cirone	2002	78%		CSF DNA	Positive	7 MS
Cirone	2002	36%	26%	Lymphoproliferative response	Negative	22 MS, 16 controls
Rodriguez Carnero	2002	0%	0%	CSF DNA	Negative	23 MS (RR), 23 controls
Tejada-Simon	2002	66%	33%	PCR DNA on serum or plasma	Positive	33 MS , 21 controls
Tejada-Simon	2002	46%	20%	CSF DNA	Positive	30 MS , 30 controls
Ablashi	2000	70%	15%	IgM Antibody titres	Positive	21 MS
Akhyani	2000	23%	0%	PCR DNA on serum or plasma	Positive	23 MS , 19 controls
Blumberg	2000	85%	0%	in situ PCR	Positive	13 MS , 8 controls
Knox	2000	54%	0%	rapid culture	Positive	41 MS , 61 controls
Knox	2000	54%	0%	rapid culture	Positive	41 MS , 61 controls
Soldan	2000	67%	32%	Lymphocyte response	Positive	18 MS , 21 controls
Taus	2000	0%	0%	CSF IgM antibodies	Negative	25 MS, 0 controls (?)
Enbom	1999	36%	20%	Lymphoproliferative response to H	Positive	14 MS, 29 controls
Enbom	1999	6%	6%	PCR DNA on serum or plasma	Negative	51 MS , 17 controls
Friedman	1999	++	+	Lymphocyte response	Positive	18 MS , 21 controls
Goldberg	1999	4%	0%	Nested PCR on serum or plasma	Negative	24 MS, 16 OND
Mirandola	1999	0%	0%	Nested PCR on serum or plasma	Negative	38 MS, 12 OND controls
Ongradi	1999	44%	0%	IgM CSF antibodies	Positive	13 MS
Ongradi	1999	67%	0%	IgM CSF antibodies	Positive	13 MS
Ablashi	1998	39%	7%	CSF antibodies	Positive	16 MS, 72 controls
Fillet	1998	6%	0%	PCR DNA on serum or plasma	Positive	32 MS, 13 controls
Soldan	1997	73%	18%	IgM Early Antigen antibodies	Positive	50 MS, 14 controls
Soldan	1997	43%	0%	Nested PCR on serum or plasma	Positive	50 MS, 14 controls
Tourtellotte*	1997	71%	18%	IgM Early Antigen antibodies	Positive	*unpublished data, UCLA
Challoner	1995	80%	0%	Early antigen immunohistochemisti	Positive	15 MS, 45 controls
Liedtke	1995	11%	0%	CSF DNA - acellular	Positive	36 MS, 24 controls
Wilborn	1994	14%	0%	DNA on CSF	Positive	21 patients, 16 controls

Studies on the association of HHV-6 & MS using assays that do not differentiate between active and latent virus: 55% positive

Lead Author	Year	MS +	Controls +	Technique used	Result	Size of Study
Tuke	2004	44%	41%	PCR brain tissue	Negative	124 MS
Cermelli	2003	58%	27%	Nested PCR on tissue	Positive	64 MS, 41 controls
Chapenko	2003	62%	29%	PCR DNA on PBMCs	Positive	26 MS , 150 controls
Alvarez-Lafuente	2002	20%	4%	rtPCR DNA on PBMCs	Positive	103 MS (RR), 46 controls
Alvarez-Lafuente	2002	49%	22%	nested PCR on PBMCs	Positive	102 MS (RR), 102 controls
Gutierrez	2002	++	+	IgG, IgM, IgA Antibody titres	Positive	139 MS
Gutierrez	2002	++	+	IgG, IgM, IgA Antibody titres	Positive	72 CSF , 31 OND
Ablashi	2000	80%	60%	PCR on PBMCs	Positive	15 MS , 10 controls
Akhyani	2000	76%	75%	PCR DNA on PBMCs	Negative	23 MS , 19 controls
Akhyani	2000	84%	88%	PCR on saliva	Negative	38 MS , 17 controls
Akhyani	2000	26%	0%	PCR on urine	Negative	38 MS , 16 controls
Alvarez	2000	49%	22%	PCR DNA on PBMCs	Positive	MS , 102 controls
Hay	2000	7%	14%	PCR DNA on PBMCs	Negative	29 MS , 14 controls
Kim	2000	21%	0%	PCR DNA on PBMCs	Positive	34 MS , 20 controls
Mayne	1998	24%	23%	PCR DNA on PBMCs	Negative	46 MS, 17 controls
Merelli	1997	0%	50%	CSF DNA- cellular	Negative	5 MS, 8 controls
Nielsen	1997	+	+	IgG Antibody titres	Negative	189 MS, 190 controls
Gordon	1996	+	+	PCR on tissue	Negative	7 MS, 27 controls
Sanders	1996	57%	43%	PCR on tissue	Positive	37 MS, 37 controls
Sola	1993	++	+	IgG Antibody titres	Positive	126 MS. 500 controls

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HHV-6

Results suggesting an association between HHV-6 and MS

- HHV-6A DNA is found in cell-free specimens from MS patients but not from healthy controls.

- More HHV-6 DNA is found during MS exacerbations than during relapses.

- More HHV-6 DNA is found in MS plaques than in non-affected white matter.

- HHV-6A DNA is detected in CSF in the early stages more than in later stages of MS.

- Lymphoproliferative response to HHV-6A is greater in MS patients than in controls.

- Researchers at UCSF have induced MS symptoms in marmoset monkeys by injecting them with HHV-6A. (unpublished data, in press)

- A portion of the HHV-6 sequence is identical to that of myelin protein suggesting molecular mimicry may be a factor in autoimmunity.

Looking for early antigen (EA) or immediate early antigen (IEA) antibodies is the most sensitive method of detecting ***active*** HHV-6A infection

- The immediate early antigen (IEA) is a protein which is produced 8-12 hours after infection and reaches a peak after 48 hours. It is not produced during the latent phase.

- The early antigen (EA) protein is produced from 12 to 18 hours after infection.

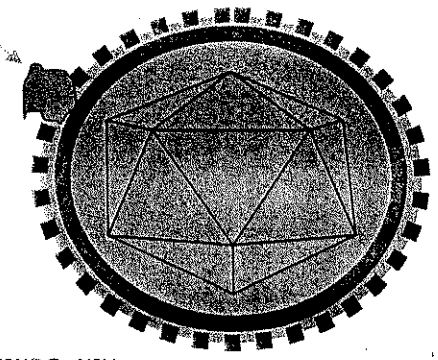
- The EA and IEA antigen is not produced when the virus is in its latent stage.

Could HHV-6 induce autoimmunity in the same manner that HHV-5 (CMV) induces autoimmunity in transplant patients?

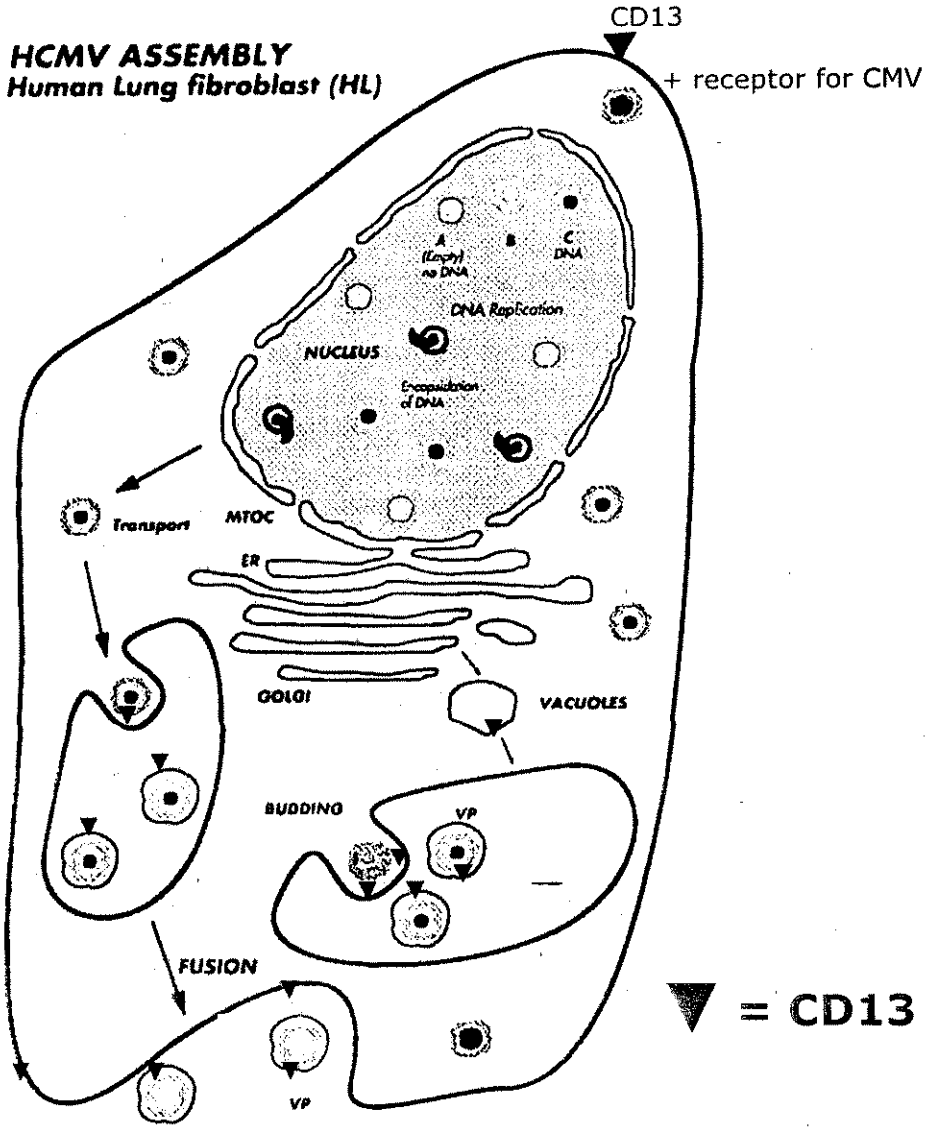
The leading theory of CMV- induced autoimmunity in transplant patients is that the CD13 cell surface protein from CMV infected tissues is incorporated into the viral envelope of CMV inducing chronic GHVD post transplant.

Similarly, myelin proteins from infected oligodendrocytes could become incorporated into the HHV-6A envelope as they enter and leave the cell, inducing CNS autoimmunity in MS and CFS patients. This is an area of current interest at the Viral Immunology Division at the NINDS at the NIH.

Myelin protein in the HHV-6A virus envelope



Illustrations courtesy of NINDS, NIH



Release of virus particles

HHV-6