Cerebrospinal Fluid EBV Replication is Associated with Compartmental Inflammation and Pleocytosis in HIV-positive naïve and Treated Individuals

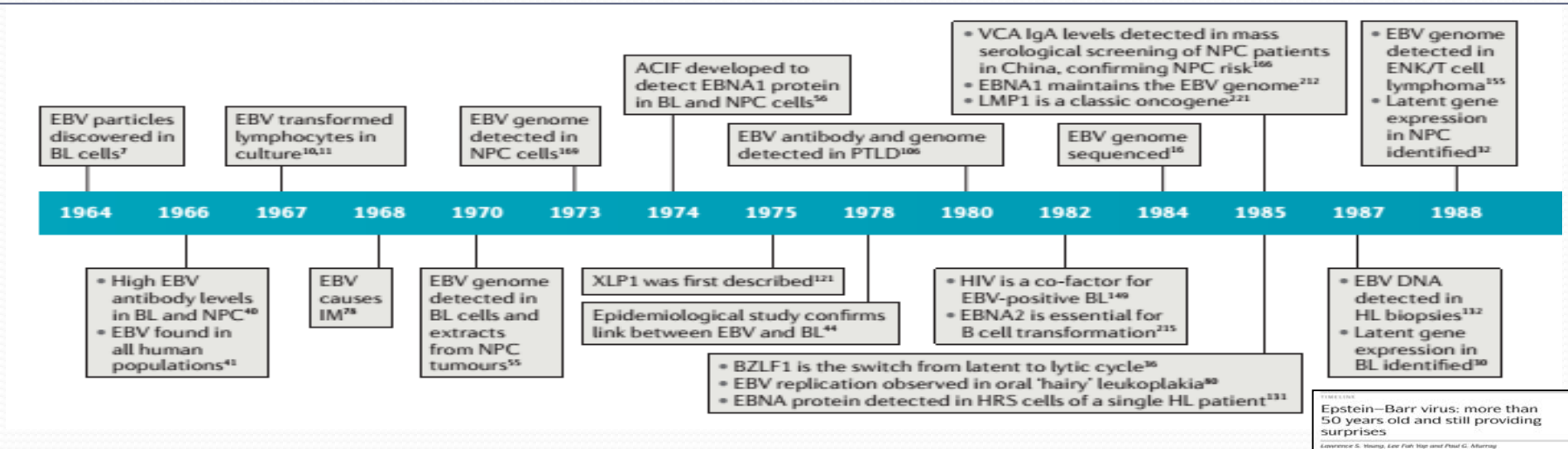
Lupia T, Milia MG, Atzori C, Audagnotto S, Imperiale D, Romito A, Scabini S, Gregori G, Lipani F, Bonora S, Di Perri G, Calcagno A.

Tommaso Lupia
University of Torino
Clinic of Infectious Diseases
Ospedale Amedeo di Savoia

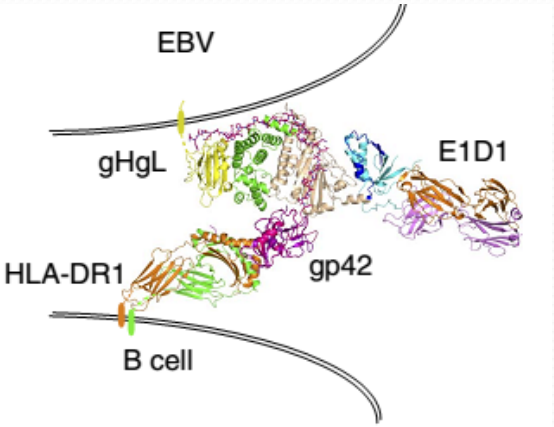
EBV (Epstein-Barr Virus)

HHV-4 (Human Herpes Virus – 4)

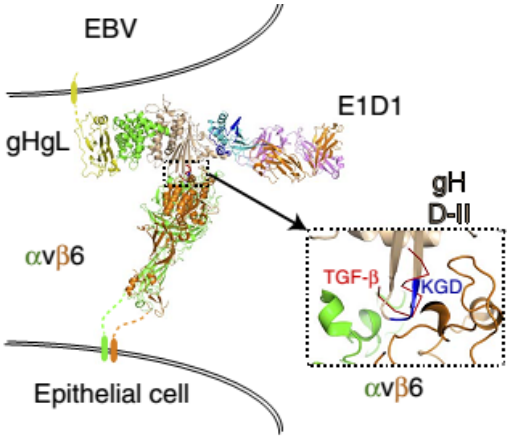
- Widely disseminated gamma-herpesvirus: approximately 90-95% of adults are seropositive, lifelong persistence.
- Replicate in several host cells (B and T lymphocytes, Epithelial and Endothelial cells, Myocites and fibroblasts).
- Can reach the CNS directly (through the BBB) or inside PBMCs (with a " trojan horse"- like mechanism)



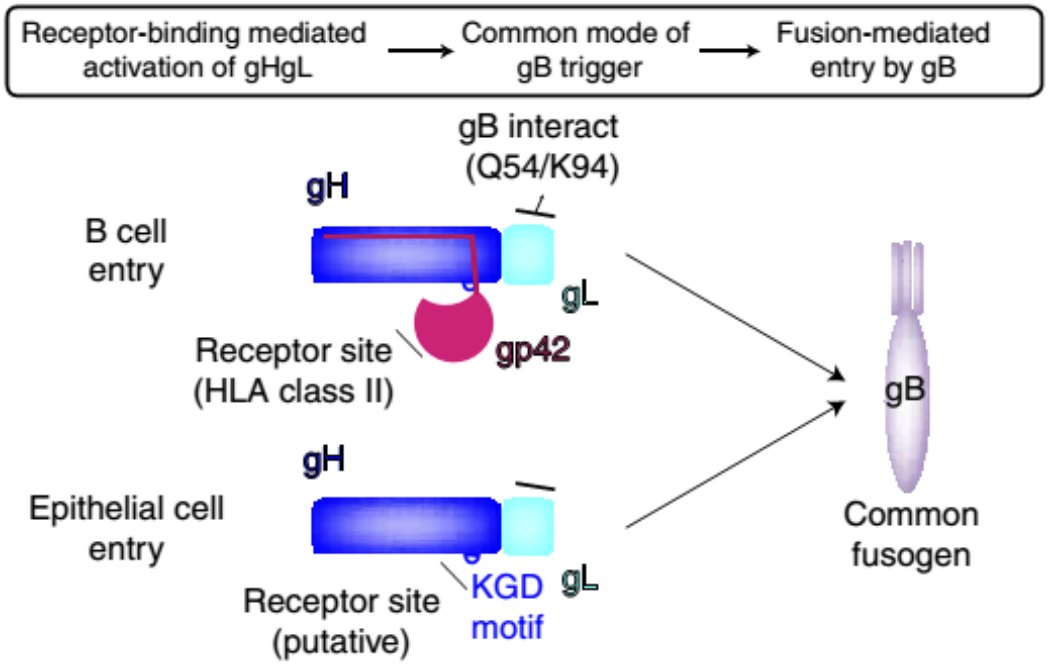
- Entry and fusion of EBV into <u>B cells</u> involves **5** different glycoproteins (gp350/220, gH, gL, gp42 and gB).
- Entry and fusion of EBV with <u>epithelial and endothelial cells</u> needs **3** gp (gH,gL and gB)
- gp42 has an <u>ambivalent role</u>



Hybrid crystal structure (gHgL/gp42/HLA)
'EBV-B cell entry triggering complex'



Docked hypothetical model (gHgL/αvβ6)



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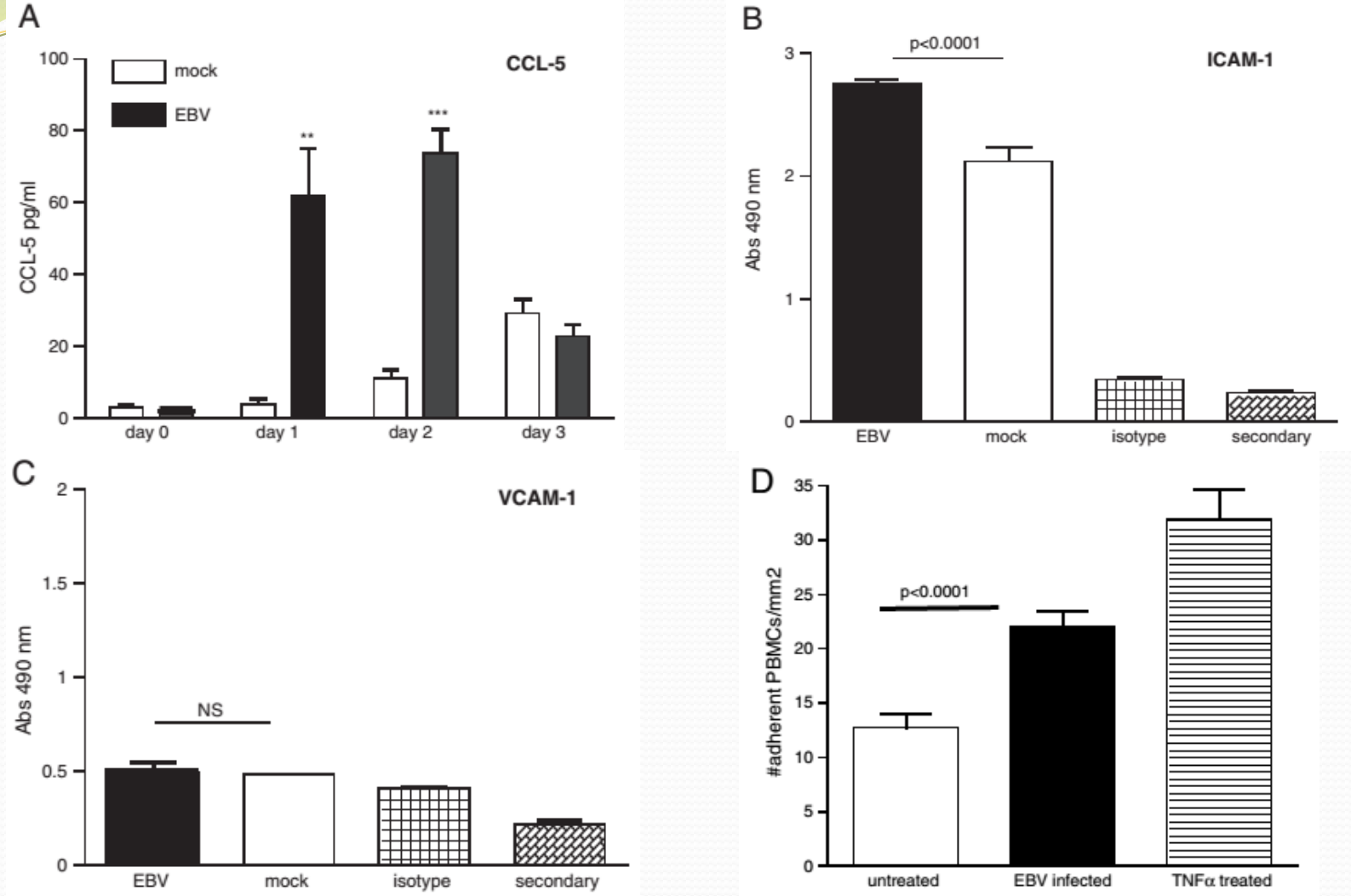
Structural basis for Epstein-Barr virus host cell tropism mediated by gp42 and gHgL entry glycoproteins

Karthik Sathiyamoorthy[1], Yao Xiong Hu[1], Britta S. Möhl[2], Jia Chen[2], Richard Longnecker[2] & Theodore S. Jardetzky[1]

Infection and reactivation in endothelial cells up-regulates the expression of cytokines, chemokines and adhesion molecules

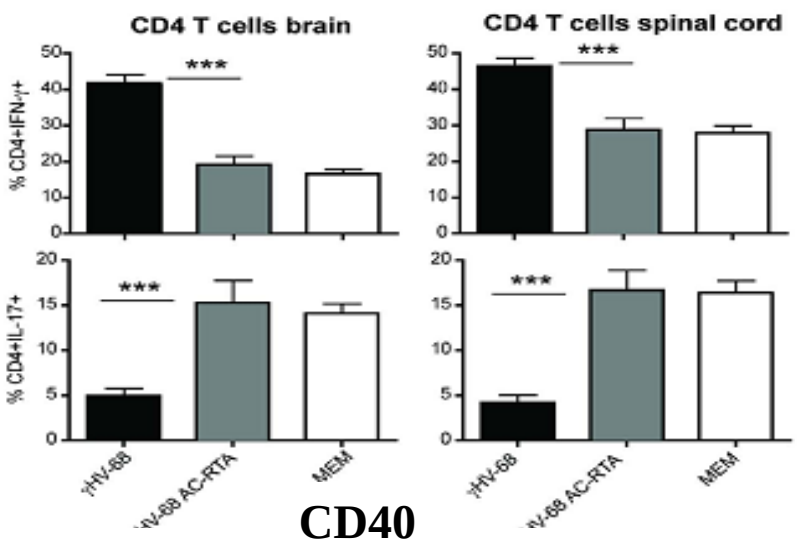


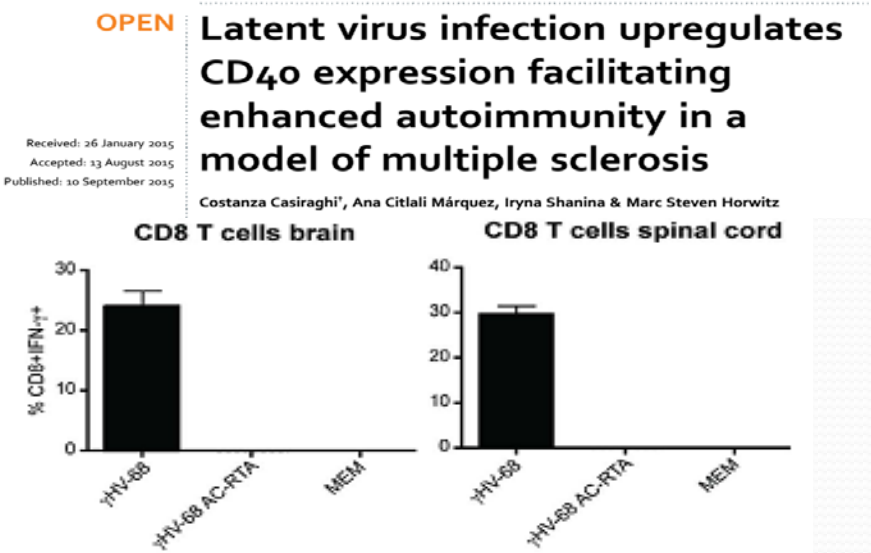
Epstein-Barr virus infection of human brain microvessel endothelial cells: A novel role in multiple sclerosis

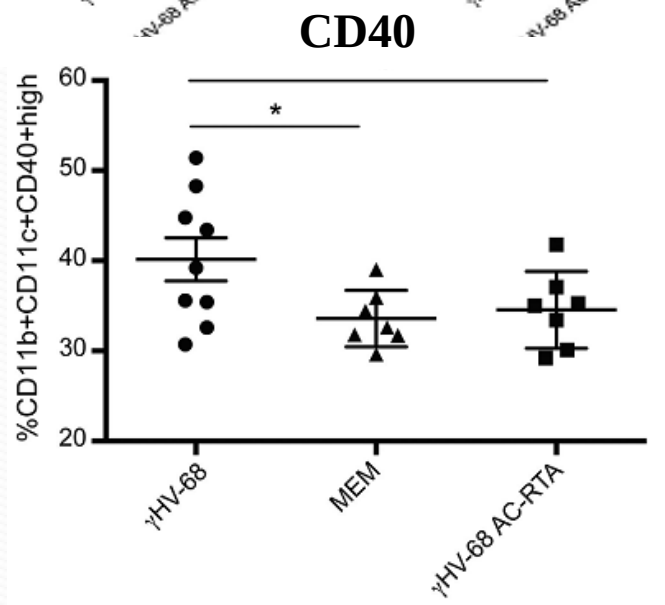
Costanza Casiraghi [a], Katerina Dorovini-Zis [b], Marc S. Horwitz [a,*]

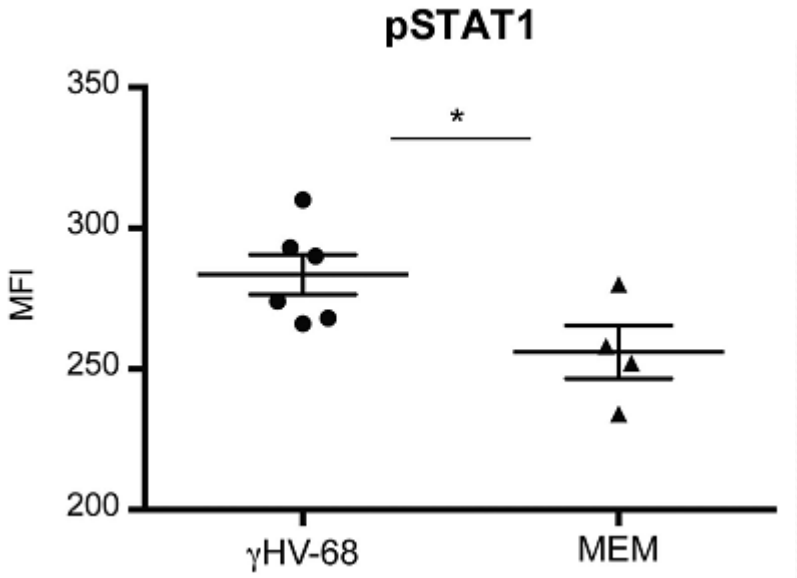
[a] *Department of Microbiology and Immunology, The University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3*
[b] *Department of Pathology and Laboratory Medicine, The University of British Columbia and Vancouver General Hospital, Vancouver, British Columbia, Canada V5Z 1M9*

- **γHV-68** latency upregulates **CD40** surface expression on APCs and the transcription factor **STAT1**
- **CD40** expression and co-stimulation is pivotal in controlling the type and strength of the adaptive immune response : <u>enhance both **CD4+** and **CD8+** effector T cells activation and reduce **Tregs**</u>









Pediatric Epstein-Barr Virus–Associated Encephalitis: 10-Year Review

Asif Doja, MD; Ari Bitnun, MD; Elizabeth Lee Ford Jones, MD; Susan Richardson, MD; Raymond Tellier, MD; Martin Petric, MD; Helen Heurter, RN; Daune MacGregor, MD

Patient	Age (yr)	Sex	Prodrome Duration (d)	Clinical Manifestations	CSF Abnormality	EEG Abnormality	Radiologic Abnormality	Outcome
1	15	M	2	Fever, headache, vomiting, seizures	↑ WBC (57 × 10⁶/L)	None	None	Mild fatigue
2	4	F	21	Fever, vomiting, headache, spasticity, weakness, ADEM	↑ WBC (62 × 10⁶/L)	ND	↑ T_2-weighted signal bilateral thalami	Mild weakness RTB
3	7	F	7	Headache	None	None	≠ T_2-weighted signal L thalamus	
4*	3	M	1	Fever, status epilepticus	None	Status epilepticus	Volume loss, ↑ T_2-weighted signal bilateral hippocampi	Died
5	13	M	7	Fever, headache, vomiting, meningismus, seizures, leg weakness	↑ WBC (275 × 10⁶/L), ↑ protein (0.66 mg/dL)	Diffuse slowing	↑ T_2-weighted signal in brain stem, basal ganglia	Mild fatigue
6	15	F	7	Fever, malaise, vomiting, cough	↑ WBC (13 × 10⁶/L)	Diffuse slowing	Diffuse bilateral abnormal signal in corpus callosum and subcortical white matter	Mild fatigue
7	16	M	21	Headache, fever, vomiting, bilateral lower limb weakness, urinary retention, word finding difficulty, staring episodes	↑ WBC (38 × 10⁶/L), ↑ protein (0.60 mg/dL)	Normal	T_2-weighted and FLAIR hyperintensity within temporal lobes, midbrain, right cerebral peduncle, pons, and upper medulla; diffuse abnormal signal in spinal cord	Mild fatigue
8	14	M	7	Chickenpox lesions, mostly crusted; generalized weakness, urinary retention	↑ WBC (69 × 10⁶/L), ↑ protein (0.65 mg/dL)	Diffuse slowing	FLAIR hyperinsity in insula bilaterally, caudate, occiptal lobe, and brain stem; increased signal in thoracic spinal cord.	RTB
9	15	M	6	Fever, headache, sore throat	↑ WBC (38 × 10⁶/L), ↑ protein (0.46 mg/dL)	ND	ND	RTB
10	2.6	F	5	Fever, vomiting, diarrhea, staring spells, unusual posturing	↑ WBC (13 × 10⁶/L)	Normal	Normal	RTB
11	15	M	21	Sore throat, lymphadenopathy, headache, vomiting	↑ WBC (21 × 10⁶/L), ↑ protein (0.99 mg/dL)	Focal left posterior slowing	Patchy diffuse subtle FLAIR and T_2-weighted hyperintensity in subcortical white matter and deep cortical white matter of occipital lobes	RTB
12	16	F	3	Mood disturbance and psychosis, seizures	↑ WBC (82 × 10⁶/L)	Diffuse slowing	R temporal lobe edema, increased signal intensity in both temporal lobes	Mood/psychiatric disturbance
13	17	F	7	Fever, headache, vomiting, diarrhea, left facial and arm weakness, slurred speech	↑ WBC (22 × 10⁶/L), ↑ protein (0.88 mg/dL)	Poor background, bitemporal epileptiform spikes	Normal	RTB
14	12	F	1	Fever, headache, seizures	↑ WBC (78 × 10⁶/L), ↑ protein (1.14 mg/dL)	Diffuse slowing	↑ T_2-weighted signal right frontal lobe, basal ganglia	RTB
15	12	F	5	Fever, headache, vomiting, ataxia, asymmetric reflexes	↑ WBC (53 × 10⁶/L), ↑ protein (1.19 mg/dL)	Diffuse slowing	L temporoparietal edema	Mild fatigue
16	8	F	14	Fever, headache, vomiting, decreased visual acuity	↑ WBC (58 × 10⁶/L)	Diffuse slowing	Bilateral optic nerve swelling, worse on right	Mild fatigue
17	11	M	14	Fever, seizures	↑ WBC (24 × 10⁶/L)	FIRDA, slowing	Mild cerebral edema	RTB
18	15	F	2	Fever, headache, seizures	↑ WBC (58 × 10⁶/L)	Diffuse slowing	Meningeal enhancement	Mild fatigue
19	13	M	30	Fever, headache, vomiting, seizures, decreased visual acuity	↑ WBC (323 × 10⁶/L), ↑ protein (1.16 mg/dL)	Epileptiform discharges bitemporally	None	RTB
20†	14	F	6	Classic infectious mononucleosis (fever, sore throat, lymphadenopathy), seizures later evolving into status epilepticus	None	R frontal lobe epileptiform activity	Edema, ischemia to thalami, basal ganglia, subinsular cortex, and R temporal lobe	Died
21	11	M	14	Fever, headache, vomiting, sore throat, seizures, intermittent hearing impairment	None	Diffuse slowing	↑ T_2-weighted signal R temporal lobe	Mild fatigue

Perivascular lymphohistiocytic infiltrates :
the inflammatory infiltrate was dominantly composed of CD3+ and CD8+ T-lymphocytes and CD4+ and CD20+

Immunostaining analysis:
antiEBER (EBV-encoded small RNA) antibody showed EBV-positive cells in the perivascular site

NEUROPATHOLOGY
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doi:10.1111/neup.12356

Case Report

Biopsy-proven case of Epstein–Barr virus (EBV)-associated vasculitis of the central nervous system

Kohei Kano,[1] Takayuki Katayama,[1] Shiori Takeguchi,[1] Asuka Asanome,[1] Kae Takahashi,[1] Tsukasa Saito,[1] Jun Sawada,[1] Masato Saito,[2] Ryogo Anei,[2] Kyousuke Kamada,[2] Naoyuki Miyokawa,[3] Hiroshi Nishihara[4] and Naoyuki Hasebe[1]

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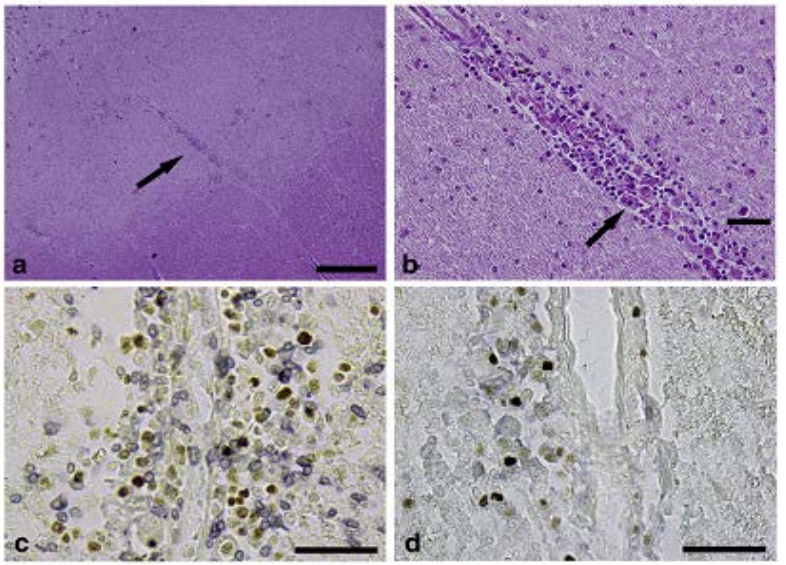
Short communication

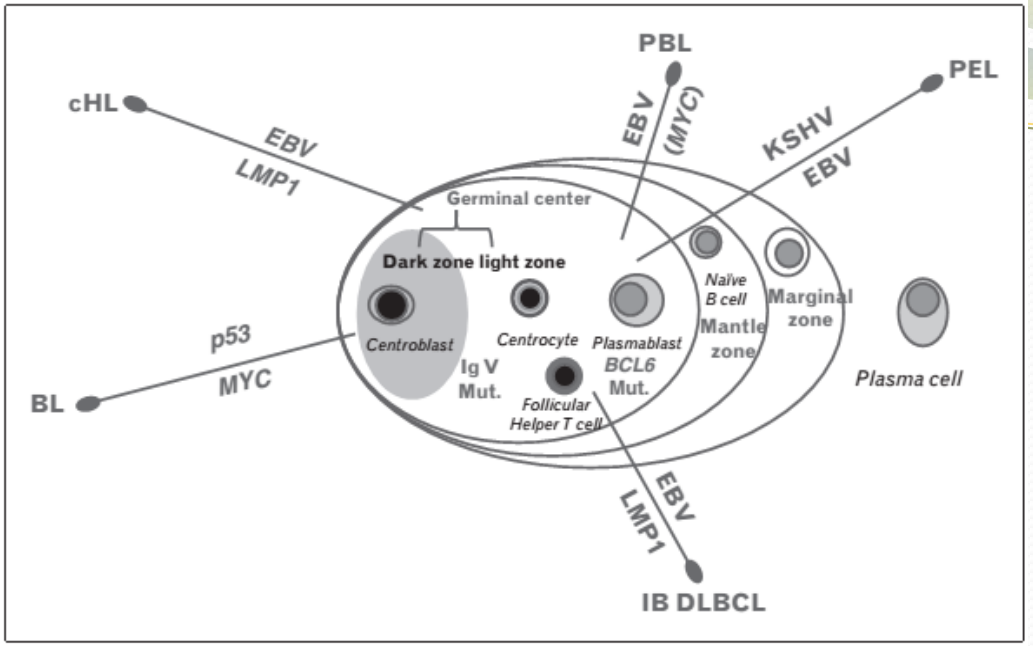
An autopsy case of chronic active Epstein–Barr virus infection (CAEBV): Distribution of central nervous system (CNS) lesions

Zen Kobayashi [a,b,c,*], Kuniaki Tsuchiya [c,d], Makoto Takahashi [b], Osamu Yokota [c,e], Atsushi Sasaki [f], Ekapot Bhunchet [g], Tetsuaki Arai [c], Haruhiko Akiyama [c], Masaharu Kamoshita [h], Minoru Kotera [a], Hidehiro Mizusawa [b]



Perivascular cellular infiltration in the substantia nigra (a, arrow) : The infiltrating cells were lymphocytes and macrophages (b)

Midbrain: showed that some of the CD3 positive cells were also EBV-encoded RNA-1 (EBER1) positive



REVIEW

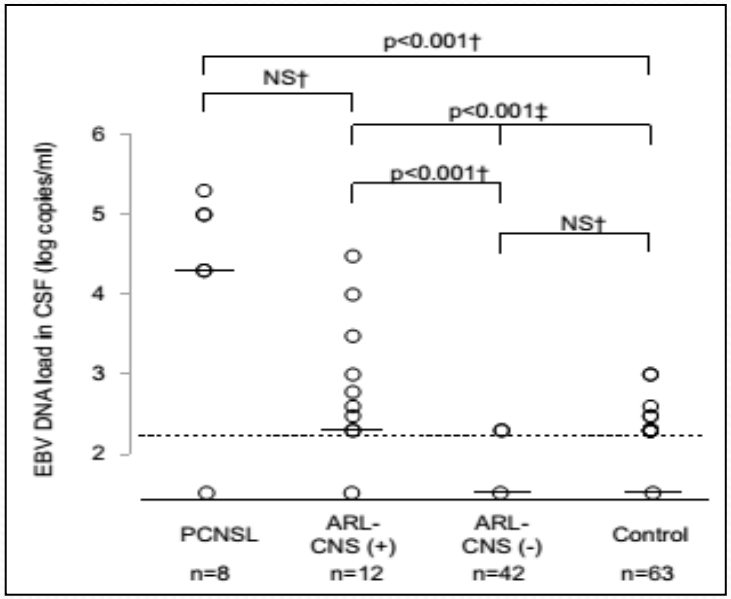
Epstein–Barr virus associated lymphomas in people with HIV

Antonino Carbone[a], Chiara C. Volpi[b], Ambra V. Gualeni[b], and Annunziata Gloghini[b]

Burkitt lymphoma-plasmacytoid
Primary central nervous system lymphoma
Diffuse large B-cell lymphoma, immunoblastic – plasmacytoid
Diffuse large B-cell lymphoma, centroblastic
Plasmablastic lymphoma of the oral cavity type
Primary effusion lymphoma and its solid variant
KSHV-associated MCD-related large cell lymphoma
Hodgkin lymphoma
Other histotypes (rare)
Polymorphic B-cell lymphoma (PTLD-like)

Epstein-Barr Viral Load in Cerebrospinal Fluid as a Diagnostic Marker of Central Nervous System Involvement of AIDS-related Lymphoma

Kunio Yanagisawa[1], Junko Tanuma[2], Shotaro Hagiwara[3], Hiroyuki Gatanaga[2],



Settings	Association with viral infections (virus)	Level of evidence
Transplantation	Post-transplant lymphoproliferative disease	Strong
	Diffuse large B-cell lymphoma (EBV)	
	Kaposi sarcoma (HHV-8)	Strong
	Non-melanoma skin cancer (HPV)	Strong
	Non-melanoma skin cancer (MCV)	Moderate
AIDS	AIDS-related lymphoma:	
	Burkitt lymphoma (EBV)	Strong
	Diffuse large B-cell lymphoma (EBV)	Strong
	Hodgkin lymphoma (EBV)	Moderate
	Primary effusion lymphoma (HHV-8+/EBV±)	Strong
	Multicentric Castleman disease	Strong
Adult T-cell leukaemia/lymphoma	HTLV-I	Strong
Cervical cancer	HPV	Strong
Anal cancer	HPV	Strong
Oropharyngeal cancer	HPV	Moderate

EBV, Epstein–Barr virus; HHV-8, human herpesvirus-8; HPV, human papillomavirus; HTLV-I, human T-cell lymphotropic virus type I; MCV, Merkel cell polyoma virus.

REVIEW

Immunodeficiency-associated viral oncogenesis

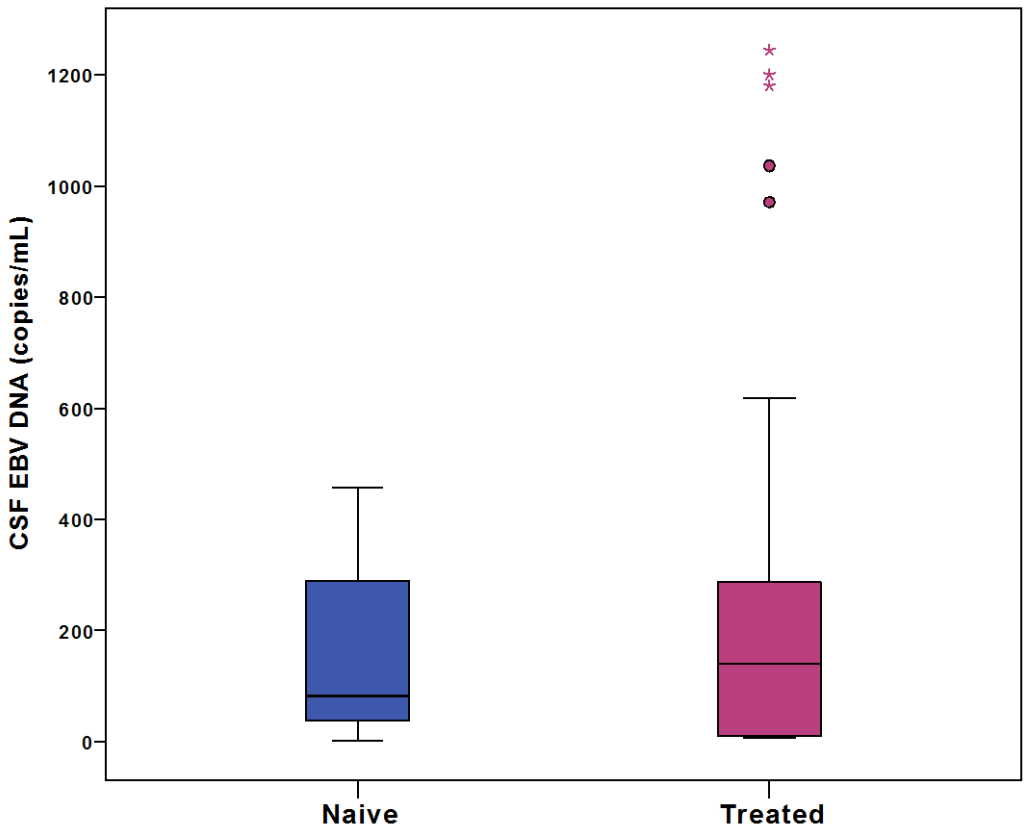
A. Pierangeli[1], G. Antonelli[1] and G. Gentile[2]

Materials and Methods

- Retrospective study from 2010 to 2017
- HIV-positive patients receiving LPs for clinical reasons
- Lymphomas/lympho-proliferative disorders excluded
- No autoimmune diseases
- Real-time PCR method was used for measuring :
  - HIV-RNA (20 copies/mL) and EBV DNA (100 copies/mL)
- CSF escape was defined as 1 Log_{10} higher than plasma VL or detectable if plasma undetectable (20 copies/mL)
- Immune-enzymatic method was used for the measurement of :
  - CSF albumin to plasma ratios (CSAR)
  - Tau, phosphorylated tau,1-42 Beta amyloid
  - Neopterin, S100 beta
- Data are expressed as medians (interquartile ranges)

n=281	Naive	Treated
n	116	170
Gender (male)	70.7%	69.1%
Age (years)	41.1 (35.3-46.7)	46 (39.2-55.3)
Current CD4+ (cell/uL)	80 (34 – 221)	261 (86 – 531)
Nadir CD4+ (cell/uL)	63 (23 – 118)	46 (13 – 194)
Plasma HIV-RNA (Log_{10} copies/mL)	5.3 (4.5 - 5.9)	<1.3 (<1.3 - 2.4)
CSF HIV-RNA (Log_{10} copies/mL)	3.8 (2.8 - 4.6)	1.5 (<1.3 - 2.3)
CSF to plasma HIV RNA ratio (Log_{10} copies/mL)	0.7 (0.6-0.9)	1.0 (0.5- 1.5)
CSF escape (%)	5%	20%

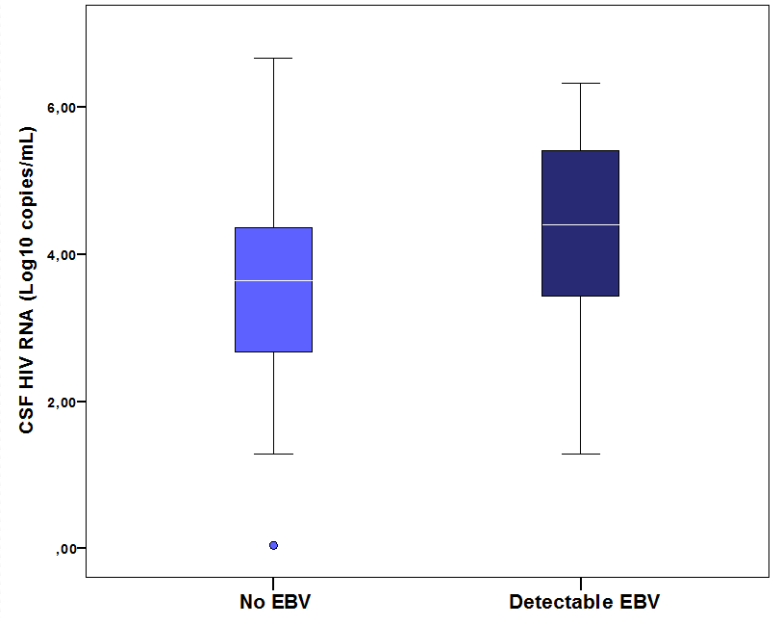
	Naive	Treated
Detectable CSF EBV DNA	20.7%	17.7%
CSF EBV DNA (copies/mL)	<100 (< 100 – 292)	141 (< 100 - 448)

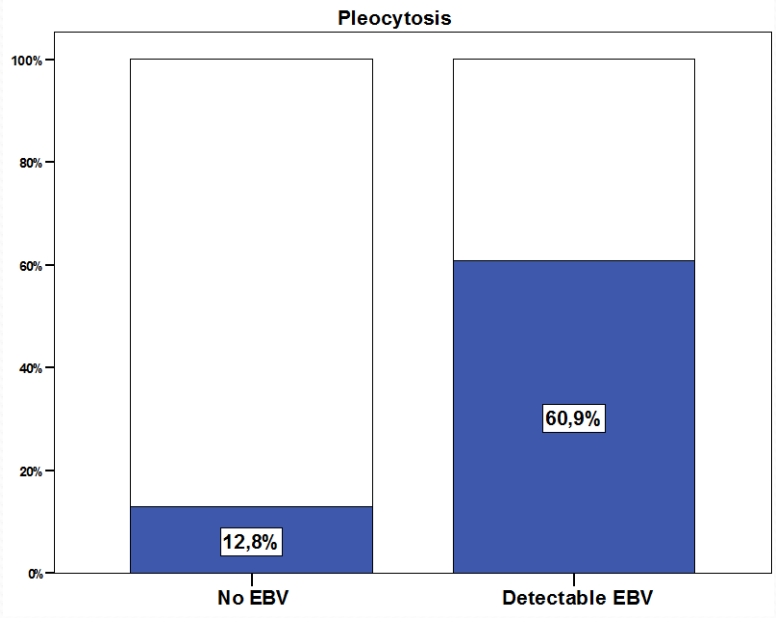


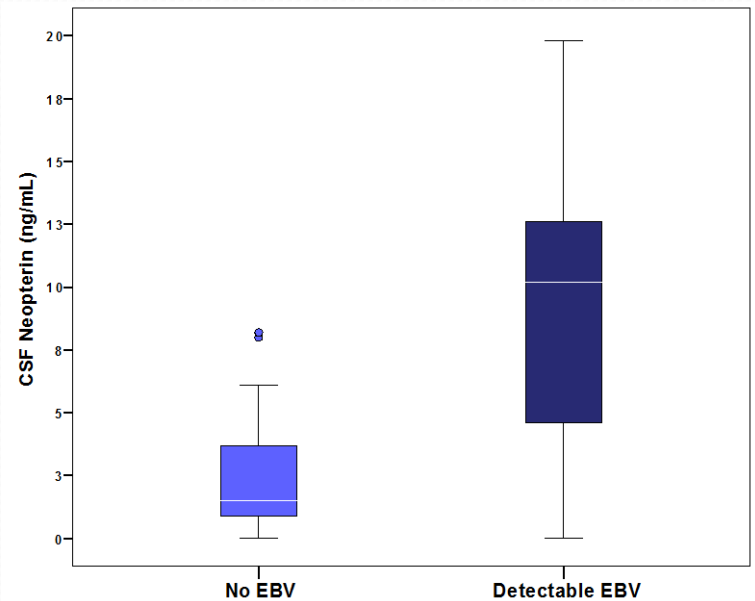
Naive	Detectable EBV-DNA	Undetectable EBV-DNA	p-values
Age (years)	40.8 (31.0-44.1)	42.4 (35.7-46.7)	n.s.
Current CD4+ (cell/uL)	84 (48-151)	80 (34-231)	n.s.
Nadir CD4+ (cell/uL)	63 (25-101)	63 (23-144)	n.s.
Plasma HIV-RNA (Log_{10} copies/mL)	5.0 (3.7-5.7)	5.3 (4.7-5.9)	n.s.
CSF HIV-RNA (Log_{10} copies/mL)	**4.4 (3.3-5.4)**	**3.6 (2.6-4.3)**	**0.010**
CSF to plasma ratio (Log_{10} copies/mL)	**0.9 (0.7-1.0)**	**0.7 (0.5-0.8)**	**0.025**
CSF discordance	**13.6%**	**3.4%**	**0.095**

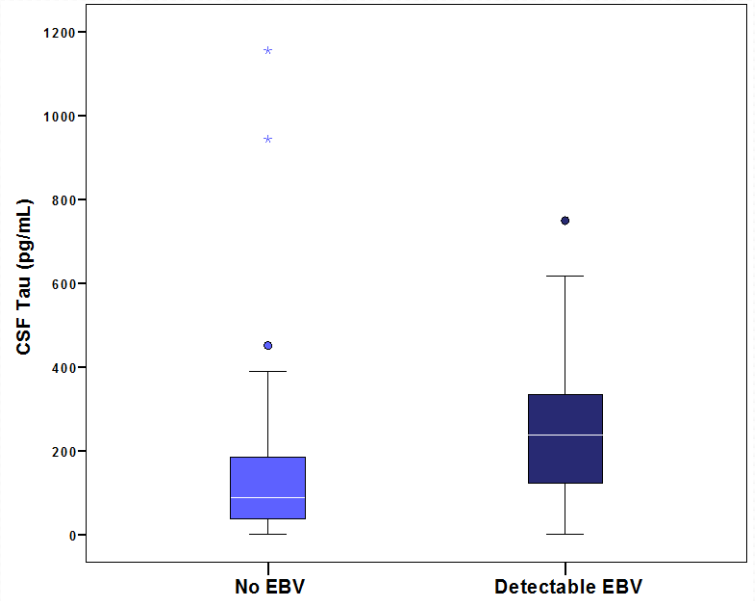
Naive	Detectable EBV-DNA	Undetectable EBV-DNA	p-values
CSAR	5.9 (4.0-8.7)	6.4 (4.25-10.4)	n.s.
Pleocytosis (>5 cell/mm3)	60.9%	12.8%	<0.001
IgG index (mg/dL)	0.4 (0.2-0.7)	0.4 (0.2-0.8)	n.s
Tau (pg/mL)	237 (127.6 – 343.4)	87 (37.5 – 184.7)	0.001
p-Tau (pg/mL)	37.5 (21.8 – 45.7)	25 (17.0 – 37.0)	0.021
1-42 Beta amyloid (pg/mL)	788.5 (435.4-1015.2)	804.0 (520.6-1142.7)	n.s.
Neopterin (pg/mL)	10.2 (3.8 -15.8)	1.5 (0.9 – 3.7)	<0.001
S-100 beta (pg/mL)	226 (140-288.6)	140 (52.7-237.5)	n.s.

Naïve subjects





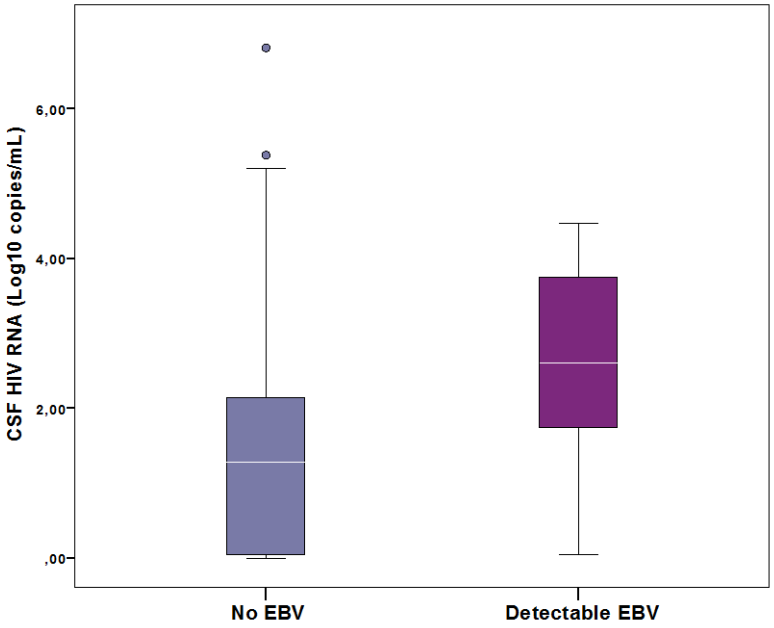


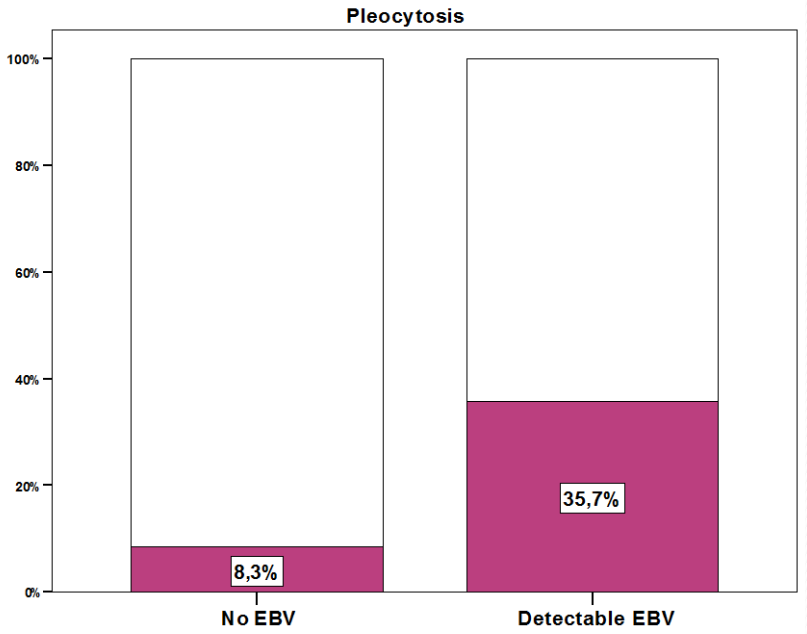


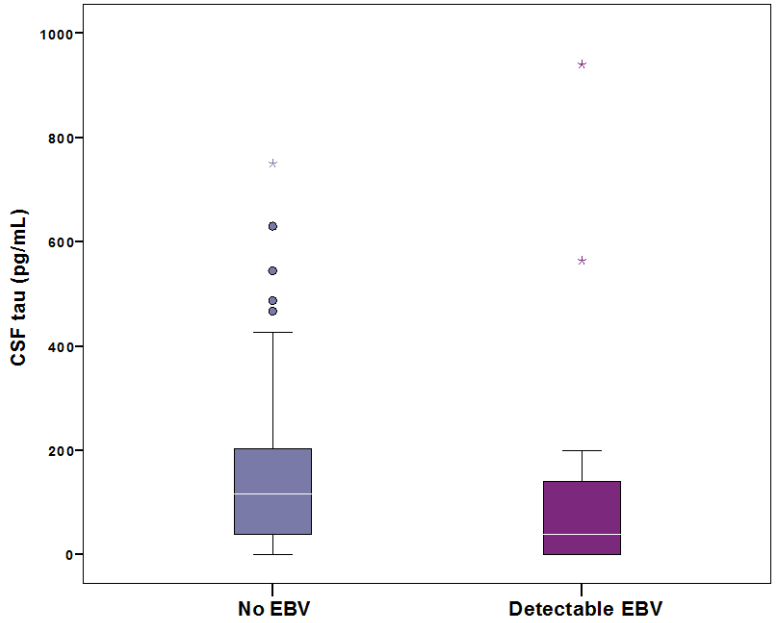
Treated	Detectable EBV-DNA	Undetectable EBV-DNA	P-values
Age (years)	45.8 (40.7-56.6)	46.3 (39.1-55.4)	n.s.
Current CD4+ (cell/uL)	**116 (35-400)**	**287 (114-554)**	**0.015**
Nadir CD4+ (cell/uL)	31 (12-131)	50 (15-203)	n.s.
Plasma HIV-RNA (Log_{10} copies/mL)	2.6 (1.4-4.1)	1.3 (0.2-2.0)	n.s.
CSF HIV-RNA (Log_{10} copies/mL)	**2.6 (1.7-3.8)**	**<1.3 (0.0-2.1)**	**<0.001**
CSF to plasma ratio (Log_{10} copies/mL)	1.0 (0.7-1.6)	1.0 (0.4-1.5)	n.s.
CSF escape	25.9%	23.3%	n.s.

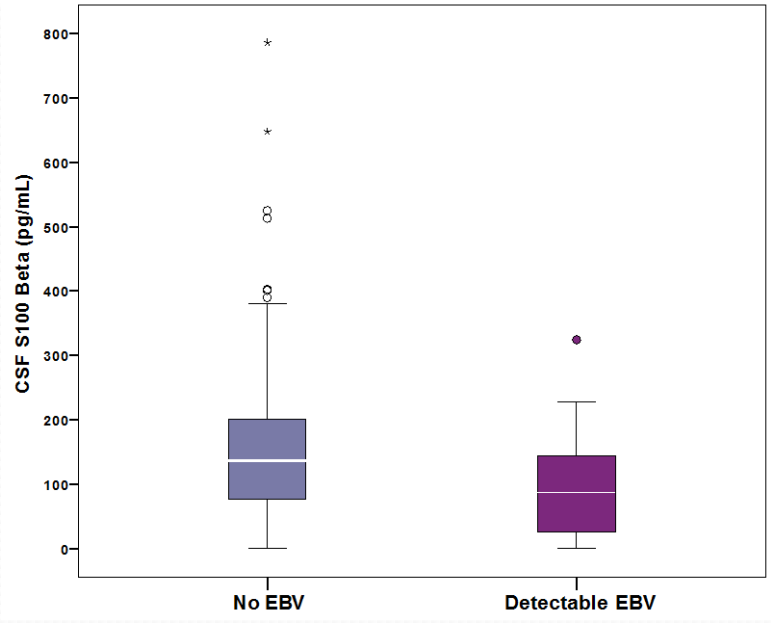
Treated	Detectable EBV-DNA	Undetectable EBV-DNA	P-values
CSAR	6.2 (4.8-11.2)	5.0 (3.7-7.0)	p=0.007
Pleocytosis (>5 cell/mm3)	35.7 %	8.3%	p<0.001
IgG index (mg/dL)	0.4 (0.2-0.7)	0.3 (0.2-0.5)	n.s
Tau (pg/mL)	37.5 (87-146)	116 (37.5-203.0)	p=0.015
p-Tau (pg/mL)	25.5 (15-35.5)	32.2 (21.0-41.7)	p=0.018
1-42 Beta amyloid (pg/mL)	666.8 (87-855.7)	869.7 (632.3-1059)	n.s.
Neopterin (pg/mL)	1.1 (0.0 -4.1)	0.7 (0.4 – 1.4)	n.s.
S-100 beta (pg/mL)	87.7 (18.0-158)	136.9 (73-200.6)	p=0.023

Treated subjects









Conclusions

- EBV was detected in a significant proportion of naïve (20.7%) and treated HIV-positive patients (17.7%)
- Naive patients with positive CSF EBV DNA, showed:
  - **pleocytosis,**
  - **higher CSF HIV RNA** and **CSF to plasma HIV-RNA ratios**.
  - higher values of **Tau, p-Tau** and **Neopterin**
- Treated patients with detectable EBV-DNA showed:
  - **pleocytosis** and higher **CSAR**
  - higher **CSF HIV-RNA**
  - lower levels of **Tau**, **p-Tau**, **S-100 Beta**
- The rate of CSF escape was similar in naïve and treated patients

Discussion (I)

- **EBV could create a BBB breach**: trafficking of infected cells across BBB enhanced **through up-regulating** of some mediators and receptors, during EBV reactivation in endothelials cells.
  - **Pleocytosis** is found in naïve and treated EBV-positive patients(60.9% vs. 35.7%)
  - **CSAR was** significatively higher in treated EBV+ subjects (6.2 vs 5.0) as well as higher **EBV DNA values**
    - it could relate to a greater barrier damage?

- EBV primary infection and reactivation could increase CNS HIV replication/trafficking **(secondary escape?)**
  - CSF HIV- RNA is significantly higher both in naïve EBV+ pts (4.4 vs 3.6 Log_{10} copies/mL) and treated pts (2.6 vs 1.3 Log_{10} copies/mL).
  - CSF to plasma HIV RNA ratio results higher in naïve (0.9 vs 0.7 Log_{10} copies/mL) with detectable DNA.

Discussion (II)

- Which is the role of EBV in the **direct infection** of neuronal cells and in the **indirect** neuronal damage?
  - Unexpected finding of lower tau, p-tau and S100beta in treated patients with detectable CSF EBV DNA

- **T-regs** down-regolation and, on the other hand, increase in **CD4+** and **CD8+** could unmask some autoinflammatory and autoimmune reactions involving CNS
  - Further studies on immune phenotype in patients with pleocytosis
  - CSF EBV longitudinal analysis (patients with symptomatic CSF escape?)

New Horizons ?

REVIEW

Epstein–barr virus vaccines

Jeffrey I Cohen

Table 2 Human trials of EBV vaccines

Vaccine	*Adjuvant*	*Results*
Prophylactic		
Vaccinia-gp350	None	Induced neutralizing antibody and may have reduced infection[35]
Recombinant gp350	None, alum, or alum/MPL	Induced neutralizing antibody[36]
Recombinant gp350	Alum/MPL	Induced neutralizing antibody; reduced rate of infectious mononucleosis, but not infection[37]
Recombinant gp350	Alum	Induced transient neutralizing antibody in a minority of patients with chronic kidney disease; did not prevent EBV post-transplant lymphoproliferative disorder[38]
EBNA-3A peptide	Tetanus toxoid in oil and water emulsion	Trend toward reduction of infectious mononucleosis but not infection[39]
Therapeutic		
Modified vaccinia Ankira expressing LMP2 and a portion of EBNA-1	None	T-cell responses to LMP2 or EBNA-1 detected in 15 of 18 NPC patients with a three to fourfold increase in T-cell responses[55]
Modified vaccinia Ankira expressing LMP2 and a portion of EBNA-1	None	T-cell responses to LMP2 or EBNA-1 detected in 8 of 14 NPC patients[56]
Autologous dendritic cells pulsed with LMP2 peptides	None	Boosted CD8 T-cell responses to LMP2 in NPC patients; tumor regression observed in two of nine patients[57]
Autologous dendritic cells transduced with adenovirus expressing LMP2 and a portion of LMP1	None	Induced LMP-specific delayed type hypersensitivity responses in 75% of NPC patients, but no increase in LMP1 or LMP2-specific T cells; transient partial response in 1 of 16 patients, and stable disease in 2[58]

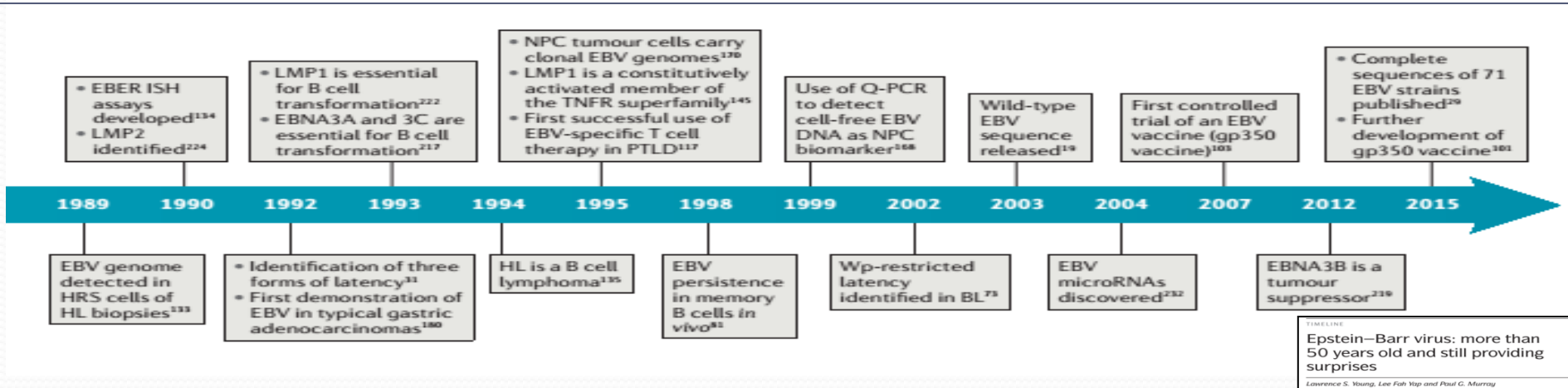
Abbreviations: EBNA, Epstein–Barr virus nuclear antigen; EBV, Epstein–Barr virus; LMP1, latent membrane protein 1; MPL, monophosphoryl lipid A; NCP, nasopharyngeal carcinoma patients.

Q-PCR assays for five EBV regions
(LMP1, LMP2, BAMH1W, EBNA1, and BZLF1):

- optimize detection
- minimizing false-negative results (related to partial genomic deletion)

Variation in risk and outcomes of Epstein–Barr virus-associated breast cancer by epidemiologic characteristics and virus detection strategies: an exploratory study

Sally L. Glaser[1,2] · Alison J. Canchola[1] · Theresa H. M. Keegan[1,3] · Christina A. Clarke[1,2] · Teri A. Longacre[4] · Margaret L. Gulley[5]



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Molecular Biology LAB

Maria Grazia Milia
Valeria Ghisetti

Neurobiology LAB

Cristiana Atzori
Alessandra Romito