Epstein-Barr Virus May Contribute to Central Nervous System Involvement in HIV-positive

Individuals

Lupia T^{1#}, Milia MG², Atzori C³, Audagnotto S¹, Imperiale D³, Mighetto L⁴, Pirriatore V¹, Gregori G²,
Lipani F¹, Ghisetti V², Bonora S¹, Di Perri G¹, Calcagno A¹.

1 Unit of Infectious Diseases, Department of Medical Sciences, University of Torino, Torino, Italy

*2 Laboratory of Virology and Molecular Biology, Ospedale Amedeo di Savoia, ASL "Città di Torino",
Torino, Italy*

3 Unit of Neurology, Ospedale Maria Vittoria, ASL "Città di Torino", Torino, Italy

4 Laboratory of Immunology, Ospedale Maria Vittoria, ASL "Città di Torino", Torino, Italy.

Running Head: EBV and HIV in the CSF

#Address correspondence to Tommaso Lupia, Tommaso.lupia89@gmail.com

*Present address: Tommaso Lupia, Unit of Infectious Diseases, Department of Medical Sciences,
University of Torino, Amedeo di Savoia Hospital, C.so Svizzera 164, 10149 Torino, Italy

Type of article: Original article

Key words: EBV; HIV; central nervous system; blood brain barrier; trafficking; biomarkers; S-100
beta.

Word count: Total 2097 (Abstract 250)

Figures: 3

Tables: 2

Conflicts of Interest and Source of Funding: AC has received honoraria from Abbvie, BMS, Gilead,
Janssen-Cilag, MSD, Viiv and he is currently receiving research grants from BMS, Gilead and Viiv.
GDP and SB have received honoraria from Abbvie, BMS, Gilead, Janssen-Cilag, MSD, Viiv. The
remaining authors have no conflict of interest to declare.

38 39 **Abstract**

40 Epstein-Barr virus (EBV) often accesses the central nervous system (CNS) where it may lead to blood
41 brain barrier (BBB) integrity disruption, facilitating the migration of immune cells into brain
42 parenchyma. Our aim was to study the association between cerebrospinal fluid (CSF) EBV DNA and
43 HIV-1 compartmental replication. 281 HIV-positive adults undergoing lumbar punctures for clinical
44 reasons (excluding those with lymphoproliferative disorders) and CSF samples were examined. CSF
45 virological, neurodamage (tau, p-tau, 1-42 beta amyloid) and immune activation (neopterin and
46 S100beta) markers were measured by immune-enzymatic, ELISA and PCR validated methods. Two
47 hundred eighty one patients were included; 111 (40.5 %) were naïve for antiretroviral treatment. CSF
48 EBV DNA was detectable in 25 (21.9%) naïve and 26 (16%) treated patients at low levels (<100 and
49 146 copies/mL). Naïve EBV+ subjects presented higher CSF HIV RNA, biomarkers (t-tau, p-tau,
50 neopterin) and higher rates of pleocytosis. Treated EBV+ individuals showed pleocytosis, higher CSF
51 HIV RNA, CSF to serum albumin ratio, IgG index and neopterin. No association was observed
52 between detectable CSF EBV DNA and the rate of CSF escape. In patients with plasma HIV RNA <20
53 copies/mL (n=97) CSF EBV DNA was detectable in 13 subjects (13.4%) and it was associated with
54 pleocytosis, higher CSF HIV RNA and neopterin levels. EBV DNA was detectable in a considerable
55 proportion of HIV-positive patients and it was associated with higher levels of CSF HIV RNA and
56 neuronal damage/inflammation biomarkers. The role of EBV reactivation in HIV-associated CNS
57 disorders warrant further studies.

58 **Importance**

59 EBV is a human gamma-herpesvirus with a seroprevalence in adults approaches 95% and the pattern of
60 clinical manifestations is very heterogeneous and varies from asymptomatic or mild viral infection to a

tightly linked with several malignancies as nasopharyngeal carcinoma, Hodgkin's lymphoma and Burkitt's lymphoma. HIV-infected and immunocompetent patients were both at risk of primary infection and complications linked to EBV.

Primary tropism of EBV is for lymphocytes (type B, T and NK), epithelial, endothelial and smooth muscle cells and establishes lifelong latent infection. Central nervous system could be affected by this herpesvirus in primary infection and reactivation and EBV-DNA is not an uncommon finding in CSF in HIV-infected population. The significance of our research is in identifying the presence of a link between HIV and EBV CNS replication.

Introduction

Epstein-Barr virus (EBV) or Human Herpesvirus 4 (HHV-4) is a widely disseminated gamma herpesvirus capable to persist, lifelong and asymptomatically, in a latent infection in adults [1]. Transmission of EBV is so efficient that by adulthood most (> 95%) of the world's population has been infected [2].

EBV may affect the central nervous system (CNS) and clinical manifestations were first noted in 1931 by Epstein and Johansen. Both primary infection and reactivation can cause neurological diseases and central nervous system involvement occurs in 1 to 10% of the cases [3]. Individual state of immunocompetence, age and comorbidities have been associated with the occurrence of neurological complications that include: meningo-encephalites, cerebellitis, optic neuritis, cranial nerve palsy, peripheral neuropathy, Alice in Wonderland syndrome, ataxia, chorea, post-infectious autoimmune disorders, including Guillain-Barrè syndrome, acute disseminated encephalomyelitis (ADEM) and transverse myelitis [3][4][5].

Biopsy proven vasculitis due to EBV infection have been reported: perivascular inflammatory infiltrate was dominantly composed of CD3+ and CD8+ T-lymphocytes and macrophages. Some of the CD3 positive cells were also EBV-encoded RNA-1 (EBER1) positive, one of the two small noncoding RNAs (EBER1 & 2) found in latently EBV infected cells [6][7][8].^[11]_{SEP}

In MS patients' brains, EBV infection in B cells seems to alters the ability of B cells to process and present a pathogenetically relevant myelin autoantigen and expression of higher levels of costimulatory molecules than healthy controls, suggesting an enhanced APC function of B cells in MS brains, leading to an higher autoimmune risk [9]. EBV-positive B lymphocytes count in normal human brain is very low, but is shown an higher cell number in HIV infected brains. In PCR-based studies and in situ hybridization studies were shown a detection of EBV in both lymphomatoid tissue and in pleomorphic lymphoid infiltrates [10].

Several works have established that EBV can infect macrovascular endothelial cells in human tissue [11] [12], human brain microvessels [13] and in culture with human umbilical vein endothelial cells (HUVECs) [14] [15]. In endothelial cells with lytic reactivation of EBV were found an increase production of pro- inflammatory molecules (CCL-2 and CCL-5) and also hyper-expression of adhesion molecules on surfaces (ICAM-1 and VCAM-1) with a potential creation of an inflammatory breach through the Blood Brain Barrier (BBB) [13] [14] [15] [16]

HIV-positive patients have a higher risk of EBV-associated diseases due to reduced immune surveillance; non-Hodgkin and Burkitt's lymphomas are among AIDS-defining conditions. Highly Active Antiretroviral Treatment (HAART) has dramatically reduced the incidence of HIV-associated dementia but milder forms of cognitive impairment as well as cerebrospinal fluid (CSF) HIV escaper persist despite treatment. Beside incomplete CSF antiretrovirals' penetration/effectiveness HIV escape may be due to enhanced blood brain barrier (BBB) permeability and secondary to other concomitant

infections (“secondary escape”) [17]. We aimed at studying the role of CSF EBV DNA in HIV-positive subjects in terms of HIV replication, BBB damage and biomarkers of neuronal damage/inflammation.

Material and Methods

Adult HIV-positive patients undergoing lumbar punctures for clinical reasons, were enrolled. Patients with primary central nervous system lymphomas (PCNSLs), lymphoproliferative diseases (Lds) and autoimmune disorders were excluded. Demographic, immunovirological, clinical and therapeutic data were recorded as well as CSF features. The protocol was approved by our Ethics Committee (Comitato Etico Interaziendale di Orbassano, n. 103/2015). Patients signed a written informed consent at enrollment.

HIV RNA was measured through the real time Polymerase Chain Reaction (PCR) assay CAP/CTM HIV-1 vs. 2.0 (CAP/CTM, Roche Molecular System, Branchburg, NJ, detection limit: 20 copies/mL of HIV-1 RNA). EBV DNA was measured through the real time Polymerase Chain Reaction (PCR) (detection limit: 100 copies/mL of EBV DNA).

Quantitative determination of albumin in serum and CSF was measured by Immunoturbidimetric methods (AU 5800, Beckman Coulter, Brea, CA, USA). CSAR, calculated as CSF albumin (mg/L)/serum albumin (g/L), was used to evaluate BBB function. BBB damage definition was derived from age-adjusted Reibergrams (normal if below 6.5 in patients aged <40 years and below 8 in patients >40 years).

CSF total tau (t-tau), phosphorylated tau (p-tau) and β -amyloid1-42 ($A\beta$ 1-42) were measured by immunoenzymatic methods (Fujirebio diagnostics, Malvern, U.S.A.) with limits_{SEP} of detection respectively of 57, 20 and 225 pg/ml. Neopterin and S100B were measured through validated ELISA methods [DRG Diagnostics (Marburg, Germany) and DIAMETRA S.r.l. (Spello, Italy), respectively].

Reference values were as follows: t-tau [<300 pg/mL (in patients aged 21–50), <450 pg/mL (in patients aged 51–70) or <500 pg/mL in older patients], p-tau (<61 pg/mL), 1–42 beta amyloid (>500 pg/mL), neopterin (<1.5 ng/mL) and S100B (<380 pg/mL) [18].

HIV RNA was measured through the real time Polymerase Chain Reaction (PCR) assay CAP/CTM HIV-1 vs. 2.0 (CAP/CTM, Roche Molecular System, Branchburg, NJ, detection limit: 20 copies/mL of HIV-1 RNA). EBV DNA was measured through the real time Polymerase Chain Reaction (PCR) (detection limit: 150 copies/mL of EBV DNA).

Data were analyzed using standard statistical methods: variables were described with medians [interquartile ranges (IQR) or ranges (minimum-maximum)] and they were compared using non-parametric tests (Mann–Whitney, Kruskal-Wallis and Spearman’s tests as specified in the text). Data analysis was performed using PASW software version 22.0 (IBM).

Results

Two hundred and eighty one adult patients were included. 111 (40.5 %) patients were naïve for combination antiretroviral treatment (cART); baseline and immune-virological characteristics, stratified by cART use, are shown in Table 1. Lumbar punctures were performed before starting antiretroviral treatment in naïve late presenting subjects ($CD4^+$ T lymphocytes $<100/uL$) or in symptomatic treated patients with cognitive disorders, headache or other neurological complaints.

CSF EBV DNA was detectable in 25 naïve (21.9%) and 26 treated (16%) patients with median values of <100 ($<100-234$) and <100 ($<100-612$) copies/mL respectively. Virological, neuronal damage and inflammation biomarkers stratified by cART use and CSF EBV detection are shown in Table 2.

149 Naïve patients with detectable EBV DNA had higher CSF HIV viral load (4.2 vs. 3.7 log₁₀ copies/mL,
150 p=0.010); CSF to plasma HIV RNA ratios (25 vs. 4%, p=0.025), higher rates of pleocytosis (52% vs.
151 12.6%, p<0.001), CSF neuronal damage biomarkers t-tau (233 vs. 114 pg/mL, p=0.002), p-tau (38 vs.
152 29 pg/mL, p=0.051) and neopterin (9.25 vs. 2.02 ng/mL, p=0.001) (Figure 1 and 2, above).

153 Treated patients with detectable EBV DNA had lower CD4 cell counts (136 vs. 287 cell/uL, p=0.015)
154 and higher CSF HIV RNA (2.1 vs. <1.3 log₁₀ copies/mL, p<0.001), higher levels of pleocytosis (40 vs.
155 8.3%, p<0.001), CSAR (6.1 vs. 5.1, p=0.011), IgG index (4.7 vs. 3.8, p=0.042) and neopterin (1.79 vs.
156 0.81 ng/mL, p=0.009) (Figure 1 and 2, below). Conversely 1-42 beta amyloid was lower in EBV-
157 positive individuals (788 vs. 893 pg/mL, p=0.007).

158 The rate of CSF escape was similar in EBV-positive naïve (8.3% vs. 4.5%, p=0.607) and treated
159 patients (28% vs. 23.3%, p=0.615).

160 In plasma controllers (HIV RNA <20 copies/mL, n=97) CSF EBV DNA was detectable in 13
161 individuals (13.4%): it was associated with pleocytosis (50% vs. 8.4%, p=0.001), higher CSF HIV
162 RNA in those with detectable viral load (2.28 vs. 1.85 Log₁₀ copies/mL, p=0.011) and higher CSF
163 neopterin levels [2.81 ng/mL vs. 0.77 ng/mL, p=0.012] (Figure 3). In CSF controllers (CSF HIV RNA
164 <20 copies/mL, n=84) CSF EBV DNA was detectable in 10 individuals (11.9%): it was associated with
165 pleocytosis (66.6% vs. 6.9%, p<0.001) and border-line higher CSF neopterin (3.74 vs. 0.78, p=0.06)
166 (Figure 3).

167

168 **Discussion**

169 In this study EBV DNA was detectable at low concentrations in up to 21% of HIV-positive patients
170 and it was associated with higher CSF HIV viral load and up to three-time higher rate of pleocytosis.

171 Such prevalence decreased in treated patients although in 13.4% of those with undetectable plasma
172 viral load EBV was still detectable and associated with worse compartmental virological and
173 immunological biomarkers.

174 Several studies reported the role of EBV in immunocompromised patients and they showed that the
175 detection of EBV DNA in the CSF is a good marker of primary central nervous system lymphoma
176 (PCNSL, with sensitivity and specificity of 70% and 80%) [19] [20] [21] [22]. Some studies reported
177 the development of PCNSL in HIV-infected EBV-positive patients [23] [24] [25] [26].

178 Weinberg and coll. reported in some patients the presence of pleocytosis, detectable CSF EBV DNA
179 and EBV related-mRNA supporting the hypothesis that EBV DNA is not carried by latently infected
180 inflammatory cells but from actively replicating virus [27]. Furthermore EBV affects the immune
181 system and it may enhance neuronal degeneration in chronic inflammatory conditions. [13][28]. Higher
182 rates of B-amyloid protein and neurofibrillary tangles have been observed in the brains of EBV-
183 positive patients diagnosed with Alzheimer's dementia as compared to controls [29]. EBV may cause a
184 sub-clinical chronic infection and facilitate inflammatory cells' trafficking through the BBB thus
185 increasing HIV entrance into the CNS. [30][31]. Our data seem to confirm this hypothesis since both
186 naïve and treated patients present higher CSF HIV viral loads (and CSF to plasma HIV RNA ratios)
187 and white blood cells. Additionally cART-treated patients with detectable CSF EBV DNA showed
188 higher CSF to albumin ratios supporting a potential role in the persistence of BBB damage. The latter
189 has been shown to be a prevalent feature of patients with dementia, to persist in some subjects despite
190 treatment and to be associated with markers of neuronal damage and inflammation [32][33][34].
191 Hosting EBV astrocytes, and microglia, theoretically could create a wide net of cell-to-cell crosstalk
192 encouraging migration of monocytic/macrophagic line cells and modifying CNS physiological
193 homeostasis [35][36][37]. This effect may be independent from HIV control and immune system

194 improvement: these conditions have been associated with the absence of neuronal damage and with the
195 lowest CSF concentrations of neopterin [38][39][40].

196 In a recent study that analyzed 108 gut biopsies collected from 19 HIV-infected and 22 HIV-
197 uninfected^[SEP] participants, CMV and EBV were detected in more than 70% of samples but more
198 commonly in HIV-positive subjects [41]. ^[SEP]While the negative effects of sporadic or continuous CMV
199 replication are well-known, there is still uncertainty on the role of EBV in chronic immune activation.

200 Additionally EBV may have a role in suppressing the CNS immune system and therefore maintain an
201 incomplete T-cell mediated inflammatory response; this may be achieved through the expression of
202 viral genes encoding for proteins with immunoevasin-like function. This may translate into higher rates
203 of pleocytosis but with less inflammatory activity [42][43]. On the other hand our EBV-positive treated
204 patients showed lower CD4 counts thus suggesting that immune control may be needed in order to
205 restore a partial control on EBV low level replication.

206 Some limitations of this study should be acknowledged including the low sample size, the lack of a
207 control group and the lack of plasma EBV DNA measurements. Additionally our cohorts include
208 several patients with very low nadir CD4 cell counts and heterogeneous clinical conditions: the same
209 effect may not be observed in less advanced individuals.

210 In conclusion we reported for the first time the prevalence of EBV detection in the CSF of HIV-
211 positive patients without lympho-proliferative disorders. Besides we observed that naïve subjects with
212 detectable CSF EBV DNA had a higher HIV viral load and higher markers of neuronal damage and
213 inflammation; in treated individuals despite a higher HIV viral load we report a higher prevalence of
214 blood brain barrier damage, pleocytosis and immune activation. Further studies are warranted for
215 understanding the contribution of EBV to HIV-associated CNS disorders.

216

217

218

219

220

221

222

223 **Tables**

224 **Table 1: Baseline characteristics**

	Naive	Treated
n	111	170
Age: years	44 (37.5-48)	48 (42.3-56.1)
Male gender: n (%)	78 (70.7%)	117 (69.1%)
BMI: Kg/m ²	24.5 (19,6 – 25,1)	22.8 (20-25.2)
CD4: Cells/uL	67 (27 – 157)	319 (116 – 570)
CD4 nadir: Cells/uL	64 (25 – 141)	46 (13 – 194)
plasma VL: Log ₁₀ copies/mL	5.3 (4.9 - 5.9)	1.3 (<1.3 – 1.9)
CSF VL: Log ₁₀ copies/mL	3.9 (2.9 - 4.7)	1.4 (<1.3 – 2.1)
Time with HIV-positive test: months	78 (47 – 205)	197 (95 – 290)
ARV classes: NNRTI	-	43
PI	-	106
INSTI	-	45
MVC	-	16
Indication for LP: Late Presentation	48	49
Opportunistic Infection	24	32
HAND	16	40
Syphilis	1	5
Follow up	0	0
Headache	0	2
CSF EBV detectable: n (%)	25 (21.9%)	

CSF EBV DNA in those with detectable levels: copies/mL	<100 (< 100 – 292)	26 (16%) 141 (< 100 - 448)
---	--------------------	-------------------------------

225

226 **Table 1:** Demographic, immunovirological and clinical variables of subjects at baseline. “BMI” body
227 mass index, “VL” viral load, “CSF” cerebrospinal fluid, “HAART” Highly Active Antiretroviral
228 Treatment, “NNRTI” non-nucleoside reverse transcriptase inhibitor, “PI” protease inhibitor, “INSTI”
229 integrase strand-transfer inhibitor, “MVC” Maraviroc, “HAND” HIV-1 associated neurocognitive
230 disorder.

231

232

233 **Table 2 Cerebrospinal fluid biomarkers characteristics of the four groups**

	Naive			Treated		
	CSF EBV-	CSF EBV+	P value	CSF EBV-	CSF EBV+	P value
CSF cells: n/uL	0 (0-3)	10 (0-43)	<0.001	0 (0-3)	0 (0-40)	<0.001
CSF pleocytosis	12.6%	52%	<0.001	8.6%	40%	<0.001
CSAR	5.9 (4 – 8.7)	7.2 (4.1 – 11.2)	n.s.	5.1 (3.7-7.0)	6.1 (5.2-10.6)	0.008
BBBi	41.2%	47.8%	n.s.	28.3%	45.8%	0.090
IgG index	0.4 (0.2-0.7)	0.5 (0.2-0.8)	n.s.	0.4 (0.2-0.6)	0.5 (0.3-0.8)	0.020
t-tau: pg/mL	114 (67.7-202)	233 (130-334)	0.001	130.1 (58-212)	62 (37.5-153)	n.s.
p-tau: pg/mL	29 (21.6-39.2)	38 (31-44.7)	0.034	35 (25-41.8)	28.9 (21.5-37.5)	n.s.
Aβ1-42: ng/mL	904 (643 – 1105)	839 (584 – 1181)	n.s.	893 (748-1075)	788 (636-882)	0.037
Neopterin: ng/mL	2.02	9.25	<0.001	0.81	1.79	0.070

	(1.1 – 3.8)	(3.3-12.1)		(0.5-1.4)	(0.6-4.3)	
S100B: pg/mL	150.7 (87 - 242)	188.4 (96 – 279)	n.s.	140 (85-206)	54 (37.5-210)	n.s.

234

235 Table 2: Immunoactivation and neurodamage markers, stratified according to presence or not of EBV

236 DNA on CSF and cART. “CSF” cerebrospinal fluid, “CSAR” Cerebrospinal fluid Serum Albumin

237 Ratio, “BBBi” Blood Brain Barrier impairment, “t-tau” total tau, “p-tau”phosphorylated tau, “Aβ1-42”

238 1-42 beta amyloid, “S100B” S100 Beta. “n.s.” non significant (p values >0.05).

239

240

241

242 **Figure 1**

243

244

Figure 1: Cerebrospinal fluid HIV RNA (A), pleocytosis (B) and cell numbers (C) in naïve (above) and treated patients (below). “CSF”, cerebrospinal fluid; “CSF EBV+”, detectable CSF EBV DNA.

Figure 2

n.s.

$p=0.001$

n.s.

$p=0.001$

Figure 2: Cerebrospinal fluid biomarkers concentrations in naïve (above) and treated patients (below); cerebrospinal fluid to serum albumin ratio (A), total tau (B), 1-42 beta amyloid (C) and neopterin (D). “CSF”, cerebrospinal fluid; “CSF EBV+”, detectable CSF EBV DNA, “CSAR”; CSF to serum albumin ratio. All scatter dot plots present a central bar (median) with lateral error bars (IQR). In all graphs white dots represent observations in the EBV-negative group (left) instead black dots picture observations in EBV-positive group (right).

Figure 3

P=0.001

P=0.011

p=0.012

Figure 3: Cerebrospinal fluid pleocytosis (A), HIV RNA (B) and neopterin (C) in patients with plasma HIV RNA < 20 copies/mL. “CSF”, cerebrospinal fluid; “CSF EBV+”, detectable CSF EBV DNA

1. Calderwood MA, Venkatesan K, Xing L, Chase MR, Vazquez A, Holthaus AM, Ewence AE, Li N, Hirozane-Kishikawa T, Hill DE, Vidal M, Kieff E, Johannsen E (2007) Epstein-Barr virus and virus human protein interaction maps. *Proc Natl Acad Sci USA* 104: 7606–7611
2. Luzuriaga K, Sullivan L. Infectious mononucleosis. *N Engl J Med*. 2010;362:1993–2000
3. Abul-Kasim K, Palm L, Maly P, Sundgren PC. The neuroanatomic localization of Epstein-Barr virus encephalitis may be a predictive factor for its clinical outcome: a case report and review of 100 cases in 28 reports. *J Child Neurol*. 2009;24:720-726
4. Doja A, Bitnun A, Lee E, . Pediatric Epstein–Barr virus-associated encephalitis – 10-year review. *J Child Neurol* 2006; 21(5): 384–391
5. Kleinschmidt-DeMasters BK, Damek DM, Lillehei KO, Dogan A, Giannini C. Epstein-Barr virus-associated primary CNS lymphomas in elderly patients on immunosuppressive medications. *J Neuropathol Exp Neurol*. 2008;67:1103–11
6. Kobayashi Z, Tsuchiya K, Takahashi M et al. An autopsy case of chronic active Epstein–Barr virus infection (CAEBV): Distribution of central nervous system (CNS) lesions. *JNeurolSci*2008; 275:170–7
7. Jha HC, Mehta D, Lu J, El-Naccache D, Shukla SK, Kovacsics C, Kolson D, Robertson ES. 2016. Gammaherpesvirus infection of human neuronal cells. *mBio* 6(6):e01844-15. doi:10.1128/mBio.01844-15
8. Kano K.,Katayama T., Takeguchi S., Asanome A.,Takahashi K., Saito T.,Sawada J., Saito M., Biopsy-proven case of Epstein–Barr virus (EBV)-associated vasculitis of the central nervous system, *Neuropathology* 2017; 37,259–264
9. Morandi E, Jagessar SA, 't Hart BA, Gran B. EBV infection empowers human B cells for autoimmunity: role of autophagy and relevance to multiple sclerosis. *J Immunol* (2017)
10. Anthony I. C., Crawford D. H., Bell J. E. (2003). B lymphocytes in the normal brain: contrasts with HIV-associated lymphoid infiltrates and lymphomas. *Brain* 126, 1058–1067
11. Ban, S., Y. Goto, K. Kamada, M. Takahama, H. Watanabe, T. Iwahori, and H. Takeuchi. 1999. Systemic granulomatous arteritis associated with Epstein-Barr virus infection. *Virchows Arch*. 434:249-254
12. Guarner J., Unger E.: Association of Epstein-Barr Virus in Epithelioid Angiomatosis of AIDS Patients, *American Journal of Surgical Pathology*. 14(10):956-960, October 1990
13. Casiraghi C, Dorovini-Zis K, Epstein-Barr virus infection of human brain microvessel endothelial cells: A novel role in multiple sclerosis, *Journal of Neuroimmunology* 2011 , Volume 230 , Issue 1 , 173 – 177
14. Jones K., Rivera C., Sgadari C., Franklin J., Max E.E., Bhatia K., Tosato G. Infection of human endothelial cells with Epstein-Barr virus. *J. Exp. Med*. 1995;182:1213–1221
15. Xiong A, Clarke-Katzenberg RH, Valenzuela G, Izumi KM, Millan MT. Epstein-Barr virus latent membrane protein 1 activates nuclear factor- κ B in human endothelial cells and inhibits apoptosis. *Transplantation*. 2004;78:41–9
16. Kanno H, Watabe D, Shimazu N et al. Adhesion of Epstein–Barr virus-positive natural killer cell lines to cultured endothelial cells stimulated with inflammatory cytokines. *Clin Exp Immunol* 2008; 151:519
17. Ferretti F., Gisslen M., Cinque P., Price RW:Cerebrospinal Fluid HIV Escape from Antiretroviral Therapy *Curr HIV/AIDS Rep* (2015) 12:280–288
18. Green AJ, Harvey RJ, Thompson EJ, Rossor MN (1997) Increased S100 β in the cerebrospinal fluid of patients with frontotemporal dementia. *Neurosci Lett* 235(1-2):5–8
19. De Luca A, Antinori A, Cingolani A, et al. Evaluation of cerebrospinal fluid EBV-DNA and IL-10 as markers for in vivo diagnosis of AIDS-related primary central nervous system lymphoma. *Br J Haematol*. 1995;90:844–849

20. Cinque, P, Vago L, Dahl H, et al. Polymerase chain reaction on cerebrospinal fluid for diagnosis of virus-associated opportunistic diseases of the central nervous system in HIV-infected patients. *AIDS* 1996;10:951–958 [SEP]
21. Bossolasco S, Cinque P, Ponzoni M, et al. Epstein-Barr virus DNA load in cerebrospinal fluid and plasma of patients with AIDS-related lymphoma. *J Neurovirol* 8: 432-438, 2002 [SEP]
22. Yanagisawa K., Tanuma J., Hagiwara S. et al.: Epstein-Barr Viral Load in Cerebrospinal Fluid as a Diagnostic Marker of Central Nervous System Involvement of AIDS-related Lymphoma, *Intern Med* 52: 955-959, 2013
23. Brink NS, Sharvell Y, Howard MR, Fox JD, Harrison MJ, Miller RF. Detection of Epstein-Barr virus and Kaposi's sarcoma-associated herpesvirus DNA in CSF from persons infected with HIV who had neurological disease. *J Neurol Neurosurg Psychiatry*. 1998;65:191–5
24. Cinque P, Brytting M, Vago L, et al. Epstein-Barr virus DNA in cerebrospinal fluid from patients with AIDS-related primary lymphoma of the central nervous system. *Lancet* 342: 398-401, 1993 [SEP]
25. Rojanawiwat A, Miura T, Thaisri H, Pathipvanich P, et al. Frequent detection of Epstein-Barr virus and cytomegalovirus but not JC virus DNA in cerebrospinal fluid samples from human immunodeficiency virus-infected patients in northern Thailand. *J Clin Microbiol*. 2005
26. Wang J, Ozzard A, Nathan M, Atkins M, Nelson M, Gazzard B, Bower M 2007. The significance of Epstein-Barr virus detected in the cerebrospinal fluid of people with HIV infection. *HIV Med* 8:306–311
27. Weinberg A. , Shaobing L., Palmer M., et al: Quantitative CSF PCR in Epstein-Barr virus infection of the central nervous system. *Ann Neurol* 2002;52:543—548
28. Mrass P, Weninger W.. Immune cell migration as a means to control immune privilege: lessons from the CNS and tumors. *Immunol Rev* (2006) 213:195–212
29. Xu S., Gaskin F., Increased incidence of anti-b-amyloid autoantibodies secreted by Epstein-Barr virus transformed B cell lines from patients with Alzheimer's disease *Mechanisms of Ageing and Development*, 94 (1997) 213–222
30. Hutt-Fletcher LM. Epstein–Barr virus entry. *Journal of Virology* 2007; 81: 7825–7832 [SEP]
31. Houldcroft CJ, Kellam P., Host genetics of Epstein–Barr virus infection, latency and disease, *Rev. Med. Virol*. 2015; 25: 71–84
32. Calcagno A, Atzori C, Romito A et al (2016) Blood brain barrier impairment is associated with cerebrospinal fluid markers of neuronal damage in HIV-positive patients. *J Neurovirol* 22(1):88–92
33. Anesten B, Yilmaz A, Hagberg L, et al. : Blood-brain barrier integrity, intrathecal immunoactivation, and neuronal injury in HIV. *Neurol Neuroimmunol Neuroinflamm*. 2016;3(6):e300
34. Carroll A, Brew B. HIV-associated neurocognitive disorders: recent advances in pathogenesis, biomarkers, and treatment. *F1000Res* 2017; 6:312
35. A. Menet, C. Speth, C. Larcher, W. M. Prodinger, M. G. Schwendinger, P. C. et al. (1999). Epstein-Barr Virus Infection of Human Astrocyte Cell Lines. *Journal of Virology*, 73(9), 7722–7733.
36. Daugvilaite, V., Arfelt, K. N., Benned-Jensen, T., Sailer, A. W., & Rosenkilde, M. M. (2014). Oxysterol-EBI2 signaling in immune regulation and viral infection. *European Journal of Immunology*, 44(7), 1904–1912. <https://doi.org/10.1002/eji.201444493>
37. Rutkowska, A., Preuss, I., Gessier, F., Sailer, A. W., & Dev, K. K. (2015). EBI2 regulates intracellular signaling and migration in human astrocyte. *Glia*, 63(2), 341–351. <https://doi.org/10.1002/glia.22757>

38. Dahl V, Low levels of HIV-1 RNA detected in the cerebrospinal fluid after up to 10 years of suppressive therapy are associated with local immune activation. *AIDS*. 2014;28:2251–2258
39. Chan P, Hellmuth J, Spudich S, Valcour V (2016) Cognitive impairment and persistent CNS injury in treated HIV. *Curr HIV/AIDS Rep* 13:209–217
40. Motta I., Allice T., Romito A., Calcagno A.:Cerebrospinal fluid viral load and neopterin in HIV-positive patients with undetectable viraemia., *Antivir Ther* 2017 Feb 15
41. Gianella S., Chaillon MA, Mutlu EA. : Effect of cytomegalovirus and Epstein–Barr virus replication on intestinal mucosal gene expression and microbiome composition of HIV-infected and uninfected individuals, *AIDS* 2017, 31:2059–2067
42. Tagawa T, et al. (2016) Epstein-Barr viral miRNAs inhibit antiviral CD4+ T-cell responses targeting IL-12 and peptide processing. *J Exp Med*, 10.1084/jem.2016024
43. Rensing, M.E., M. van Gent, A.M. Gram, M.J.G. Hooykaas, S.J. Piersma, and E.J.H.J. Wiertz. 2015. Immune evasion by Epstein-Barr virus. *Curr. Top. Microbiol. Immunol.* 391:355–381. ^[1]_{SEP}