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Differential Genotypic Evolution of HIV-1 Quasispecies in Cerebrospinal Fluid and Plasma: A Systematic Review

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Abstract

HIV-1 enters the central nervous system by passing the blood-brain barrier during primary infection. Despite the introduction of combination antiretroviral therapy, the prevalence of HIV-associated neurocognitive disorders remains high and is probably related to ongoing viral neuropathological processes. The central nervous system forms a distinct physiological, cellular, and pharmacological environment. We aimed to systematically review whether the central nervous system also constitutes a distinct virological compartment, allowing differential genotypic evolution of HIV-1. Only original research papers that compared paired plasma/cerebrospinal fluid samples for drug resistance associated mutations as defined by the IAS-USA Drug Resistance Mutations Panel or compared viral envelope (env) patterns or coreceptor prediction were included. If available, HIV RNA levels were included in the analysis, with a relevant difference defined as 0.5 log_{10} copies/ml. Data from 35 reports with heterogeneous study design and methods was pooled and statistical analysis was performed as appropriate. A total of 555 subjects with 671 samples could be included in this review. We observed that compartmentalization of the central nervous system occurs frequently as reflected by differences in HIV viral load, resistance associated mutations, and viral coreceptor tropism preference. **(AIDS Rev. 2013;15:152-61)**

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Key words

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Introduction

The central nervous system (CNS) and its surrounding cerebrospinal fluid (CSF) are separated from the circulatory system by the blood-brain barrier (BBB). During primary HIV infection, the integrity of the BBB is affected by viral components such as transcriptional activator (Tat) and the structural envelope glycoprotein 120 (gp120), enabling HIV to enter the CNS[1]. HIV infection also induces the release of proinflammatory cytokines, rendering the BBB more permeable. As such, leukocytes, including those which are HIV infected, can migrate towards chemoattractants released in the CNS[1]. Other proposed mechanisms of viral penetration of the BBB include HIV infection of endothelial cells and astrocytes[1,2].

Once HIV has entered the CNS it is situated in an environment distinct from blood and lymphoid tissue. Within the CNS, perivascular macrophages and microglia rather than T lymphocytes are the main source of infection[3-10]. Although it was originally thought that HIV variants using the CCR5 coreceptor mainly infect macrophages and microglia, it is now known that viruses using the CCR5 coreceptor can be either T-cell (T)-tropic or macrophage (M)-tropic, depending on the density of CD4 levels on the cell surface. Lower CD4 density is associated with M-tropism and higher CD4 densities with T-tropism[11]. Viruses using CXCR4 are most often T-tropic since the CXCR4 coreceptor is most abundantly

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present on T lymphocytes[12], although infection via CXCR4 of macrophages has been reported as well[13]. Additionally, alternative coreceptors such as CCR3 (in conjunction with CCR5) and CXCR6 may play a more pronounced role in CNS target cells than in peripheral cells[14]. These differences in CD4 and coreceptor expression set the boundaries in which M-tropic strains are preferentially replicating in the CNS and T-tropic strains in plasma and lymphoid tissue[15,16]. This implies that besides the V3 region, which determines coreceptor usage, other determinants within the viral envelope gene *(env)* also play an important role in determining neurotropism[17]. The specific viral characteristics determining neurotropism are unknown but are reflected by genotypic patterns in *env*[10,18], and possibly by *tat*[19] or other genes.

Differences in evolution between HIV quasispecies in CNS and plasma may be reinforced upon initiation of antiretroviral drugs, since not all antiretroviral drugs pass the BBB and enter the CSF in equal concentrations[20]. In an attempt to quantify and categorize these differences, the CNS Penetration Effectiveness (CPE) score has been introduced[21]. The CPE score is a measure of estimated penetration and effectiveness in the CNS. A higher CPE score is generally associated with more inhibition of HIV replication and subsequent lower viral loads in CSF[22]. The efficacy of antiretroviral drugs may also be influenced by differences in cell surface drug efflux transporters on macrophages and possibly microglia as compared to T lymphocytes[23]. A general observation is that after initiation of combination antiretroviral therapy (cART), viral loads in CSF tend to decline at a slower pace than the viral load in plasma. This may be explained by less drug penetration and the lower death rate of HIV-infected cells in CNS or CSF as compared to the death rate of HIV-infected $CD4^+$ T-cells in plasma[24,25].

Based upon these anatomical, cellular, and pharmacological differences, it has often been suggested that the CNS serves as a potential viral reservoir, which is clinically relevant as this may be associated with neurological symptoms. Although the prevalence of major opportunistic CNS infections has diminished due to cART, the prevalence of HIV-associated neurocognitive disorders has remained high[26]. It is hypothesized that cognitive impairment is a result of ongoing neuropathological processes involving viral production, viral replication, immune activation, effects of toxins and drugs, vascular-related problems, or accelerated aging of the brain[27]. The presence of HIV RNA in CSF, either derived from viral production or replication, is often, but not always, reported in the presence of neurological symptoms[28].

Several studies and case reports show that detectable HIV RNA in CSF is not always associated with detectable viremia in plasma[28-33]. The CNS may as such be an unnoticed source for low-level viremia[30,32,34,35] and selection of antiretroviral resistance affecting non/nucleoside reverse transcriptase inhibitors (N/NRTI)[29,34,35], protease inhibitors (PI)[29,31], integrase inhibitors[33], and fusion inhibitors[32].

Strategy and definitions

We systematically reviewed cases with paired CSF and plasma samples from literature to investigate to what extent the CNS constitutes a distinct virological compartment by comparing host cell tropism and resistance associated mutations in relation to HIV RNA levels and neurological symptoms (for methodological details see Supplementary data). After critical appraisal, 35 studies describing a total of 671 samples from 555 subjects could be included in this review (Fig. 1). Depending on the analysis, different numbers of paired samples and subjects were compared. Discordance was defined as one or more resistance associated mutations (RAM) in blood not present in CSF or *vice versa* in a paired sample. Only drug resistance associated mutations defined by the IAS-USA drug resistance panel were considered relevant (IAS USA, 2011). In this study, genetic divergence was defined as the accumulation of independent genetic changes (RAM) in CSF and plasma. A relevant difference in viral load was defined as a 0.5 $\log_{10}$ copies/ml difference between CSF and plasma. In accordance with a previous report, a CPE cutoff value > 7 was used to define effective penetration in the CNS[22].

Compartmentalization of HIV RNA levels

A total of 509 samples from 397 subjects could be used to compare HIV-1 RNA viral loads (Table 1 and 2). The HIV RNA levels in CSF and plasma of all paired samples are depicted in figure 2 and show moderate correlation ($r = 0.40$; $p < 0.001$). Median plasma viral load (4.70 $\log_{10}$ copies/ml; IQR: 3.90-5.33) was significantly higher than CSF viral load (3.67 $\log_{10}$ copies/ml; IQR: 2.84-4.50) ($p < 0.001$). Similar results were observed if only the first sample pair of a patient was analyzed: median plasma viral load 4.83 $\log_{10}$ copies/ml; median CSF viral load 3.76 $\log_{10}$ copies/ml. Overall, 60% (305/509) of the sample pairs had a higher viral

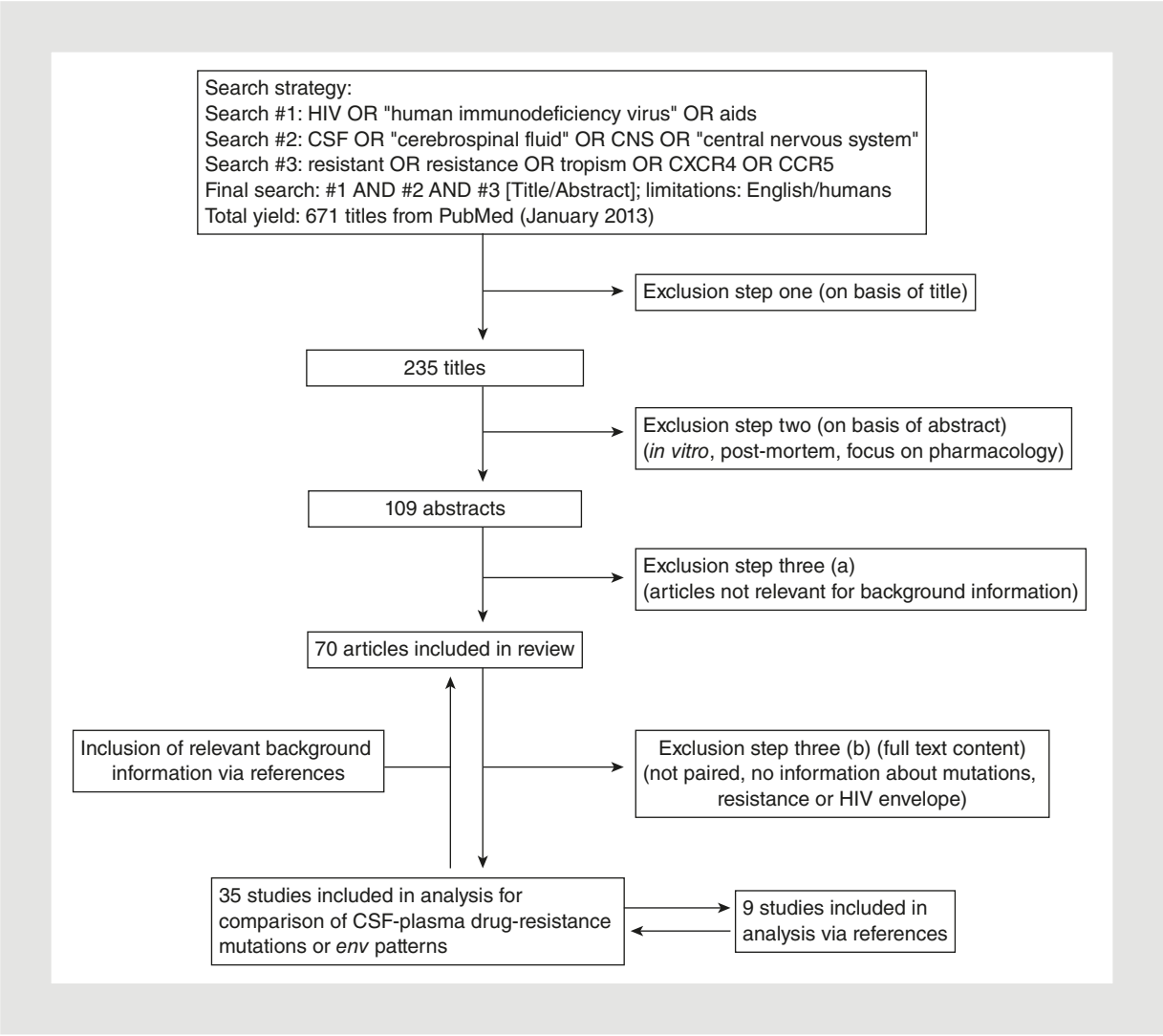

Figure 1. *Search strategy and study selection. Out of 671 titles, 35 studies are selected for the analysis. CSF: cerebrospinal fluid; CNS: central nervous system.*

load in plasma than in CSF. Only in 13% (67/509) of the pairs was a higher viral load seen in CSF.

The presence or absence of neurological symptoms was reported for 274 out of 397 subjects (69%) and these cases were included in the analyses regarding neurological symptoms. Neurological symptoms were reported as present in 181 out of 274 subjects (66%). Within this subgroup, the median viral load in plasma was 4.90 $\log_{10}$ copies/ml compared to 4.18 $\log_{10}$ copies/ml in CSF, resulting in a CSF/plasma viral load ratio of 0.85. In the remaining 93 patients without neurological symptoms (34%), a greater difference between median viral load in plasma (4.69 $\log_{10}$ copies/ml) and CSF (3.17 $\log_{10}$ copies/ml) was observed, leading to a CSF/plasma viral load ratio of 0.68. The difference in median CSF viral load between subjects with or without neurological symptoms was statistical significant ($p < 0.01$), but the difference in median plasma viral load was not ($p = 0.123$). The differences in viral load ratio were also significant between subjects with and without neurological symptoms ($p < 0.01$). In subjects with detectable viral loads in CSF, the most frequent diagnoses associated with neurological symptoms were HIV encephalitis, progressive multifocal leukoencephalopathy, and HIV-associated dementia.

In the cases where the CSF viral load exceeded that of plasma (n = 67; 13%), a majority of the samples (n = 49; 73%) was taken from subjects with neurological symptoms. Only one sample (1%) was taken from a subject without neurological symptoms and, for the remaining, the presence or absence of symptoms were not reported (n = 17; 25%) (Fig. 2). In the group

Table 1. Overview of included studies comparing mutations in cerebrospinal fluid and plasma

Author, year	Virological characteristic compared	N.° subjects with plasma - CSF HIV RNA comparison (sample pairs)	No compartmentalization N.° samples (% of samples)		Compartmentalization N.° of samples (% of samples)			Total subjects in resistance comparison (samples)
			CSF = Plasma	CSF = Plasma				
			RAM involved*	No RAM involved†	RAM in CSF not in plasma‡	RAM in plasma not in CSF§	Distinct RAM in CSF and plasma¶	
Cornelissen, et al. 2011[35]	RT/PR, HIV RNA	1 (1)	1 (100)	0	0	0	0	1 (1)
Canestri, et al. 2010[36]	RT/PR, HIV RNA	11 (11)	2 (100)	0	0	0	0	2 (2)
Lelyveld, et al. 2010[32]	RT/PR/ENV, HIV RNA	0 (0)	0	0	1 (100)	0	0	1 (1)
Watanabe, et al. 2010[33]	RT/PR/INT, HIV RNA	1 (1)	1 (100)	0	0	0	0	1 (1)
Bergroth, et al. 2009[38]	RT, HIV RNA	13 (22)	10 (45)	4 (18)	3 (14)	5 (23)	0	13 (22)
Hightower, et al. 2009[48]	RT, HIV RNA	26 (26)	3 (12)	18 (69)	2 (8)	2 (8)	1 (4)	26 (26)
Soulié, et al. 2009[49]	RT/PR, HIV RNA	22 (22)	16 (76)	0	1 (5)	0	4 (19)	21 (21)
Caragounis, et al. 2008[40]	RT/PR, HIV RNA	3 (10)	6 (67)	0	0	1 (11)	2 (22)	3 (9)
Price, et al. 2008[50]	ENV, HIV RNA	1 (1)	1 (100)	0	0	0	0	1 (1)
Spudich, et al. 2006[51]	RT/PR, HIV-RNA	34 (34)	9 (26)	16 (47)	5 (15)	4 (12)	0	34 (34)
Strain, et al. 2005[10]	RT/PR, HIV RNA,	18 (18)	6 (33)	3 (17)	1 (6)	6 (33)	2 (11)	18 (18)
Bestetti, et al. 2004[39]	RT/PR, HIV RNA	12 (18)	6 (43)	1 (7)	1 (7)	1 (7)	5 (36)	12 (14)
Brew, et al. 2004[52]	RT	0 (0)	5 (21)	10 (42)	2 (8)	7 (29)	0	24 (24)
Eggers, et al. 2003[53]	RT/PR, HIV RNA	40 (80)	4 (27)	10 (67)	0	1 (7)	0	15 (15)
McCoig, et al. 2002[42]	RT, HIV RNA	16 (20)	3 (15)	6 (30)	2 (10)	6 (30)	3 (15)	16 (20)
Tashima, et al. 2002[54]	RT/PR, HIV RNA	6 (6)	3 (50)	0	2 (33)	0	1 (17)	6 (6)
Cinque, et al. 2001[37]	RT/PR, HIV RNA	15 (29)	7 (50)	3 (21)	3 (21)	1 (7)	0	14 (14)
Lanier, et al. 2001[41]	RT	0 (0)	0	0	0	7 (70)	3 (30)	7 (10)
Stingele, et al. 2001[55]	RT/PR, HIV RNA	22 (22)	6 (26)	3 (13)	7 (30)	2 (9)	5 (22)	23 (23)
Cunningham, et al. 2000[56]	RT, HIV RNA	25 (25)	8 (26)	13 (42)	3 (10)	7 (23)	0	31 (31)
Tang, et al. 2000[57]	RT/PR, HIV RNA	5 (9)	0	0	0	0	0	0 (0)
Venturi, et al. 2000[58]	RT/PR	0 (0)	11 (46)	4 (17)	4 (17)	0	5 (21)	24 (24)
Chien, et al. 1999[59]	RT	12 (12)	4 (57)	1 (14)	0	2 (29)	0	7 (7)
Total		283 (367)	112 (35%)	92 (28%)	37 (11%)	52 (16%)	31 (10%)	300 (324)

RAM: resistance associated mutations; CSF: cerebrospinal fluid; RT: mutations in reverse transcriptase coding region; PR: mutations in protease coding region; INT: mutations in integrase coding region, ENV: mutations in envelope gene.
*Number and percentage of paired samples with similar genotypic patterns in CSF and plasma with RAM. †Number and percentage of paired samples with similar genotypic patterns in CSF and plasma without RAM. ‡Number and percentage of paired samples with RAM seen in CSF that are not found in plasma/blood. §Number and percentage of paired samples with RAM seen in blood/plasma that are not found in CSF. ¶Number and percentage of paired samples that have both RAM in CSF not found in plasma as well as RAM in plasma not found in CSF.

Table 2. Overview of included studies that comparing env patterns and coreceptor tropism in cerebrospinal fluid and plasma

Author, year	Part of env compared	N.° subjects HIV RNA (samples)	N.° subjects env (samples)	N.° coreceptor comparisons	C	R5	R5	R5	DM	DM	DM	X4	X4	X4	Method
					P	R5	DM	X4	R5	DM	X4	R5	DM	X4	
Parisi, et al. 2011[46]	V3	7 (7)	33 (33)	33		21	–	5	–	–	–	2	–	5	Genotypic (FPR 10%)
Schnell, et al. 2011[15]	gp160	11 (16)	11 (16)	11		10	–	–	–	–	–	–	–	1	Phenotypic (env-pseudotyped reporter virus), SGA, phylogenetic analysis
Schnell, et al. 2010[45]	V1/V2; V4/V5	11 (26)	11 (26)	0		–	–	–	–	–	–	–	–	–	HTA, SGA, phylogenetic analysis
Harrington, et al. 2009†[44]	V1/V2; V4/V5; C2-V3	*	66 (25)†	25†		24†	–	–	–	–	–	–	–	1†	Genotypic, HTA
Karlsson, et al. 2009[60]/2012[14]	Full virus	28 (28)	28 (28)	28		21	3	–	–	3	1	–	–	–	Phenotypic (U87.CD4 glioma cell line and NP2.CD4 cell line)
Smith, et al. 2009[61]	C2-V3	4 (12)	4 (12)	4		3	–	1	–	–	–	–	–	–	Genotypic (Wetcat SVM)
Soulié, et al. 2009[49]	V3	22 (22)	22 (22)	22		17	3	–	–	2	–	–	–	–	Genotypic (G2P, FPR not reported, PSSM)
Caragounis, et al. 2008[40]	V3	3 (10)	3 (10)	3		2	–	1	–	–	–	–	–	–	Phenotypic (MT-2 assay), phylogenetic analysis
Pillai, et al. 2006[62]/ Strain, et al. 2005[10]	C2-V3	18 (18)	18 (18)	18		14	2	–	–	1	–	1	–	–	11/25 rule, phylogenetic analysis
Abbate, et al. 2005[63]	V3	5 (5)	5 (5)	5		2	3	–	–	–	–	–	–	–	Genotypic analysis of clones (amino acid algorithm from [64] and [65]
Ritola, et al. 2005†[43]	V1-v2; V3	27 (27)	27 (27)	24		22	–	–	–	–	–	–	–	2	HTA, Genotypic (V3 sequence analysis)
Spudich, et al. 2005[47]	gp160	*	46 (46)	46		36	3	–	2	5	–	–	–	–	Phenotypic (PhenoSense HIV entry assay; env-pseudotyped reporter virus)
DiStefano, et al. 1998[66]	(V3)	21 (21)	22 (22)	22		7	–	10	–	–	–	–	–	5	Phenotypic (MT-2 assay)
Kuiken, et al. 1995[67]	V3	0 (0)	12 (12)	12		9	–	1	–	–	–	1	–	1	Genotypic, based on amino acid interpretation by [68] and [69]
Keys, et al. 1993[70]	V3	0 (0)	10 (10)	0		–	–	–	–	–	–	–	–	–	Sequence comparison, no coreceptor interpretation
Total		157 (192)	252 (287)	228		164 72%	14 6%	18 8%	2 1%	11 5%	1 < 1%	4 2%	–	14 6%	

R5: HIV using the CCR5 coreceptor; X4: HIV using the CXCR4 coreceptor; DM: dual-mixed tropism (both usage of CCR5 and CXCR4 coreceptor) or mixed tropism (HIV populations of R5 and X4 coreceptor preference); MT-2 assays: NSI (non-syncytium inducing) is reported as R5 and SI (syncytium inducing) as X4; HTA: heteroduplex tracking assay; SGA: single genome amplification.

*Information about HIV RNA levels is published, but not traceable per subject and therefore not included in analysis. †Study population of [44] has overlap with [43] of which only results of [43] are included in total count.

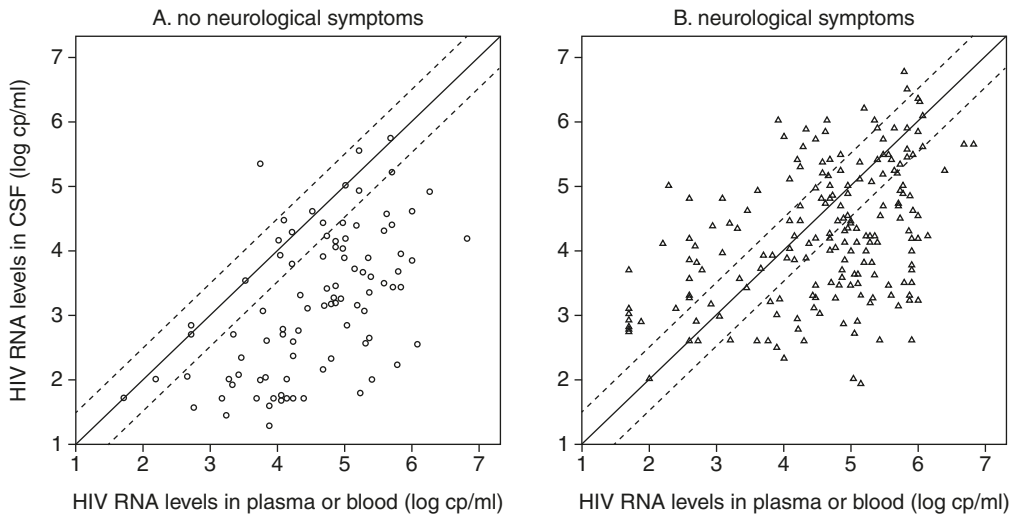

Figure 2. *HIV-RNA levels in plasma and CSF in relation to neurological symptoms. Paired samples are depicted as a dot **(A)** in the absence of neurological symptoms and as a triangle **(B)** in the presence of neurological symptoms. The middle line resembles identical values in cerebrospinal fluid and plasma. The two dotted lines resemble the borders of 0.5 log_{10} difference (r = 0.40).*

of 305 samples taken from 234 subjects in whom the viral load in plasma exceeded that of CSF, a more equal distribution of neurological symptoms was seen. A third (31%; n = 96) of the samples were from subjects with neurological symptoms, while 25% (n = 75) were from patients without such symptoms, and for 44% (n = 134) of the samples it was not reported (Fig. 2).

A total of 12 subjects (3%) had detectable CSF viral load without a detectable load in plasma (< 50 copies/ml[36] or < 400 copies/ml[37]. All these 12 patients had neurological complaints and were on antiretroviral therapy, of which four (33%) on a regimen had a CPE score ≤ 7. When all samples with detectable HIV RNA in CSF, irrespective of plasma values, were analyzed, the proportion having a CPE score ≤ 7 was 70% (70/100 samples). In the group with suppressed CSF HIV RNA levels, irrespective of plasma values, the proportion with a CPE score ≤ 7 was also high (78%; 21/27).

It would be of interest to relate HIV RNA levels in CSF and plasma with certain antiretroviral regimens; however, no fair comparison of HIV RNA levels in CSF or plasma and type of antiretroviral regimen could be made based on available literature. Of the subjects, 33% were not receiving antiretroviral treatment at sampling time and 13% of the subjects were on therapies that are not considered appropriate in current clinical practice (e.g. monotherapy). In 11%, a PI-based regimen was reported, compared to only 1% receiving a NNRTI-based regimen. Additionally, 1% used advanced combination therapy with four or more compounds or a combination including fusion-, entry-, or integrase inhibitors. In 40%, the use of antiviral therapy or its exact combination was not reported.

Compartmentalization of resistance associated mutations

Genotypic resistance associated mutation patterns were reported for 324 samples from 300 subjects out of 23 studies (Table 1). A majority of the samples (63%; 204/324) showed similar mutation patterns, with 35% of the samples (114/324) having concordant resistance associated mutations and 28% (90/324) having no resistance associated mutations in both compartments. A total of 37% (120/324) had different mutation patterns in plasma and CSF. Overall, resistance mutations were more often detected in plasma than in CSF; 16% had resistance mutations in plasma which were not found in CSF and 11% had resistance mutations in CSF which were not present in plasma. Divergent resistance patterns were observed in 10%; in these patients, specific resistance mutations were only observed in plasma

while other resistance mutations were solely seen in CSF (Table 1). As can be expected, most discordant mutations were seen in subjects under treatment, but no significant correlation between CPE score of the regimen at sampling time and the number of discordant mutations in either CSF (p = 0.08) or plasma (p = 0.38) could be found. Discordant or concordant mutation patterns were not significantly linked to neurological disease. In samples taken from subjects with neurological symptoms, 60% (104/174) concordant resistant or wild-type viral populations were observed, which was comparable to samples taken from neurologically asymptomatic subjects (66%; 45/68) (p = 0.36).

The most frequently reported mutations were reverse transcriptase mutations on position 41, 184, 210, 215, and protease mutations on position 36 and 63 in both CSF and plasma. These mutations were not always found within the same sample pair as several of these mutations were also the mutations most often found to be discordant. Hence, discordance in *pol* was most often seen at the following resistance related positions: 184 (20 only in plasma, 9 only in CSF); 215 (19, 12); 41 (13, 11); 70 (13, 7); and 69 (15, 0) in reverse transcriptase; and the subtype-related position 36 (0, 5) in protease.

Of the 67 samples with a higher viral load in CSF than in plasma, information on resistance patterns was available for comparison in 29 sample pairs. Within this subgroup, 86% (25/29) of the pairs had similar mutation patterns, whereas discordance was observed in four pairs with mutations in CSF that were not present in plasma; one of those also had mutations in plasma not seen in CSF. None of the subjects with an undetectable viral load in plasma and a detectable viral load in CSF could be evaluated for discordant mutations in the sample pair since resistance analysis could not be performed on the plasma samples. Of interest, 75% (9/12) of these pairs had resistance mutations in CSF either in this paired sample or a previous CSF sample.

Longitudinal data regarding resistance associated mutations were reported in 18 subjects[38-42]. The majority (61%) of these patients had similar viral evolution patterns in plasma and CSF. In two subjects, resistance associated mutations in CSF emerged later than in plasma[38,41]. In another two patients, samples showed signs of divergence, reflected by accumulation or persistence of more resistance mutations in plasma[38,41]. True independent evolution with major differences in evolution patterns was observed in only three out of 17 subjects[42].

Compartmentalization of HIV coreceptor preference

Results of studies that compare HIV *env* in CSF and plasma are depicted in table 2. Fifteen studies are included, with a total of 252 subjects and 287 sample pairs. Most studies included in this review compared coreceptor preference (n = 228), but some studies also investigated broader genotypic compartmentalization demonstrated by phylogenetic trees and Slatkin-Maddison tests. All studies show that in a subgroup of patients, signs of *env* compartmentalization can be observed most often associated with HIV-associated dementia during advanced disease[43,44], but occasionally also early in infection[45].

For 228 samples, coreceptor comparison was performed, either genotypically or phenotypically (Table 2). In 164 samples (72%), the viral populations were reported to be R5-tropic in both CSF and plasma, while 25 subjects (11%) had dual-mixed or X4 tropism in both compartments. In 86% of the samples, R5-tropic virus populations were detected in CSF. Discordant tropism was observed in 39 samples (17%), with mostly R5-tropic virus (82%; 32/39) in CSF and X4 or dual-mixed virus in plasma. The presence of X4-tropic virus in CSF was occasionally reported or predicted based on genotype, mainly in cases with low CD4 levels and advanced disease stage. Discordant tropism prediction, with X4 in CSF, was also reported during primary infection[46]. There was no significant difference between the viral load levels of X4 or R5 viral populations in CSF (p = 0.24).

Differential evolution of HIV quasispecies

This systematic review combined all published data on HIV resistance associated mutations and tropism from paired plasma and CSF samples. The results confirms the existence of a distinct HIV compartment in the CNS, as reflected by differences in HIV RNA levels, resistance associated mutations, and envelope characteristics.

In general, viral loads were higher in plasma than in CSF, most probably due to the relative abundance of HIV-susceptible $CD4^+$ cells in plasma compared to less plentiful microglia and perivascular macrophages in the CNS. Thus, the subset of patients with higher viral loads in CSF than in plasma is of particular interest. In patients that are not on effective therapy, this may be associated with HIV-associated neurological conditions and an influx of $CD4^+$ T-cells with active HIV

production or replication in the brain, resulting in higher concentrations of HIV RNA in CSF compared to plasma[47]. This influx might also explain the existence of a low but significant correlation between plasma and CSF viral load. In subjects on antiretroviral treatment, detectable HIV RNA levels in CSF are mostly, but not exclusively[28], seen in those with neurological symptoms, but these symptoms tend to be milder than in the untreated group. In these patients, the discrepancy in viral loads between plasma and CSF may be due to differences in effective concentrations of antiretroviral drugs or slower decay rates of HIV-infected cells in the CNS compared to plasma upon initiation of therapy. Activated immune cells in the CNS could also contribute to local viral production and higher viral loads in CSF. Finally, the selection of resistance associated variants within the CNS could also contribute to a higher viral load in CSF.

In more than half of the subjects, the same resistance associated mutations could be observed in plasma and CSF, reflecting comparable selective pressures in CSF and plasma or a more permeable BBB, allowing the exchange of viral populations and T lymphocytes between CSF and plasma. However, in a substantial part of the subjects, the barriers were seemingly intact as discordant genotypic mutation patterns were observed, with resistance mutations more often in plasma than in CSF. A correlation between CPE score and the number of discordant mutations could neither be established for CSF nor plasma. Although rare, it has been shown that a resistant variant can arise in the CSF with subsequent high CSF viral loads in the context of undetectable plasma viremia, ultimately resulting in virological failure in both compartments[32]. The overall findings suggest that distinct evolution patterns in blood and CSF cannot exclusively be attributed to inadequate pharmacological pressure, but can also arise by a founder effect in the CNS or *env* based selection due to target cell differences.

Tropism was mostly concordant in both CSF and plasma, with a great majority of R5-tropic virus in the CNS. This reflects the predominance of microglia and perivascular macrophages in the CNS, which all possess the CCR5 coreceptor and are most susceptible to M-tropic virus. However, dual tropic and X4 variants could also be found in the CSF, probably reflecting T-cell influx in the CNS. The presence of X4 variants was not reflected by a higher viral load in CSF. Genotypic partitioning of *env* sequences in CSF and plasma was often reported[15,45]. It has been described that a trend of more segregation between CSF and plasma sequences is seen during advanced disease and during chronic inflammation[10], with signs of independent HIV-1 replication and evolution regarding *env* in the CNS, especially during HIV dementia, but not during less severe forms of HIV-1 neurological disease[40,44]. It has also been postulated that astrocytes and endothelial cells, that lack the CD4 receptor, can be infected by HIV. This would imply other mechanisms of *env* binding and cell fusion or uptake than in immune cells. This review has some limitations. Firstly, most reports describe analyses in subjects who suffered from neurological symptoms, reflecting the selection criteria of most studies that are included in this review. Therefore it seems that neurocognitive complaints are more likely when there is viral replication in the CNS, but selection bias may be of influence here. Also, in current clinical practice, the prescribed cART is often not similar to the regimens reported in the included literature. Our goal was to provide a comprehensive overview on the topic of plasma/CSF compartmentalization and therefore we accepted that studies with different methodologies and study designs were combined as long as they compared paired plasma and CSF samples and analyzed resistance or *env* characteristics in both samples. As a consequence of this strict and conservative selection method, we may have missed subjects with signs of HIV compartmentalization because no results on paired samples were reported.

Conclusions

The CNS forms a distinct compartment in which the host's integrity of the BBB, its cellular compartment, external pharmacological influences, and viral determinants all play a role. On the one hand, compartmentalization can be reduced by a more permeable BBB or by adequate therapeutic management and full suppression of HIV RNA in both compartments. On the other hand, a well-functioning BBB, differences in target cells, and different pharmacological pressures between the CNS and plasma are likely to contribute to more HIV compartmentalization. HIV compartmentalization should therefore be considered a dynamic process, influenced by the course of the disease and its management, and is likely to be reflected by differences in viral load, resistance associated mutations, and *env* characteristics. This dynamic nature explains the differences seen at an individual level with, on one side of the spectrum, subjects without clear signs of compartmentalization and, on the other side of the spectrum, subjects with virological failure due to

the selection of a drug-resistant variant in the CNS. Within this continuum, varying degrees of CNS compartmentalization may exist, depending on the involved viral determinants and selective pressures. In clinical practice there should be awareness that monitoring HIV in plasma does not always correspond with the situation in the CNS and that deterioration of neurological or cognitive status could imply a clinically relevant compartmentalization with active HIV replication.

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Supplementary Data

Supplementary data is available at AIDS Reviews journal online (http://www.aidsreviews.com). This data is provided by the author and published online to benefit the reader. The contents of all supplementary data are the sole responsibility of the authors.

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