Differences between Cerebrospinal Fluid (CSF) and Blood Biomarkers of Inflammation in HIV Infection

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Introduction

HIV central nervous system (CNS) infection is associated with local inflammation that evolves over the course of systemic disease and impacts neurological function. To compare the evolution of CNS and systemic inflammation with disease progression, we assessed 10 inflammatory biomarkers in cerebrospinal fluid (CSF) and blood across a spectrum of subject groups.

Methods

This exploratory cross-sectional study measured 10 inflammatory biomarkers (TNF-α, MMP-9, CXCL10, sCD14, sCD163, sVCAM, CCL2, IL-6, TIMP-1 and neopterin) by EIA in 9 subject groups: HIV uninfected controls (HIV-, N=20); primary HIV infection (PHI, 24); untreated neuroasymptomatic (NA) subjects in 4 blood CD4+ cell strata, >350, 200-349, 50-199 and <50 cells/μL (NA, 20 each); untreated HIV-associated dementia (HAD, 12); treated, virally-suppressed (Rx, 19) and elite controllers (EC, 8). The Kruskal-Wallis test was used for overall comparisons across groups and in *a priori* defined between-group comparisons defined by pathobiological questions: PHI vs. HIV-; over the 4 NA groups; HAD vs. combined groups with CD4<200 cells/μL; Rx and EC vs. HIV-. Correlations among CSF and blood biomarkers in the entire sample were assessed by Spearman correlation. ANOVA with linear and quadratic orthogonal trends across the 4 NA groups were explored by appropriate contrasts.

Results

Subject information is listed in **Table 1** and the results of the analyses of the CSF and blood inflammatory markers are shown in **Figure 1**. Marker concentrations are shown on a 3-$\log_{10}$ scale. When the scale differs for CSF and blood, the latter are marked by an * and an arrow showing the direction of the difference. Trend analyses are shown in **Table 2**.

- Increases in CSF and blood markers above HIV- levels were common in PHI implying early immune activation in both compartments.
- In the four NA groups, the pattern differed in CSF and blood: with decreasing CD4 counts, increases in plasma biomarker concentrations were common and often linear (Table 2) while in CSF an inverted 'U' pattern was seen in some of the biomarkers.
- Inflammation in CSF and blood was reduced by treatment and was lower in elite controllers than viremics, but in both these groups inflammatory biomarker levels remained above those of the HIV- group.

Table 1. Subject characteristics

Variable	Units	GROUPS								
		HIV negative	PHI	NA, CD4 >350	NA, CD4 200-349	NA, CD4 50-199	NA, CD4 <50	HAD	RX suppressed	Elites
Subjects	N	20	24	20	20	20	20	12	19	8
Site	SF:GOT	20:0	19:5	12:8	12:8	12:8	11:9	6:6	11:8	8:0
Gender	M:F	20:0	24:0	16:4	15:5	17:3	18:2	12:0	16:3	5:3
		median (IQR)								
Age (years)	years	36 (29-48)	34 (28-44)	42 (35-44)	45 (35-55)	43 (38-55)	43 (37-49)	43 (38-54)	43 (38-50)	49 (41-55)
Time since HIV diagno.	"		0.14 (0.10-0.19)	1.6 (0.7-12)	4 (0.7-14)	1.0 (0.4-11)	4.7 (0.6-13)	2 (0.0-8)	12 (6-17)	19 (14-23)
Blood CD4	cells/μL	762 (688-989)	559 (401-749)	491 (409-572)	240 (222-306)	122 (96-145)	20 (8-39)	55 (35-160)	548 (283-770)	869 (721-1070
Blood CD8	"	534 (351-683)	1200 (708-1623)	909 (640-1142)	827 (731-1057)	762 (502-930)	495 (285-684)	650 (307-976)	757 (620-1094)	702 (401-828)
CSF WBC	"	1.0 (1.0-3.0)	6.0 (3.3-13.3)	6.5 (3.3-9.0)	6.0 (3.3-13.5)	3.5 (1.3-9.0)	0.0 (0.0-1.0)	5.5 (0.5-17.5)	1.0 (0.0-2.0)	2.0 (2.0-2.8
HIV plasma RNA	$\log_{10}$ copies/mL		4.59 (4.19-5.51)	4.15 (3.35-4.62)	4.82 (4.22-5.27)	4.89 (4.49-5.20)	5.39 (4.45-5.84)	5.29 (4.79-5.27)	1.59 (1.59-1.59)	1.59 (1.59-1.59)
HIV CSF RNA	"		2.71 (1.77-3.40)	3.20 (2.47-3.63)	4.10 (3.41-4.61)	3.85 (3.50-4.38)	3.08 (2.30-3.76)	4.59 (2.71-5.02)	1.59 (1.59-1.59)	1.59 (1.59-1.59)
CSF: blood Albumin	ratio	4.32 (3.44-6.16)	5.38 (4.46-7.40)	5.34 (3.94-6.39)	5.10 (4.14-7.43)	5.38 (3.65-7.50)	4.38 (3.74-5.1)38	9.76 (5.84-12.75)	5.09 (4.51-6.40)	4.39 (3.92-5.98)
CSF NFL	$\log_{10}$ ng/L	2.70 (2.65-2.79)	2.88 (2.80-3.21)	2.78 (2.66-2.89)	2.75 (2.63-2.93)	2.90 (2.77-3.14)	3.36 (2.89-3.79)	4.29 (3.36-4.57)	2.85 (2.64-2.88)	2.76 (2.73-2.87)

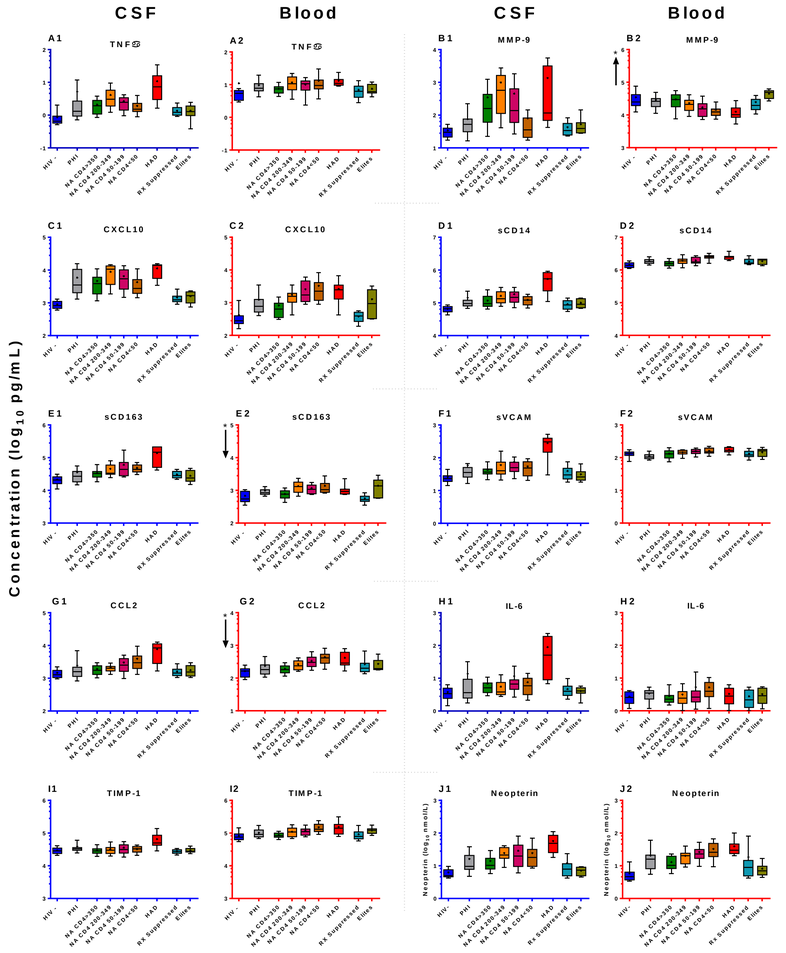
Main findings and conclusions

- HIV infection is accompanied by a complex evolving inflammatory process in both CSF and blood.
  - Changes in different inflammatory biomarkers diverged in the two sampled fluids indicating evolving compartmentalization of responses in the CNS.
- ***Primary infection*** is characterized by an early and broad CSF and blood inflammatory response which is largely (though not entirely) similar in the two compartments.
- ***Untreated systemic disease*** progression defined by blood CD4+ T cell loss across the four non-HAD NA groups shows important divergence of inflammatory response:
  - **Blood** showed variable progressive increase in most of the inflammatory biomarkers.
    - MMP-9 was a conspicuous exception with progressive decrease.
  - By contrast, **CSF** showed ***two*** *patterns of change.*
    1. **An inverted U with initial rise and then fall, e.g. TNFα, MMP-9, CXCL10.**
    2. **Progressive increase, e.g., sCD163, CCL2.**
  - **We hypothesize that these indicate predominantly lymphocytic (1.) and macrophage (2.) responses in CSF, respectively.**
- ***HAD*** was characterized by compartmentalized increase in all CSF inflammatory biomarkers.
  - High, often highest, levels of all CSF biomarkers were noted in HAD group.
  - This was not mirrored in blood concentrations of these biomarkers.
- CSF inflammation, like that in blood, was markedly attenuated by ***treatment-induced*** and ***endogenous viral suppression (elites).***
  - Though concentrations remained higher than HIV- controls, indicating **residual systemic and CNS immunoactivation** in these subjects**.**

Table 2. Trend analysis across NA groups. Linear and quadratic trends in the biomarker levels with increasing CD4 counts were assessed in the four NA groups . A linear ***increase*** was common in blood, (except MMP-9). An inverted 'U' (quadratic) trend was seen in several CSF markers. We hypothesize that in CSF a linear fit designates macrophage-predominant inflammation and a quadratic fit indicates lymphocyte-predominant inflammation. Intensity of red fill designates level of significance: dark red shows full Bonferroni correction, lighter red p<0.01 and very light red p<0.05.

Biomarker	CSF (p value)		Blood (p value)	
	linear	quadratic	linear	quadratic
TNFα	0.2751	0.0012	0.2103	0.4839
MMP-9	0.0009	0.0003	0.0001	0.6319
CXCL10	0.4623	0.0014	<0.0001	0.0769
sCD14	0.4923	0.0078	<0.0001	0.7082
sCD163	0.0052	0.1126	0.0016	0.0449
sVCAM	0.2858	0.1765	0.0688	0.3897
CCL2	0.0004	0.6074	<0.0001	0.6785
IL-6	0.2978	0.8786	0.0464	0.3667
TIMP-1	0.3604	0.3158	<0.0001	0.6913
neopterin	0.0585	0.0251	<0.0001	0.3319

Figure 1. CSF and blood inflammatory biomarker concentrations.



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