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Soluble membrane attack complex in the blood and cerebrospinal fluid of HIV-infected individuals, relationship to HIV RNA, and comparison with HIV negatives

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ABSTRACT

The soluble membrane attack complex (sMAC) represents the terminal product of the complement cascade. We enrolled 47 HIV + adults (12 of whom underwent a second visit at least 24 weeks after starting therapy) as well as 11 HIV negative controls. At baseline, cerebrospinal fluid (CSF) sMAC was detectable in 27.7% of HIV + individuals. CSF sMAC correlated with CSF HIV RNA levels and was more likely to be detectable in HIV + individuals on cART compared to HIV negative controls. In HIV + participants, there were negative association trends between sMAC and neurocognitive performance but these did not reach statistical significance.

1. Introduction

In spite of virologic suppression achievable through combination antiretroviral therapy (cART), infection with the human immunodeficiency virus (HIV) has been linked to a persistent expansion of systemic inflammation (Neuhaus et al., 2010). Of particular significance is the finding that higher systemic inflammation is associated with unfavorable clinical outcomes in HIV-infected individuals, including increased mortality (Duprez et al., 2012). HIV-associated neurocognitive disorders (HAND) remain prevalent in the cART era, and recent research suggests that the development of HAND despite cART may also be in part inflammatory mediated (Heaton et al., 2010; Zayyad and Spudich, 2015).

Dysregulation of adaptive immunity during HIV infection has been long established and aberrant T-cell activation is independently associated with HIV clinical disease progression (Hunt et al., 2011; Liu et al., 1997). While innate immunity dysregulation during HIV infection is less defined, there is mounting evidence that this arm of the immune system is abnormally activated as well. Natural killer cell activation is present in the setting of HIV and does not normalize despite virologic suppression during cART (Lichtfuss et al., 2012). Additionally, activation of the complement system has been recognized since the early years of the HIV epidemic (Liu et al., 2014; Senaldi et al., 1990). It is possible that the complement system plays a role in HIV neuropathogenesis. Non-human primate models have demonstrated upregulation of both C1q and C3 in brain tissue during simian immunodeficiency virus (SIV) infection (Speth et al., 2004). Recent research focusing on young adults with HIV showed a possible association between C1q levels in cerebrospinal fluid (CSF) and both neurocognitive impairment and CSF levels of neurofilament light chain (NFL), an established marker of neuronal damage (McGuire et al., 2016).

The membrane attack complex (MAC) is a large macromolecular protein complex composed of five complement proteins (C5b, C6, C7, C8, and C9) that together generate a pore-forming structure capable of lysing bacteria and other microorganisms (Sonnen and Henneke, 2014). The MAC is assembled when the terminal complement pathway is activated through any of the early pathways (alternative, classical, or lectin) or the extrinsic protease pathway. Thus, the level of MAC in either soluble (sMAC) or membrane bound form is a marker of terminal complement cascade activity. The MAC also contributes to inflammation through production of ion fluxes and activation of pro-inflammatory signaling pathways in host cells (Morgan, 2016). Elevated CSF sMAC levels have been found to be common in pyogenic bacterial infections of ventricular shunts and to a lesser extent in other neuro-

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inflammatory diseases such as multiple sclerosis (Ramos et al., 2016; Sellebjerg et al., 1998). In this study, we examined sMAC levels in HIV-infected individuals with varying degrees of neurocognitive impairment in comparison to HIV-negative individuals as well as sMAC change over time in a subset of HIV-infected participants.

2. Methods

2.1. Assessment of participants

Participants were enrolled between March 2011 and January 2017 at the Emory University Center for AIDS Research (CFAR) clinical core site in Atlanta as part of ongoing studies on HIV and neurocognition. Individuals with and without chronic HIV between 18 and 59 years of age were eligible for participation. Individuals were excluded from the study for any of the following: 1) history of any neurologic disease known to affect memory (including stroke, malignancy involving the brain, traumatic brain injury, and AIDS-related opportunistic infection of the central nervous system); 2) current ongoing substance use (marijuana use in the last 7 days OR cocaine, heroin, methamphetamine, or other non-marijuana illicit drug use in the last 30 days); 3) heavy alcohol consumption in the last 30 days (defined as > 7 drinks per week for women and > 14 drinks per week for men); or 4) serious mental illness including schizophrenia and bipolar disorder (depression was not excluded if participants were well controlled on treatment). HIV + participants with a history of treated syphilis and a persistently positive rapid plasma regain (RPR) titer of 1:8 or less were eligible for the study if there was a decrease in RPR of at least fourfold at six months after treatment and there were no neurological symptoms at initial syphilis presentation. Lastly, participants were excluded in the event that significant cognitive symptoms had occurred precipitously in the last 30 days in order for further medical workup to be undertaken.

A neuropsychological (NP) battery was administered to the HIV + participants that included the following nine tests used commonly in studies of cognition and HIV infection(Robertson and Yosief, 2014): 1) Trailmaking Part A; 2) Trailmaking Part B; 3) Hopkins Verbal Learning Test total recall; 4) Hopkins Verbal Learning Test delayed recall; 5) Grooved Pegboard (dominant) 6) Grooved Pegboard (non-dominant); 7) Stroop Color Naming, 8) Stroop Color-Word; and 9) Letter Fluency (Controlled Oral Word Association Test). These tests were selected in order to examine at least five domains as recommended in the most recent nosology of HAND criteria (Antinori et al., 2007). Scores were adjusted for demographic characteristics using published norms (Heaton et al., 2004). Score adjustment for practice effects was also made for longitudinal visits when available by using median practice effect data from previous work (Cysique et al., 2011). A composite neuropsychological test score (NPT-9) was then calculated by average of individual T scores. Global Deficit Score (GDS), a validated measure of neurocognitive impairment in HIV based on demographically corrected T scores, was calculated and neurocognitive impairment was judged to be present for scores of 0.5 or higher (Carey et al., 2004). The study was approved by the Emory University Institutional Review Board and written consent was obtained from all participants.

2.2. Laboratory assessment

Routine laboratory studies including CD4 + T-lymphocyte counts were performed at the hospital clinical laboratory while HIV RNA levels from plasma and CSF were performed at the Emory Center for AIDS Research (CFAR) virology core using the Abbott laboratories m2000 Real Time HIV-1 assay system (reverse transcriptase polymerase chain reaction). Lowest limit of HIV detection was 40 copies/milliliter (ml). Individuals with no history of HIV infection were confirmed to be negative with the fourth generation Abbott antigen/antibody assay. Soluble MAC was quantitated using the MicroVue complement sC5b-9 Plus enzyme immunoassay (Quidel Corporation) according to the manufacturer instructions. Levels were calculated from a standard curve and the lower limit of detection was 3.7 nanograms (ng)/ml. Samples below the lower limit of detection were assigned a value of one-half lower than the limit of detection as previously described (Ramos et al., 2016). Samples were assayed in duplicate and were performed by personnel blinded to all demographic and disease characteristics including whether the sample was from an HIV positive or negative participant.

2.3. Statistical analyses

Analyses were performed with SAS JMP software version 12 and Graphpad prism version 6.07. Normality of variable distribution was assessed with the Shapiro-Wilk test. Given the skewed distribution of most variables, comparisons between continuous variables were performed with the Wilcoxon rank sum test. Comparisons between categorical variables were performed with the chi square likelihood ratio test. For longitudinal paired variable comparison, the Wilcoxon signed rank test was used. For correlations, Spearman's rho test was used for variables that did not meet normality criteria and Pearson's correlation coefficients (r) were generated for variables that met normality criteria. p values for correlation results were adjusted for multiple comparisons using the Bejamini-Hochberg false discovery rate (FDR) correction (Bejamini, 1995). Alpha level for significance was set at < 0.05.

3. Results

3.1. HIV + participants at baseline

There were 47 HIV + individuals at study entry, 12 of whom had a second longitudinal visit at least 24 weeks after starting cART (total of 59 visits). At baseline (see Table 1), 40.4% were already on cART with plasma and CSF HIV RNA level < 40 copies/ml. Forty one of 47 participants (87.2%) had a negative RPR. Twenty one participants (44.7%) had neurocognitive impairment with GDS of greater than or equal to 0.5. Thirteen participants (27.7%) had detectable CSF sMAC at baseline. 10.6% had confirmed hepatitis C virus (HCV) infection with positive serum antibody and detectable plasma HCV RNA, while a separate 4.3% had confirmed hepatitis B virus (HBV) infection with positive HBV surface antigen and detectable plasma HBV DNA. However, the presence of chronic HBV or HCV was not associated with an increased likelihood of detectable CSF sMAC (37.5% for participants with either hepatitis virus versus 25.6% for participants with neither, $p = 0.5$).

3.2. Longitudinal assessment of HIV + participants

Twelve of the participants off cART had a second visit at least 24 weeks after starting therapy (median 27 weeks, interquartile range 25–30 weeks). Seven of these achieved suppression of plasma HIV RNA and CSF HIV RNA to < 100 copies/ml. Despite the fact that not all participants achieved full virologic suppression, there was a significant decrease in plasma sMAC level at the second time point (median 171 ng/ml at time point 1 versus median 153 ng/ml at time point 2, $p = 0.034$, see Fig. 1 for dot plots). Corresponding to this was an improvement in NPT-9 (mean 41.4 for time point 1 versus mean 46.5 for time point 2, $p = 0.007$). There was a trend towards decrease in CSF sMAC level at the second time point, but given that a significant proportion of participants had undetectable CSF sMAC at baseline, this did not reach statistical significance ($p = 0.11$, see Fig. 2).

3.3. HIV negative participants compared to virologically suppressed HIV + subgroup

For this comparison, only HIV + individuals with a visit on cART for at least 24 weeks with plasma and CSF HIV RNA < 100 copies/ml were considered. Additionally, participants who were positive for HCV,