Immune Activation of the Central Nervous System Is Still Present after >4 Years of Effective Highly Active Antiretroviral Therapy

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Highly active antiretroviral therapy (HAART) effectively reduces human immunodeficiency virus (HIV) RNA in cerebrospinal fluid (CSF), as well as in plasma. The effect on intrathecal immunoactivation is less well studied. We had earlier found that a substantial number of patients still have evidence of intrathecal immunoactivation after up to 2 years of treatment. We identified 15 patients treated with HAART for ≥4 years who had plasma HIV-RNA levels of <50 copies/mL for ≥3.5 years. CSF samples were available from 10 patients before treatment. We measured white-blood-cell count, HIV-RNA level, neopterin level, and IgG index. During treatment, all patients had HIV-RNA levels of <50 copies/mL in plasma and CSF. In CSF, both neopterin level and IgG index decreased significantly. After 4 years, 9 (60%) of the 15 patients still had neopterin levels in CSF that were above the upper normal reference value (5.8 nmol/L). During HAART, 9 (60%) of the 15 patients had an abnormal IgG index (>0.63). HAART significantly decreases intrathecal immunoactivation, but, despite effective treatment for >4 years, with HIV-RNA levels <50 copies/mL for ≥3.5 years, a substantial proportion of patients continue to show signs of macrophage/microglia activation and intrathecal immunoglobulin production in the CNS.

HIV-1 (hereafter referred to as "HIV") invades the central nervous system (CNS) early in the infectious course, with detectable virus in cerebrospinal fluid (CSF) [1] and the brain [2]. It establishes a low-grade chronic process with signs of intrathecal immunoactivation as well as detectable HIV RNA persisting in CSF during the entire course of infection [3]. At later stages of the disease, ~20% of untreated patients develop an encephalopathy known as “AIDS dementia complex” (ADC) [4, 5], in which the inflammatory response may contribute to neuronal damage [6]. Evidence of CNS immunoactivation triggered by HIV includes frequent elevation of CSF white blood cell (WBC) count, increased level of neopterin in CSF, and increased production of intrathecal immunoglobulin [3, 7–9]. The main sources of neopterin are activated macrophages and microglia stimulated by interferon-γ [10]. HIV also triggers a humoral immune response in the CNS, which can be measured as either an increase in the IgG index or the detection of CSF-specific oligoclonal IgG bands [7, 11–13].

The introduction of highly active antiretroviral therapy (HAART) has led to a significant decline in HIV-associated morbidity and mortality, as well as in the incidence of ADC, when medication is available [14]. HAART also has an effect on the immune response within the CNS, although ongoing intrathecal immunoactivation has previously been shown to still be present despite otherwise effective treatment [15]. To evaluate the long-term effect that antiretroviral combination treatment has on the CNS immune response, we measured neopterin level, WBC count, and IgG index in CSF in neurologically stable patients successfully treated with HAART for several years.

Patients, materials, and methods. We retrospectively identified patients from 2 centers who had received treatment with HAART for ≥4 years, with HIV-RNA levels in plasma that were <50 copies/mL for ≥3.5 years, and who had undergone lumbar punctures in separate local protocols evaluating CSF responses to changes in antiretroviral treatment. Treatment of these patients was initiated between 1996 and 2000. One blip (i.e., a single HIV-RNA value of 50–1000 copies/mL) was allowed during the period, and HAART was defined as a combination of at least 3 active drugs from at least 2 different drug classes—that is, 2 nucleoside reverse-transcriptase inhibitors plus 1 protease inhibitor or nonnucleoside reverse-transcriptase inhibitor. All patients had regimens containing at least 2 CNS-penetrating drugs. A total of 15 HIV-1–infected...
patients were identified, 10 from the Department of Infectious Diseases of Sahlgrenska University Hospital (Göteborg, Sweden) and 5 from the Department of Neurology of the University of California, San Francisco. Follow-up clinical and neurological evaluation was performed, on average, every third month. All patients were neurologically asymptomatic, except for 2 patients who presented with ADC stage 1 or 2 at initiation of treatment and who had ADC stage 1 (residual static-gait spasticity) during follow up. Characteristics of the 15 patients are shown in table 1.

Paired samples of plasma and CSF were analyzed for HIV-RNA level, WBC count, neopterin level, and IgG index. In addition, CSF and plasma from 10 patients were available before treatment. A previous report has described CSF characteristics after 2 years of treatment of 7 of the patients in the present study [15]. The protocols followed in the present study were approved by the Research Ethics Committee of Göteborg University and by the University of California San Francisco Committee on Human Research. All patients provided informed consent.

Paired samples of blood and CSF were collected from each of the 15 patients. After cell counts were performed on the CSF samples, they were centrifuged and the supernatants were aliquoted and frozen at −70°C for later analysis. Pleocytosis was defined as a CSF WBC count > 4 × 10⁶ cells/L. Quantitative determinations of albumin and IgG in serum and in CSF were performed by nephelometry (Behring Nephelometer Analysers; Behringwerke AG). CSF and serum samples were analyzed in the same analytical run and with reference to the same standard curve, to obtain method-independent high sensitivity and specificity in CSF and serum.

Intrathecal IgG synthesis was determined on the basis of the IgG index, defined as [CSF IgG (mg/L)/serum IgG (g/L)]/[CSF albumin (mg/L)/serum albumin (g/L)] [16]. The reference value for the IgG index was <0.63 [17].

Neopterin levels were measured by use of a commercially available radioimmunoassay (Henningtest Neopterin; BRAHMS) with a normal reference value of <8.8 nmol/L in serum and <5.8 nmol/L in CSF [18, 19].

Levels of HIV-1 RNA in CSF and plasma were quantified in cell-free CSF and plasma, by use of quantitative polymerase chain reaction (HIV-1 monitor assay, version 1.5; AmpliCor) (Roche Diagnostic System; Hoffman–La Roche) with a dynamic range down to 50 (1.70 log₁₀) copies/mL and a detection limit of ~20 copies/mL.

Descriptive group statistics are presented as median (range) values. Paired pre- and during-treatment values were compared by use of paired-samples t test. P < .01 was considered to be statistically significant.

Results. The effect that HAART has on HIV-RNA level, WBC count, neopterin level, and the IgG index is shown in figure 1. The median level of HIV RNA before treatment was 3.74 log₁₀ copies/mL (range, 1.90–5.07 log₁₀ copies/mL) in CSF and 4.85 log₁₀ copies/mL (range, 3.83–5.39 log₁₀ copies/mL) in plasma. According to study-inclusion criteria, all patients had HIV-RNA levels of <50 (1.69 log₁₀) in CSF and plasma while being treated with HAART. The median CSF WBC count before treatment was 5 × 10⁶ cells/L (range, 0–111 × 10⁶ cells/L) before treatment and 1 × 10⁶ cells/L (range, 0–3 × 10⁶ cells/L) during treatment. Notably, 1 patient had a markedly elevated level of CSF monocytes when treatment was initiated. This patient had

Table 1. Characteristics of included HIV-infected patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, years</th>
<th>CDC classification</th>
<th>Antiretroviral therapy</th>
<th>Period of treatment, months</th>
<th>CD4 nadir</th>
<th>CD4</th>
</tr>
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<tr>
<td>1</td>
<td>64</td>
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<td>710</td>
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<td>500</td>
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<td>3</td>
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<td>544</td>
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<td>200</td>
<td>579</td>
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<tr>
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<tr>
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<td>1035</td>
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<tr>
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NOTE. 3TC, lamivudine; abc, abacavir; azv/r, atazanavir/ritonavir; CDC, Centers for Disease Control and Prevention; ddi, didanosine; efv, efavirenz; idv, indinavir; lopiv, lopinavir/ritonavir; nf, nelfinavir; nvp, nevirapine; rit, ritonavir; sqv-sgc, saquinavir in soft-gel capsule; tfv, tenofovir disoproxil fumarate; tfv, tenofovir; zdv, zidovudine.
suffered from herpes zoster and facial palsy shortly before treatment, which likely contributed to marked CSF pleocytosis.

Neopterin levels decreased significantly in CSF ($P < .001$) and plasma ($P < .001$) during treatment. The median level of neopterin in CSF was 17.4 nmol/L (range, 6.7–55.3 nmol/L) before treatment (pretreatment data were available for 10 of the 15 patients) and 6.6 nmol/L (range, 3.7–10.7 nmol/L) after >4 years of HAART. However, 9 (60%) of the 15 patients still had neopterin levels in CSF that were above the upper normal reference value (5.8 nmol/L) after >4 years of treatment, compared with 10 (100%) of 10 patients before treatment. In plasma, the median level of neopterin was 14.6 nmol/L (range, 8.3–37.1 nmol/L) before treatment and 7.5 nmol/L (range, 2.8–21.6 nmol/L) after >4 years of HAART, with 10 (67%) of the 15 patients having normal levels during treatment and with 1 (10%) of 10 patients having normal concentrations before treatment.

The median IgG index was 0.73 (range, 0.43–1.99) before treatment and 0.70 (range, 0.46–3.05) during HAART ($P < .01$); 9 (60%) of the 15 patients had an elevated index value (0.63) during HAART, and 7 (70%) of 10 had an elevated index value before HAART. During follow-up, 13 (87%) of the 15 patients remained neurologically asymptomatic; the remaining 2 patients, who presented with ADC and gait spasticity, were stable and showed no signs of progress during treatment.

**Discussion.** It is well established that HAART reduces HIV-RNA levels in CSF as well as in plasma [20]. Previously, it has been shown that HAART reduces intrathecal immunoactivation in treated patients, although a significant proportion of patients still have signs of ongoing inflammatory activity [15]. The results of the present study show that, even after successful treatment (i.e., that which reduces HIV-RNA levels to below the limit of detection) for several years, the majority of the 15 patients studied still had elevated levels of inflammatory activity in the CNS, with signs of macrophage/microglial activation, measured as elevated levels of neopterin and of production of intrathecal IgG, albeit at levels significantly lower than those observed before initiation of treatment.

HIV establishes an inflammatory response within the CNS, resulting in macrophage activation. The inflammatory response is considered to be the main mediator of neuronal damage in HIV-related neurodegenerative disease. Viral products have also been implicated in neuronal-cell damage [6]. Ongoing immu-

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**Figure 1.** Effect of highly active antiretroviral therapy on human immunodeficiency virus (HIV)-RNA level, white blood cell (WBC) count, neopterin level, and IgG index. CSF, cerebrospinal fluid.
noactivation might reflect a low level of persisting viral replication within the brain or CSF, below the limit of detection of HIV-RNA in CSF [21]. However, when ultrasensitive PCR assays with limits of detection that are 2–2.5 copies HIV RNA/mL have been used on samples from successfully treated patients, the majority have been found to be negative for HIV RNA in CSF [22, 23]. In light of these findings, it seems less likely that viral replication within or close to the CSF drives the inflammatory response. Whether viral replication still occurs within the brain is not known. Even in patients in whom therapy has stabilized HIV infection, low-level replication may continue systematically [22–24], related to activation of infected memory CD4+ T cells. Entry of virus from the blood, via trafficking CD4+ T cells or mononuclear cells infected before they become resident in the CNS, may continue to expose the CSF compartment to low levels of virus.

It has been proposed that an inflammatory response, once established, may give rise to a self-sustaining state of cellular activation [6]. Patients with herpes simplex virus type 1 (HSV-1) encephalitis have been found to have persisting intrathecal immunooactivation [25], whereas rapid neutralization of increased levels of neopterin has been observed in patients with aseptic meningitis [26]. Continuing replication of HSV-1 within the brain has been suggested as the cause of the prolonged inflammatory response in patients with encephalitis.

CSF represents an accessible compartment for the monitoring of HIV infection of the CNS. However, it is not easily determined how well CSF measurements reflect various processes in the brain itself [3, 27]. The CNS is separated from blood by the blood-brain and blood-CSF barriers. Some previous studies have suggested a correlation between virus in CSF and brain [28, 29], whereas others have shown differences between the viral pool in CSF and that in the periphery [30, 31]. It is likely that CSF, in part, a compartment separate from the systemic infection, with virus originating both from brain and from blood. Very early in systemic infection, HIV infection of CSF is similar to that of plasma and likely reflects replication in trafficking CD4+ T cells. Subsequently, infection in CSF diverges, to varying degrees, from that in plasma, and the “autonomous” component of infection likely originates from infected CNS cells of monocyte lineage—mainly perivascular and meningeal macrophages as well as microglia—that are more long-lived and that may be able to sustain infection within the CNS, with or without replenishment from blood [32, 33].

High levels of neopterin are found in HIV-infected patients with dementia and opportunistic infections, but increased levels are also frequently found in asymptomatic HIV-infected individuals [3, 34, 35]. Intrathecal immunoglobulin and neopterin production increase with the duration of HIV infection, as long as treatment is not initiated [12, 36]. The HIV infection in the CNS triggers an intrathecal immune response even in the absence of neurological symptoms or signs of neurological injury [37, 38]. It has been estimated that only 2.5% of neopterin in CSF originates from outside the CNS [35]. A majority (60%) of the patients in the present study had elevated levels of neopterin in CSF, indicating macrophage activation within the CNS despite effective HAART.

More than half (60%) of the patients still showed signs of intrathecal immunoglobulin production after effective HAART, indicating ongoing humoral immune activation. Immunoglobulin production, as well as increased levels of neopterin, might reflect low-grade viral replication within the brain, replication that was not detected when HIV-RNA levels in CSF were measured. The humoral response might also be the result of a non-specific immunological reaction, because HIV-specific antibodies constitute only a minor part of the intrathecally produced antibodies [39], as well as a consequence of autoimmune reactions triggered by HIV [40, 41].

The significance of intrathecal immunooactivation remains unclear. Other markers have been studied in relation to HIV disease in the CNS. β-2-microglobulin has been shown to correlate with CSF viral load and with disease progression, but it is frequently within the normal range in patients receiving HAART, and a recent study found no clear association with progressive neurological disease in such patients [42]. Similarly, no clear association between neurological status and other immune activation markers has been found [43].

HAART has had a profound impact on the incidence of CNS disease, including ADC [14], and has proved to be effective in reducing CSF viral load, even resulting in clinical improvement in some patients with this dementia [27, 38, 44–46]. ADC typically occurs late in the infectious course, with immune-system failure and AIDS, and treatment of the systemic infection appears to be effective in the prevention of dementia. There is concern, however, about the possible future increase in the incidence of neurological complications [6, 47], as patient survival is prolonged. In the present study, patients showed no signs of progressive neurological symptoms, which would suggest that, at least in the shorter term, ongoing low-grade immunooactivation is benign. However, the number of patients included in this study was limited, and continued investigation will be necessary to evaluate the long-term effects that HIV infection has in the brain.

References