

SHORT REPORT

Tumour necrosis factor (TNF- α) and neurological disorders in HIV infection

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Abstract

Tumour necrosis factor (TNF- α) concentrations were determined in the CSF from 42 HIV-infected patients, with or without CNS involvement. In addition, 14 subjects with various neurological disorders but without HIV antibodies were included as controls. Raised CSF concentrations of TNF- α (>40 ng/l) were detected both in patients with AIDS dementia complex (ADC) (6/9) and with CNS opportunistic infections (10/19) and, less commonly, in HIV infected subjects without CNS diseases (2/14) and in anti-HIV negative controls (1/14). The highest CSF concentrations of TNF- α (>100 ng/l), however, were found in seven out of eight patients with cryptococcal meningitis. Although a role for TNF- α in demyelinating lesions associated with ADC has been suggested, our results indicate that a clear elevation of TNF- α in the CSF from HIV positive patients mostly occurs in acute inflammatory disorders, such as cryptococcal meningitis.

The course of HIV infection is often complicated by neurological diseases, whose pathogenesis are not well known. HIV has been shown to infect several cell types in the brain of infected patients. Electron microscopy and hybridisation studies have shown that HIV replication occurs predominantly in macrophages and microglial cells.^{1,2} On the other hand, HIV has also been detected in astrocytes, basal ganglial neurons, and capillary endothelial cells.³ The precise mechanism by which infected cells mediate neurological dysfunction and destruction has not been elucidated. Recently, it has been suggested that HIV activation of monocytes/macrophages invading the CNS, as well as resident microglia, may result in an enhanced production of cytotoxic factors, which may be involved in the neuropathogenesis of HIV infection.⁴ In particular, one of the monocyte/macrophage-derived cytokines, tumour necrosis factor (TNF- α), has been shown to have multiple toxic effects on neural cells in vitro.⁵

To investigate the relation between TNF- α and HIV-related neurological disorders, we determined the concentrations of this cytokine in the CSF of HIV-infected patients.

Patients and methods

A total of 42 HIV-infected patients, admitted to the Istituto of Malattie Infettive of University "La Sapienza" of Rome, were enrolled in this study. Fourteen had no neurological disorders at the time of CSF handling, while nine had AIDS dementia complex (ADC), eight cryptococcal meningitis, seven cerebral toxoplasmosis, three tuberculous meningitis, and one had a bacterial meningitis. The neurological diseases were diagnosed on the basis of clinical examination, routine serological assays, CSF cultures, and CT or MRI of the brain, or both. In addition, 14 patients negative for anti-HIV with the following neurological disorders were studied: viral meningitis (three), tuberculous meningitis (three), pneumococcal meningitis (one), bilateral optical atrophy (one), cerebral abscess (one), cerebral neoplasma (two), multiple sclerosis (three).

Samples of CSF were collected by lumbar puncture and analysed for cell count and routine protein and glucose chemistry values. Bacterial and fungal cultures were also done with routine methods. Cryptococcal antigen assay was done by standard latex agglutination. CSF samples were immediately placed in a refrigerated centrifuge (4°C) and spun at 3000 rpm for 10 minutes. Cell supernatants were stored at -80°C until use.

TNF- α was quantified by a sandwich enzyme immunoassay (Biokine, T Cell Sciences, Cambridge, MA, USA). Samples and standards were incubated in anti-TNF- α monoclonal antibody-coated polystyrene microtitre wells. TNF- α binds to antibody on the coated well. The unbound sample components were removed by washing. A horseradish peroxidase conjugated anti-TNF- α monoclonal antibody with neutralising properties against TNF- α was then added to bind the TNF- α captured by the first antibody, thereby completing the sandwich. After washing, substrate solution was added to the wells. The reaction was stopped by addition of 2N H₂SO₄, and optical density (OD) at 492 nm was measured with an enzyme linked immunosorbent assay (ELISA) microreader (Sclavo, Siena, Italy). A standard curve was constructed from five TNF standards and unknown values were determined from the standard curve and expressed as ng/l. The detection limit of the assay was 10 ng/l, and values greater than 40

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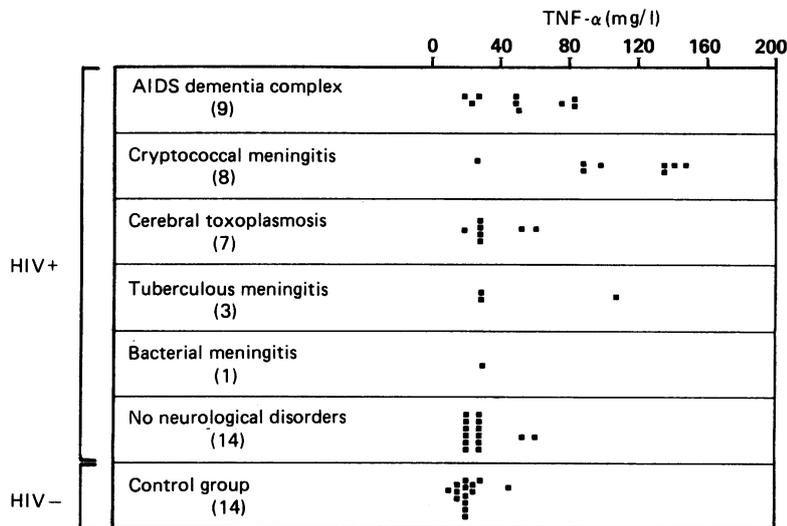


Figure Distribution of CSF TNF- α concentrations in 42 HIV-infected patients and in 14 controls negative for anti-HIV.

ng/l were considered elevated.

Statistical evaluation was performed by analyses of variance.

Results

The distribution of CSF TNF- α concentrations in the 42 HIV-infected patients and in the 14 subjects negative for anti-HIV but with other neurological disorders is shown in the figure. Raised CSF concentrations of TNF- α (>40 ng/l) were detected in six out of nine ADC patients, 10 out of 17 patients with CNS opportunistic infections (7/8 with cryptococcal meningitis, 2/7 with cerebral toxoplasmosis, 1/3 with tuberculous meningitis, 0/1 with bacterial meningitis), and in two out of 14 HIV-infected subjects with no neurological manifestations. CSF concentrations of TNF- α greater than 40 ng/l were also found in one anti-HIV negative patient with tuberculous meningitis.

CSF TNF- α concentrations (mean (SEM)) in the patients with ADC (52.3 (7.9)) and cryptococcal meningitis (108.7 (14.2)) were significantly higher than those observed in the HIV-positive subjects with no CNS involvement (29.6 (3.4)); $p < 0.01$ and $p < 0.001$ respectively) and HIV-negative controls (20.9 (2.0)); $p < 0.001$ for both). Endotoxin was not detected in any of the subjects by means of the Limulus amoebocyte lysate assay. The highest CSF concentrations of TNF- α were found in patients with cryptococcal meningitis and in one individual with tuberculous meningitis. In seven patients with cryptococcal meningitis a follow up examination of TNF- α concentrations was conducted by analysing CSF samples taken at different time intervals during the course of the disease. Results showed a progressive decline in TNF- α concentrations which were closely related to the improvement of neurological manifestations and to the decline of cryptococcal antigen titres.

Discussion

Our findings showed the presence of TNF- α in the CSF of HIV-infected patients, especially in those with neurological disorders. Recent investigations report that TNF- α produces oligodendroglial cell death, large dilatations of the myelin sheath, and in vitro demyelination.⁶⁻⁸ Furthermore, stimulated rat astrocytes release a cytotoxic factor that destroys rat oligodendrocytes in vitro as does recombinant human TNF (r-Hu-TNF). In this respect, some of the neuropathological abnormalities related to the HIV infection of the brain may be due to an increased production of TNF- α which mediates various neurotoxic effects.⁹ In particular, this cytokine might be involved in the process of white matter destruction associated with ADC.⁹

In our study, elevated concentrations of TNF- α were observed in the CSF of patients with cryptococcal meningitis and, to a lesser but still significant extent, in ADC individuals. This discrepancy may depend on the acute phase of opportunistic infections in which more TNF- α is released, while in ADC the lower concentrations of this cytokine might be related to the longer evolution of this pathological condition. Nevertheless, in ADC lower amounts of TNF- α , which are present over a longer period of time, might exert a much greater detrimental effect on the host. Interestingly, we observed in patients with cryptococcal meningitis a decline in CSF TNF- α concentrations associated with the recovery of inflammatory status and the improvement of laboratory results, in terms of cryptococcal antigen titre reduction. These findings seem to support the correlation between infection and TNF- α concentration in CSF.

The two HIV-infected patients who were asymptomatic but had raised concentrations of TNF- α showed no evidence of subclinical HIV encephalopathy as assessed by neuropsychometric analysis. They did, however, have elevated concentrations of β_2 microglobulin with detectable HIV antigen p24 in the CSF. In agreement with other investigations, the detection of these two markers in the CSF of patients without ADC may reflect a presymptomatic stage of neurological involvement by HIV.^{10 11}

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