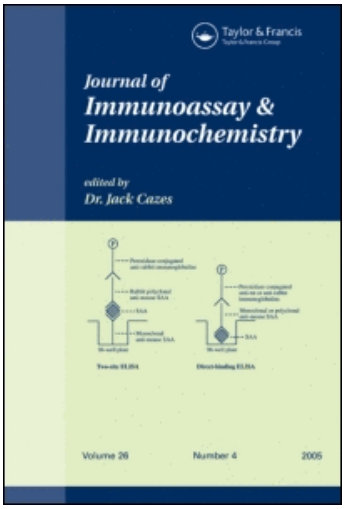

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Immunodetection of a Hepatitis C Virus (HCV) Antigen and Th1/Th2 Cytokines in Cerebrospinal Fluid of Meningitis Patients

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ABSTRACT

Infection with hepatitis C virus (HCV) has become the most important public health problem in Egypt. HCV infection has been implicated in diseases of the central nervous system. Cerebrospinal fluid (CSF) and serum samples from 91 patients with meningitis (62 males and 29 females, mean age of 37 years) were investigated. Anti-HCV antibodies and HCV antigen were evaluated in patients CSF and serum using enzyme linked immunosorbent assay. The levels (mean $\pm$ SD pg/ml) of Th1 cytokines (IFN-γ and TNF-α) and Th2 interleukines (IL-10 and IL-4) were also determined. The anti-HCV antibodies were detected in high percentages both in CSF samples (71%) and in sera (90%). Also, the HCV antigen was detected in about 60% of tested CSF and serum samples. The levels of IFN-γ and IL-10 cytokines were significantly higher ($P<0.05$) in both serum and CSF of patients positive for HCV antigen than those negative.

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HCV antigen was detected in the CSF of meningitis patients with a significant upregulation of Th1 and Th2 responses. The high incidence of HCV infection may draw light on the etiological role of HCV in the pathogensis of meningitis diseases in our study group.

Key Words: HCV; CSF; HCV-NS4 antigen; Serum; Dot-ELISA.

INTRODUCTION

Hepatitis C virus (HCV) is a single strand RNA hepatotrophic virus infecting 170 millions around the world[1] and more than 20% of Egyptian blood donors.[2] Patients with chronic HCV are likely to have significant changes in their physical and mental well-being, most commonly manifested as fatigue and depression;[3,4] HCV itself could affect cerebral function.[5] HCV infection has been implicated in diseases of the central nervous system (CNS). It was speculated that HCV replication in CNS occurs in cells of macrophage/monocyte lineage similarly to the mechanism postulated for HIV-1 neuroinvasion.[6,7] Radkowski et al.[8] detected HCV RNA negative strand, which is a viral replicative intermediate, in brain tissue in HCV-infected patients. In a recent study, Laskus et al.[9] found HCV sequences in cerebrospinal fluid (CSF) in 8 of 13 HCV-positive patients, suggesting that HCV can enter the brain, and peripheral blood mononuclear cells (PBMC) may be carrying HCV into the CSF. However, mechanisms underlying viral persistence in chronic HCV are not yet clarified, but a complex interplay of virological and immunological factors is implicated.[10] T-cell immunoregulatory cytokines may play a crucial role in the host response to HCV infection. While T-helper type 1 cytokines are required for host antiviral immune responses, T-helper type 2 cytokines can inhibit the development of these effector mechanisms.[11] The present study was undertaken to detect anti-HCV antibodies and an HCV antigen[12,13] in CSF and to evaluate the influence of HCV infection on the CSF levels of Th1 cytokines (IFN-γ and TNF-α) and Th2 interleukines (IL-10 and IL-4).

EXPERIMENTAL

Serum and CSF Samples

Serum and CSF samples of 91 Egyptian individuals (62 males and 29 females aged 21–57 years; mean age of 37 years) kindly provided by staff of Abbassia Fever Hospital, Cairo, Egypt were included in the present study

after approval from the hospital ethics committee. They were considered likely to have meningitis on the basis of clinical criteria, patient history, physical signs, and symptoms. The laboratory criteria were based on CSF examination (aspect, cytology, biochemistry, Gram staining, and culture). Patients who had received intravenous antibiotic were excluded from our analysis. Sera of HCV infected individuals (10 males and 6 females aged 20–58 years; mean age of 36 years) and healthy volunteers (10 males and 6 females aged 20–58 years; mean age of 35 years) were used as controls. An informed consent was obtained from all individuals participated in the present study and they were fully informed concerning the nature of the disease and the diagnostic procedures involved.

Enzyme Linked Immunosorbent Assay (ELISA) for Anti-HCV Antibodies

Anti-HCV antibodies were detected in serum and CSF using Ortho HCV Ab ELISA test III kit (Ortho Clinical Diagnostics Systems, Raritan, NJ) according to the manufacture instruction.

ELISA for HCV-NS4 Antigen

CSF (1 : 2) or serum (1 : 10) in coating buffer (pH 9.6) were tested for HCV antigen according to Attallah et al.[12,13] In brief, sample coated ELISA plate was sealed with an acetate plate sealer and incubated overnight at 2–8°C. After blocking of free binding sites, specific anti-HCV-NS4 antibody diluted in PBS-T20 was added (50 μL per well) and incubated at 37°C for 2 hr. After washing, anti-rabbit IgG alkaline phosphatase conjugate was added and incubated at 37°C for 1 hr. The amount of coupled conjugate was determined by incubation with *p*-nitrophenyl phosphate substrate for 30 min at 37°C. The reaction stopped and absorbance was read at 405 nm using ELISA reader (Σ960; Axiom, Burstadt, Germany). The cutoff level of ELISA above or below which the tested sample is considered positive or negative was calculated as the mean ELISA optical densities of 36 serum samples from healthy volunteers $\pm$ 3 standard deviation [i.e., $0.224 \pm (3 \times 0.025)] = 0.299$.

Cytokine Measurements

TNF-α, IL-4, and IL-10 were quantified by commercially available ELISA kits (Quantikine Kit, R&D Systems, Inc., Minneapolis, USA),

according to the manufacturers' instructions. After 2 hr of sample incubation, plates were washed 3 times, then 200-μL of an enzyme linked polyclonal antibody were added to each well. Following a wash to remove any unbound antibody, enzyme reagent a substrate solution is added to the wells and color develops in proportion to the amount of cytokines. The color development is stopped and the intensity of the color is measured. IFN-γ Th1 cytokines were quantitied by commercially available ELISA (ABC Diagnostics, New Damietta, Egypt), according to the manufacturers' instructions. In brief, after 30 min incubation of standard and sample, the coated plate with monoclonal antibody to IFN-γ was washed 5 times then 50-μL of polyclonal antibody to IFN-γ were added to each well. Following a wash to remove any unbound antibody 50-μL per well of anti-rabbit alkaline phosphatase conjugate (Sigma) were added to each well. Enzyme reagent; a substrate solution is added to the wells and color develops in proportion to the amount of IFN-γ.

Statistical Analyses

Data were expressed as mean ± SD and were analyzed by using the statistical analysis program package Instate Software for Science, version 2.3 (Graphpad Software, Inc., San Diego, CA). *P*-values (two-tailed) of <0.05 were considered significant.

RESULTS

Detection of Anti-HCV Antibody and HCV Antigen in Serum and CSF

Of tested serum samples, 66/73 (90%) were positive for anti-HCV antibody, and 44/73 cases (60%) were positive for HCV antigen. Of tested CSF samples from meningitis patients, 65/91 (71%) were positive for anti-HCV antibodies, and 53/91 (58%) were positive for HCV antigen.

Determination of Th1/Th2 Cytokines in Serum and CSF

Serum levels of IFN-γ (83.05 ± 80.3 pg/mL) and IL-10 (70.6 ± 55.6 pg/mL) in patients positive for HCV antigen were significantly higher ($P = 0.03$ and 0.0009) than those (37.9 ± 37.7 and 22.0 ± 18.9) in patients negative for HCV antigen, respectively. Patients positive for HCV antigen

Table 1. Detection of Th1 and Th2 cytokines in CSF of meningitis patients positive and negative for HCV antigen.

	CSF levels (mean ± SD, pg/ml) of T-helper cytokines		
Cytokines	HCV antigen positive ($n = 21$)	HCV antigen negative ($n = 19$)	P-value[a]
Th1			
IFN-γ	78.02 ± 71.29	36.55 ± 31.07	0.025
TNF-α	123.29 ± 117.8	63.42 ± 61.20	0.055
Th2			
IL-4	37.57 ± 19.43	30.21 ± 14.23	0.184
IL-10	46.14 ± 34.21	24.21 ± 19.79	0.019

[a]Two sided P-values <0.05 between HCV antigen positive and negative considered significant.

had higher levels of TNF-α (88.6 ± 91 pg/mL) compared with those patients negative for HCV antigen (65.3 ± 63.3 pg/mL), but did not reach a significant level ($P > 0.05$).

The CSF levels of IFN-γ (78.02 ± 71.29 pg/mL) and IL-10 (46.14 ± 34.21 pg/mL) in patients positive for HCV antigen were significantly higher ($P = 0.025$ and 0.019) than those (36.55 ± 31.07 and 24.21 ± 19.79) in patients negative for HCV antigen, respectively. Patients positive for HCV antigen had higher CSF level of TNF-α and IL-4 (123.29 ± 117.8 pg/mL; 37.57 ± 19.43) compared with those patients negative for HCV antigen (63.42 ± 61.2 pg/mL); 30.21 ± 14.23 but did not reach a statistical significant difference ($P > 0.05$) (Table 1).

All healthy controls showed very low serum levels of Th1 (IFN-$\gamma < 31.2$ pg/ml and TNF-$\alpha < 15.6$ pg/mL) or Th2 (IL-4 < 31.2 pg/mL and IL-10 < 7.8 pg/mL) cytokines, whereas positive HCV controls showed significantly high levels of TNF-α (110.7 ± 16.2); IFN-γ (55.4 ± 45.9); IL-4 (37.3 ± 2.5); IL-10 (43.3 ± 5.7).

DISCUSSION

HCV infection appears to be endemic in most parts of the world, with an estimated prevalence of 24.3% in Egypt.[14] HCV infection has been implicated in diseases of the CNS. Laskus et al.[9] found HCV sequences in CSF of HCV infected patients suggesting that HCV can enter the brain, and PBMC may be carrying HCV into the CSF. Also, the presence of anti-HCV antibody

and HCV RNA in CSF indicated that HCV had reached the intrathecal compartment, suggesting the direct viral involvement in the pathogenesis of chronic sensory neuropathy.[15] Moreover, HCV RNA has been detected in CSF from both HIV-positive and HIV-negative patients.[16] In the present study, an HCV antigen and anti-HCV antibodies were detected in high percentages in both CSF and serum samples. It has been reported that in the case of HBV or HCV associated postinflammatory liver cirrhosis purulent, bacterial meningoencephalitis, might be connected with rapid liver disease progression and even the death of a patient.[17] Eggers et al.[18] suggested a distinct pattern of viral replication in the CNS in HIV-1 encephalopathy. Our results show a high incidence of anti-HCV antibodies and HCV antigen in the CSF and sera of our patients with background of meningitis in a country with high prevalence of HCV infection.[14] This suggests the presence of viral replication in the CSF and may draw a light on an etiological role of HCV in the pathogenesis of meningitis of our study group. In addition, the levels of investigated Th1 cytokines and Th2 interleukins were higher in patients positive for HCV antigen than in patients negative for HCV antigen in both serum and CSF. These results suggest that the significant upregulation of Th1 and Th2 responses are highly associated with chronic HCV infection. Spanakis et al.[19] reported that both Th1/Th2 responses are highly associated with chronic HCV infection irrespective of the hemodialysis status, HCV viremia, and liver biochemistry parameters. Helper-T cell subpopulations, Th1 and Th2, seem to contribute to progression of chronic HCV in a reciprocal fashion.[11,20] Protective anti-inflammatory cytokines are also secreted during the course of meningitis. IL-10 inhibits the release of proinflammatory cytokines including TNF-α.[21] There is an evidence that IL-12 together with TNF-α as a costimulator are probably responsible for the production of IFN-γ and contribute to local host defense in the CSF compartment.[22] In conclusion, our results may draw light on the etiological role of HCV in the pathogensis of meningitis in our study group. The high incidence of HCV infection and the significant up-regulation of different cytokine profiles of T-cells within CSF of patients positive for HCV antigen with meningitis background illustrate a different behavior of immune response, that may have pathogenic implications.

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