LETTER TO THE EDITOR

CYTOKINE PROFILES IN PERIPHERAL BLOOD MONONUCLEAR CELLS AND SERA FROM PATIENTS WITH ACUTE SELF-LIMITED HEPATITIS A

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Hepatitis A, caused by Hepatitis A virus (HAV), is an enterically transmitted self-limiting disease that does not lead to chronicity (*1*). The infection with HAV provides a life long immunity. The mechanisms of viral clearance are poorly understood (*2*). Cytokines are critical components of both humoral and cell-mediated immune responses that determine the course of the disease (*3*). In order to understand the role of cytokines in HAV infection, Th1 (interleukin 2 (IL-2) and interferon γ (IFN-γ)) and Th2 (IL-4 and IL-10) cytokine profiles in peripheral blood mononuclear cells (PBMCs) and sera from patients with acute self-limited hepatitis A were studied.

Forty-two serologically confirmed (positive for IgM anti-HAV antibodies) pediatric acute self-resolving hepatitis A patients (mean age of 6.3 years) were analyzed. The male to female ratio was 1.8:1. Five apparently healthy age-matched control patients without any apparent infection were also included. All the patients were negative for hepatitis B surface antigen (HBsAg) and none had autoimmune, metabolic or drug-associated liver disease.

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Abbreviations: ALT = alanine transaminase;CTLs = cytotoxic T lymphocytes; HAV = Hepatitis A virus; HBsAg = hepatitis B surface antigen; HIV-1 = Human immunodeficiency virus 1; IFN-γ = interferon-γ; IL = interleukin; PBMCs = peripheral blood mononuclear cells; PHA-P = phytohemaglutinin P; PMA = phorbol-12-myristate-13-acetate

An informed consent was obtained from the parents/guardians of patients and control patients. Th1 and Th2 cytokine levels were estimated in serum samples (n=32) and supernatants of mitogen-stimulated lymphocyte cultures (n=10). Briefly, PBMCs at a density of $1x10^6$ cells/ml were stimulated with phytohemagglutinin P (PHA-P, 5 μg/ml, Sigma) and phorbol-12-myristate-13-acetate (PMA, 1 μg/ml, Sigma) at 37°C in 5% CO_2. Unstimulated resting cells were used as controls. To assess the background release of cytokines, the culture supernatants from resting cells were collected after the incubation at 37°C for 24 hrs. The supernatants from cells stimulated with PHA and PMA were collected after an optimum period of 48 hrs and 72 hrs, respectively, and were further assayed for the concentration of individual cytokines using the ELISA SETS (BD Pharmingen, USA). The sensitivities of the ELISA SETS were 7.8 pg/ml for IL-2, IL-4 and IL-10 and 4.6 pg/ml for IFN-γ. The Wilcoxon Signed- Ranks statistical test was used for evaluation of significance of differences.

The obtained results are summarized in the table. As regards the serum cytokines, their levels in control children were below the detection limits of the assay. In the patients, the IFN-γ levels was higher than that of IL-10 ($p < 0.001$). Also IL-2 levels were higher than that of IL-4 ($p < 0.01$). As regards the PBMC cytokines in control children, the levels of IL-2 and IL-4 were below the detection limit, while the levels of IFN-γ and IL-10 levels were signifiacant. In the patients, the IFN-γ level was higher than that of controls

	Cytokines ± SD (pg/ml)			
	IFN-γ	IL-2	IL-4	IL-10
Serum				
Controls (n = 5)	<4.6	<7.8	<7.8	<7.8
Patients (n = 32)	106.6 ± 197.6	91.5 ± 233.9	34.8 ± 72.2	54.3 ± 113.7
PBMCs				
Controls (n = 5)	13.9	<7.8	<7.8	21.5
Patients (n = 10)	184 ± 313	128 ± 123	15.3 ± 16.1	12.4 ± 14.5

($p < 0.05$), while the IL-10 level was normal, the IL-2 level was higher than that of IL-4 level ($p < 0.01$) and the FN-γ level was higher than that of IL-10 ($p < 0.01$).

The role of cellular immunity in determining the course of HAV infection or in protection against reinfection has not been studied. The extent to which interferons contribute towards the resolution of HAV is also unknown (*4*). IFN-γ is released from virus-infected cells and confers antiviral protection on neighbor cells. In our study, all the acute resolving hepatitis A patients have universally released IFN-γ, suggesting its possible involvement in the elimination of HAV. There appeared a noteworthy report on a sharp rise in IFN-γ levels among HBV DNA-positive carriers super-infected with HAV, leading to suppression of HBV replication (*5*). In conclusion, our results demonstrate for the first time that acute resolving hepatitis A patients produce a type I biased cytokine response.

References

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