# Intracellular expression of pro-inflammatory cytokines (IL-1α, TNF-α, and IL-6) in chronic hepatitis C

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## Abstract

The study aimed at localizing TNF-α, IL-1α, IL-6 at light and electron microscope levels in patients with chronic hepatitis C, using the immunocytochemical techniques in biopsy material from patients with chronic hepatitis C and at comparing the expression of the cytokines with histopathological changes. Our studies demonstrated an augmented expression of all cytokines in liver biopsies in chronic hepatitis C, in comparison with respective values, obtained in control biopsy material. The highest expression of the cytokines was observed in hepatocytes. That was confirmed by electron microscopy, which demonstrated the cytokines mainly in altered ER cisterns and in the cytoplasm. In children, the expression of IL-1α was negatively correlated with staging, while in adult patients; the staging was positively correlated with the expression of TNF-α. The new element involves demonstration of cellular and subcellular expression of TNF-α, IL-1α and IL-6 in hepatocytes in in vivo infection.

**Key words:** chronic hepatitis C; pro-inflammatory cytokines; immunocytochemistry.

## Introduction

The role of pro-inflammatory cytokines has been documented in acute phase reactions in the liver, in normal proliferation of hepatocytes, in autoactivation of Kupffer cells and proliferation of Ito cells, in cirrhotic processes in the liver and in regeneration of the organ in chronic hepatitis C [1-3]. In chronic hepatitis C, augmented serum levels of TNF-α, IL-1 and IL-6 have been detected [4-6]. Controversies prevail as to the amounts of the cytokines, detected in tissues in chronic hepatitis C and as to the correlations between the expression of the cytokines on one hand, and grading and/or staging of intrahepatic viral load on the other [2, 4, 7, 8]. Cellular expression of pro-inflammatory cytokines in the liver has been observed mainly in Kupffer cells, activated stellate cells and in the endothelium of liver sinusoids [3, 6, 8]. Individual studies have shown that also hepatocytes are capable to produce cytokines in response to the infection with hepatitis C virus (HCV) [6, 7, 8].

The aim of the study was to investigate the intrahepatic localization of TNF-α, IL-1α and IL-6 in liver specimens from patients, chronically infected with HCV, to determine the cellular and subcellular localization of these cytokines. Attempts were also made to correlate the number of cells with cytokine expression with inflammation activity (grading) and the advancement of fibrosis (staging) and to compare cytokine expression in groups of children and adults with chronic hepatitis C.

## Material and Methods

Thirty-one patients were studied (19 children, and 12 adults) with chronic hepatitis C confirmed serologically (anti-HCV and HCV RNA positive), in whom liver biopsies were performed before anti-viral therapy. The control group consisted of six liver biopsies of serologically HCV, HBV, HCMV, EBV and HIV negative patients with non-specific changes in the liver. Tissue specimens were fixed in 10% formalin and embedded in paraffin for purposes of light microscopy. For electron microscopy, the material was fixed in paraformaldehyde/glutaraldehyde, embedded in epon, and ultrathin sections became subject of ultraimmunocytochemistry. Histopathological lesions were evaluated, following the classical H+E staining, as well as

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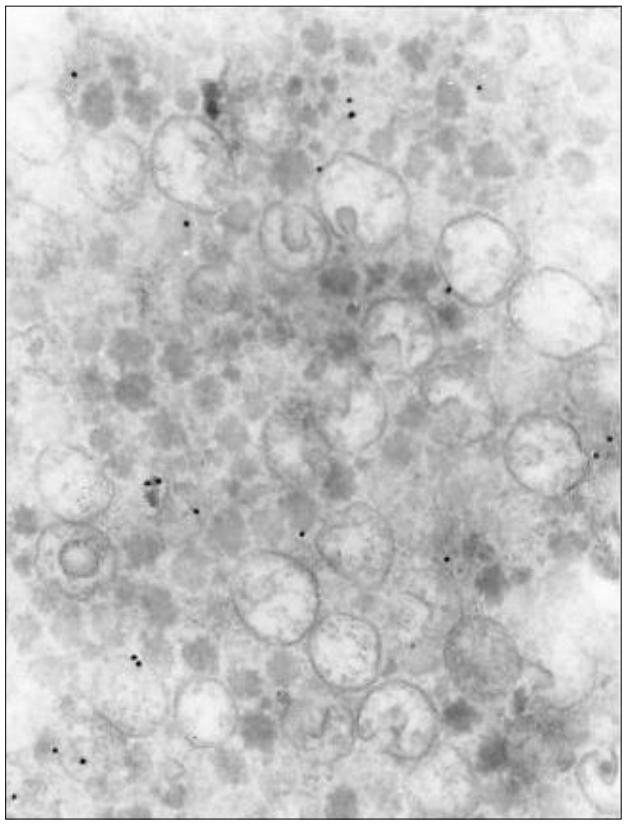
*Figure 1.* Localization of IL-6 in hepatocyte from patient with chronic hepatitis C. Original magn.x400

*Figure 2.* Localization of IL-6 in hepatocyte from patient with chronic hepatitis C. Gold particles overlay mainly dilated endoplasmic reticulum (ER) and cytoplasm. Streptavidin-colloidal gold. TEM x30.000



the tri-chromate technique, according to Masson, and periodic acid-Schiff with diastase pre-treatment. Each biopsy specimen was evaluated, using a simple numerical scoring system for the grade of portal/periportal necroinflammation (G1) (0-4), the grade of lobular necroinflammation (G2) (0-4), and for the stage of fibrosis (S) (0-4). Biopsies from chronic HCV patients were compared first between the two groups of patients (children and adults) and, then, in the entire group of patients (n=31), the expression of each cytokine was compared between lobules and portal spaces, as well as the total expression of a cytokine in the patients was compared with the total expression of the cytokine in control biopsies. The following specific MAbs were used: (a) mouse anti-human TNF-α antibody, (b) goat anti-human IL-1α antibody (c) mouse anti-IL-6 antibody (all from R&D Systems), (d) mouse anti-human macrophage antibody, CD68 (DAKO) and (e) anti-human Von Willebrand Factor antibody (DAKO EPOS A/S, Denmark). The studies followed the classical ABC technique alone or associated with the ImmunoMax amplification. Ultrathin sections were subjected to a labelling with 15 nm colloidal gold-coated streptavidin. The contents of three cytokines in liver biopsies, demonstrated by the ImmunoMax technique, were calculated by the semiquantitative technique, relating the score of 0 to 4 points to the fraction of stained cells (score 0 - 0% cells, 1 - less than 5% cells, 2 - 5-20% cells, 3 - 20-40% and 4 - more than 40% positive cells). The preparations were examined under a light microscope, at 400x magnification. In each section of the liver biopsy, five fields in hepatic lobules and periportal area and, at least, five different portal spaces in each specimen were examined. Statistical analysis took advantage of the Mann-Whitney U test for nonparametric independent data and the Wilcoxon test for nonparametric dependent data. Correlations between data rows were determined, employing Spearman's rank correlation index.

## Results and Discussion

Both the grade of inflammation and the stage of fibrosis were significantly lower in children than those in adults (G1+G2, 1.8±0.2 vs. 3.7±0.4, respectively, $p<0.004$ and S, 1.1±0.2 vs. 2.5±0.3, respectively, $p<0.008$). Our studies demonstrated an augmented expression of all pro-inflammatory cytokines and of IL-1α and IL-6, particularly in the livers of chronic hepatitis C patients of either age group, as compared to their expressions in the control ($p<0.05$). This has corroborated our earlier observations and results of other investigators [6, 7, 8]. The augmented expression of the three cytokines was noted in hepatic lobules, as compared to portal spaces ($p<0.0001$). No difference was detected in the total expression of studied cytokines between the affected children and adult patients. In children, significantly higher numbers of cells with IL-6 expression were noted, as compared to cells with TNF-α ($p<0.05$) and IL-1α ($p<0.02$) expression. In adult patients cells with IL-1α expression and those with IL-6 expression were more numerous than those with TNF-α expression ($p<0.05$). In the lobules, expression of the studied cytokines pertained mainly the cytoplasm of hepatocytes (Fig. 1), which corroborated by ultrastructural studies. Apart from the cytoplasm, ultrastructural studies

documented presence of the cytokines also in altered cisterns of endoplasmic reticulum (Fig. 2). This confirmed the observations of other investigators related to IL-6 [6]. The novel element in the present results involved ultrastructural demonstration of the TNF-α and IL-1α and their demonstration, first of all, in hepatocytes. The other cells with the expression of cytokines included individual cells of liver sinusoids (macrophages and endothelial cells) and individual lymphocytes in inflammatory infiltrates. In the control material, a scanty expression of the cytokines was noted in individual hepatocytes and cells of liver sinusoids. In children, a negative correlation was disclosed between the expression of IL-1α protein and staging (Spearman's correlation coefficient of -0.557; $p<0.05$), in adults, a positive correlation was detected between the expression of TNF-α protein and staging (Spearman's correlation coefficient of 0.689; $p<0.05$). The results confirmed the literature data, related to the correlation between serum cytokine levels and histopathological evaluation of the biopsies [5, 6]. Our results have pertained the expression of TNF-a, IL-1a and IL-6 in HCV infection in vivo and may point to a direct involvement of the cytokines, both in the development of inflammation and fibrosis of the organ, as well as the ineffective anti-viral defence.

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