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Hepatic Expression of CXC Chemokines Predicts Portal Hypertension and Survival in Patients With Alcoholic Hepatitis

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Background & Aims: **Alcoholic hepatitis (AH) is characterized by hepatocellular damage, inflammation, and fibrosis. We performed a prospective study to associate hepatic expression of the CXC subfamily of chemokines with histology findings and prognosis of patients with AH.** ***Methods:*** **Liver biopsy samples from 105 patients with AH and 5 normal liver samples (controls) were evaluated for steatosis, inflammation, fibrosis, and cholestasis. Computer-based morphometric analysis assessed the numbers of infiltrating $CD3^+$ T cells and $CD15^+$ cells (neutrophils); terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling staining was used to quantify apoptosis. Expression of CXC and CC chemokines and selected signaling components were assessed by quantitative reverse-transcription polymerase chain reaction; protein levels of interleukin (IL)-8 and Gro-α also were determined by immunohistochemistry. Serum levels of IL-8 and Gro-α were measured by enzyme-linked immunosorbent assay. The Cox regression model identified variables associated with mortality.** ***Results:*** **Most patients (75%) had severe AH; their 90-day mortality rate was 21.9%. In AH liver samples, expression of the CXC subfamily members IL-8, Gro-α, CXCL5, CXCL6, CXCL10, and platelet factor 4 was up-regulated and compared with controls. The CC chemokine CCL2, but not CCL5, also was up-regulated. Higher expression levels of IL- 8, CXCL5, Gro-γ, and CXCL6 were associated with worse prognosis. Expression of CXC components correlated with neutrophil infiltration and the severity of portal hypertension. In the multivariate analysis, IL-8 protein levels were an independent predictor of 90-day mortality. IL-8 and Gro-α serum levels did not correlate with prognosis.** ***Conclusions:*** **Hepatic expression of CXC components correlates with prognosis of patients with AH. Reagents that target CXC chemokines might be developed as therapeutics.**

Alcoholic hepatitis (AH) occurs in patients with heavy alcohol intake and it is characterized by hepatocellular damage; inflammatory cell infiltrate, predominantly by neutrophils; and rapidly progressive fibrosis.[1] The severe forms are associated with liver failure and portal hypertension, leading to a short-term poor prognosis.[2,3] Current therapies for this condition fail in many patients. Prognosis of patients with AH has been assessed using the Maddrey's Discriminant Function.[4] Recently, we described the ABIC score, a new scoring system that allows prognostic stratification of these patients.[5] The ABIC score identifies patients with low (<6.71), intermediate (6.71–8.99), and high risk (≥9) of death at 90 days and 1 year.

The pathogenesis of AH is poorly understood. An interaction between neutrophils and cytokines may play a role.[6] Patients with chronic alcohol abuse have increased endotoxin serum levels leading to Kupffer cell activation, neutrophil recruitment, and increased production of cytokines.[6,7] The degree of neutrophil infiltration as well as serum levels of proinflammatory cytokines such as tumor necrosis factor α, and interleukins (ILs) 1, 6, and 8, are associated with disease severity.[7–9] However, the use of monoclonal antibodies against tumor necrosis factor α in patients with severe AH is associated with more severe infections and a higher mortality rate.[10,11]

Chemokines are classified into 4 subfamilies: CC, CXC, CX3C, and C. CXC chemokines can be subclassified into ELR^+ or ELR^- based on the presence of a tripeptide motif ELR (Glu-Leu-Arg) at the NH_2 terminus. The ELR motif appears to be important in ligand/receptor interactions on neutrophils. IL-8, CXCL5, CXCL6, CXCL7, Gro-α, Gro-β, and Gro-γ are members of the ELR^+ CXC family. In contrast, ELR^- CXC chemokines have a reduced ability to induce neutrophil chemotaxis. This sub-

Abbreviations used in this paper: ALD, alcohol-induced liver disease; AH, alcoholic hepatitis; HR, hazard ratio; HVPG, hepatic venous pressure gradient; IL, interleukin; CXCR, chemokine receptor; PF4, platelet factor 4; ENA-78, epithelial-derived neutrophil-activating peptide 78; GCP-2, granulocyte chemotactic protein 2; γIP-10, interferon-γ–induced protein 10; IFN, interferon; MCP-1, monocyte chemoattractant protein 1; STAT-1, signal transducer and activator of transcription factor 1; RANTES, regulated upon activation, normally T-expressed, and presumably secreted.

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group includes interferon-γ–induced protein (γIP-10) and PF4 (platelet factor 4). Importantly, ELR$^+$ chemokines bind CXC receptor 1 (CXCR1) and CXCR2, whereas γIP-10 and PF4 interact with CXCR3.[12,13]

We hypothesize that CXC chemokines, in particular ELR$^+$ CXC chemokines, may play an important role in the pathogenesis of AH. This assumption is based on the following data. First, the CXC family of chemokines includes a number of ligands and receptors that play a major role in neutrophil infiltration of chronically damaged tissues.[14–16] Second, neutrophil infiltration is a hallmark histologic finding in patients with AH and has prognostic significance.[17–23] Third, livers from patients with AH have overexpression of IL-8 and Gro-α compared with normal livers.[23,24] Moreover, studies in patients with AH have shown that IL-8 is present in vascular endothelium, inflammatory cells, intrahepatic bile ducts, and in fibrous septa.[25] Fourth, it is likely that the biological effects of CXC chemokines on liver cells may play a role in AH.[2]

The current study was undertaken to investigate the pathogenic role of CXC chemokines in AH. For this purpose, we investigated the impact of hepatic gene expression of CXC chemokines on disease severity and survival in patients with AH.

Materials and Methods

Patients

We prospectively included patients admitted to the Liver Unit (Hospital Clínic, Barcelona) between January 2000 and September 2007 with clinical, analytic, and histologic features of AH. Inclusion criteria were as follows: patients with active alcohol abuse were defined according to the Diagnostic and Statistical Manual of Mental Disorders IV[26] and excessive ethanol consumption (>60 g/day) for at least 3 months before admission; increased aminotransferase levels (aspartate aminotransferase [AST] > alanine aminotransferase [ALT]), high γ-glutamyl transpeptidase and bilirubin serum levels, and histologic diagnosis of AH characterized by the presence of hepatocellular damage (hepatocellular ballooning and presence of Mallory bodies), inflammatory infiltrate (neutrophils), and pericellular fibrosis. Patients with hepatocellular carcinoma or any other potential cause of liver disease were excluded from the study. Liver biopsy was obtained using a transjugular approach because most patients with AH have severe coagulation disorders, and to measure portal pressure gradient. All patients received nutritional and psychological support for achieving alcohol abstinence. Patients with severe AH (ABIC score, ≥6.71) were treated with 40 mg/day prednisone for 4 weeks followed by a 2-week taper, unless they had any contraindication (severe bacterial infection or diabetes mellitus with poor metabolic control) to corticosteroid treatment. The study was approved by the Ethics Committee of the Hospital Clínic and all patients gave informed consent.

Histologic Analysis and Hepatic Hemodynamic Measurements

Liver biopsy specimens were formalin-fixed and paraffin-embedded. Liver specimens (3-μm thick) were stained with H&E and Masson's trichrome. Biopsy specimens were assessed blindly by the same pathologist (R.M.). Histologic analysis was performed as follows: (1) degree of hepatocellular damage/ballooning (0, none; 1, mild; 2, moderate or severe) and presence of Mallory bodies, giant mitochondria, and cholestasis (0, no; 1, yes); (2) degree of lymphocytic infiltration (0, none; 1, mild; 2, moderate; 3, severe); (3) degree of neutrophil infiltration (0, none; 1, mild; 2, moderate; 3, severe); (4) degree of steatosis (0, <10%; 1, 10%–33%; 2, 33%–66%; 3, >66%); (5) steatosis type (0, macrovesicular; 1, microvesicular; 2, mixed); (6) steatosis distribution (0, focal; 1, diffuse); (7) degree of lobular fibrosis (0, none; 1, mild; 2, moderate; 3, severe); (8) lobular fibrosis distribution (0, zone 3; 1, zones 2 and 3; 2, panlobular); (9) fibrosis stage (0, no fibrosis; 1, portal; 2, portal fibrosis and few septa; 3, septal fibrosis without cirrhosis; 4, cirrhosis), (10) cholestasis type (0, canalicular; 1, mild hepatocellular; 2, severe hepatocellular; 3, canalicular and hepatocellular).

Hepatic hemodynamic assessment was performed within 48 hours of admission. The portal pressure was estimated on the hepatic venous pressure gradient (HVPG), as described in detail previously.[27]

Hepatic Gene Expression Analysis

Hepatic gene expression was evaluated in 49 patients. In the remaining patients, the amount of liver tissue obtained only allowed histologic analysis. Liver biopsy specimens were submerged in an RNA stabilization solution (RNAlater; Ambion, Austin, TX) and stored at −20°C until RNA extraction. Total RNA was extracted with TRIzol (Life Technologies Inc, Rockville, MD). RNA integrity and concentration was assessed with a microfluidic glass chip platform (Bioanalyzer 2100; Agilent, Palo Alto, CA). Five hundred micrograms of total RNA were retrotranscribed with a high-capacity complementary DNA Archive Kit (Applied Biosystems, Foster City, CA). Eleven predesigned Taq-Man assays for target genes of CXC chemokine family were assessed in patients with AH: CXCL1 or Gro-α, CXCL2 or Gro-β, CXCL3 or Gro-γ, CXCL4 or PF4, CXCL5 or epithelial-derived neutrophil-activating peptide 78 (ENA-78), CXCL6 or granulocyte chemotactic protein 2 (GCP-2), CXCL7 or proplatelet basic protein, IL-8, CXCL10 or γIP10, CXCR1 or IL-8 receptor A, and CXCR2 or IL-8 receptor B. These genes were selected and distributed into a 384-well Taq-Man Low Density Array cards (Applied Biosystems). Samples were analyzed for quadruplicate using an ABI PRISM 7900 (Applied Biosystems) as described in detail else-