Balance between pro and anti-inflammatory cytokines in patients with acute alcoholic hepatitis

Sylvie NAVEAU (1, 4), Axel BALIAN (1), Frédérique CAPRON (2), Bruno RAYNARD (1), David FALLIK (1), Hélène AGOSTINI (1), Liliane GRANGEOT-KEROS (3), Alain PORTIER (4), Pierre GALANAUD (4), Jean-Claude CHAPUT (1), Dominique ÉMILIE (4)

(1) Service d’Hépato-Gastroentérologie, (2) Service d’Anatomie et Cytologie Pathologiques, (3) Service de Virologie-Immunologie, Hôpital Antoine Béclère, 92141 Clamart Cedex, France, Assistance Publique-Hôpitaux de Paris ; (4) INSERM U131, Institut Paris-Sud sur les cytokines, 92140 Clamart.

SUMMARY

The ability of endogenous IL-10 to modulate inflammatory response and to limit hepatotoxicity has been shown in several models of liver injury.

Aims — The objectives of this study were to evaluate the relationship between liver disease and the balance between pro and anti-inflammatory cytokines in acute alcoholic hepatitis.

Methods — Twenty-five patients with pure steatosis, 17 with cirrhosis and mild acute alcoholic hepatitis (discriminant function value < 32) and 41 patients with cirrhosis and severe acute alcoholic hepatitis (discriminant function value ≥ 32) were studied. Plasma levels of interleukin 10 (IL-10) and soluble TNF receptors (TNFsRp75 and 55) were analyzed using ELISA assays. Hepatocyte proliferative activity was assessed with proliferating cell nuclear antigen labeling index (PCNA-LI) on formalin-fixed paraffin embedded liver biopsy specimens.

Results — In patients with steatosis, cirrhosis with mild and severe acute alcoholic hepatitis, the plasma levels of IL-10 were higher (P < 0.05) than in healthy controls. Between day 1 and day 8, the TNFsRp55/IL-10 ratio increased by 137 ± 47 in the 10 patients with severe acute alcoholic hepatitis treated with prednisolone who died within 2 months and by 9.3 ± 14 in the 19 patients still alive at 2 months (P = 0.031). In patients with severe acute alcoholic hepatitis, PCNA-LI on liver biopsy was negatively correlated with the TNFsRp55/IL-10 ratio increase from day 1 to day 8 (r = -0.42, P = 0.11). PCNA-LI was positively correlated with TNFsRp75/TNFsRp55 ratio increase from day 1 to day 15 (r = 0.52; p < 0.05).

Conclusion — Our data suggest the anti-inflammatory system is up-regulated in patients with alcoholic liver disease. Nevertheless, in patients with severe acute alcoholic hepatitis, IL-10 production seems insufficient to modulate TNF-α cytotoxicity mediated by TNFRp55.

Introduction

Regulation of necrosis and inflammation by cytokines involves an intricate balance between pro and anti-inflammatory cytokines. Tumor necrosis factor α (TNF-α) is one of the pro-inflammatory cytokines likely to play an important role in alcoholic liver injury [1].

The multiple activities of TNF-α are mediated through two high-affinity receptors, TNF receptor p55 (TNFRp55) and TNFRp75. Soluble TNF receptors p55 and p75 are separated by proteolytic cleavage from the extracellular portions of their respective membrane associated TNF receptors [2].

Leist et al. [3] have reported that TNFRp55 activation was both necessary and sufficient for TNF-induced hepatocyte toxicity in mouse liver. Moreover, Yin et al. [4] have recently reported...
that long-term ethanol feeding caused liver injury in wild type and TNFRp75 knockout mice but not in TNFRp55 knockout mice providing solid evidence in support of the hypothesis that TNF-α plays an important role in the development of early alcohol-induced liver injury via the TNFRp55 pathway.

Furthermore, we have previously shown in cirrhotic patients that the plasma levels of TNFsRp55 and the TNFsRp75/TNFsRp55 ratio were correlated more strongly with parameters of hepatocellular injury than the TNFsRp75 plasma levels, suggesting that the preferential increase of TNFsRp55 reflects the direct hepatotoxicity of TNF-α in alcoholic liver disease [5]. The decreased TNFsRp75/TNFsRp55 ratio was a specific marker of severe acute alcoholic hepatitis (AAH) and we have reported the favorable prognostic value of TNFsRp75/TNFsRp55 ratio increase between day 1 and day 15 (Δ TNFsRp75/TNFsRp55) during prednisolone treatment in patients with severe AAH [5]. Between day 1 and day 15, the decrease in TNFsRp55 plasma levels was significantly greater in the patients still alive at 2 months than in patients who died within 2 months (P < 0.05). In contrast, the time course of TNFsRp75 plasma levels was not different between the two groups [5].

IL-10 is a potent anti-inflammatory cytokine that inhibits the synthesis of pro-inflammatory cytokines by T helper type 1 T cells, mono/macrophages, and neutrophils [6]. IL-10 can be synthesized by several cell types within the liver, including Kupffer cells and hepatocytes. The ability of endogenous IL-10 to modulate the inflammatory response and to limit hepatotoxicity is shown in several models of liver injury [7].

The ultimate effect of TNF-α on hepatocytes in vivo is strongly influenced by other cytokines in liver tissue. For instance interruption of the gene for the anti-inflammatory cytokine interleukin-10 exacerbates TNF-mediated liver injury in mice [8]. IL-10 has been shown to protect mice from polymorphonuclear-mediated hepatotoxicity induced by LPS plus galactosamine or concanavalin A [9, 10].

Since direct hepatotoxicity of TNF-α is principally mediated by TNFRp55, we studied TNFsRp55 plasma level as an indicator of TNF-α system activation. We also studied the anti-inflammatory cytokine IL-10.

To evaluate the relationship between liver disease and the balance between pro and anti-inflammatory cytokines in acute alcoholic hepatitis, we assessed the plasma levels of IL-10, TNFsRp55 and of TNFsRp55/IL-10 ratio in patients with different stages of alcoholic liver disease and determined the evolution and the prognostic value of dysregulation of these cytokines in cirrhotic patients with severe AAH treated with prednisolone. We also investigated the role of pro and anti-inflammatory cytokines in modulating the proliferative activity of hepatocytes in patients with severe AAH.

**Patients and methods**

We prospectively studied 83 consecutive patients, 52 men, 31 women, mean age 49 ± 0.9 years with histologically proven alcoholic liver disease between October 1997 and October 2000. To be included in the study, alcoholic patients had to have consumed ≥ 50 g of alcohol daily over the previous year, have negative hepatitis B surface antigen and hepatitis C viral serologies and have given their appropriate consent. Their daily consumption of alcohol over the last 5 years was 115 ± 4 g (mean ± SE) and the total duration of alcohol abuse was 21 ± 0.6 years. Information on alcohol consumption was recorded using a specific questionnaire [11]. Exclusion criteria were gastrointestinal bleeding, bacterial infection, hepatocellular carcinoma, acute pancreatitis, severe associated disease and presence of anti-HIV antibodies. Three groups were defined by liver histology and by discriminant function (DF) value based on measurements of prothrombin time and serum total bilirubin [12]. Twenty-five patients had pure steatosis, 17 patients had cirrhosis with mild AAH (DF value < 32) and 41 patients had cirrhosis with severe AAH defined by a DF value ≥ 32. Morphological criteria were those accepted internationally [13] and alcoholic hepatitis was defined by liver cell damage, typically as ballooning degeneration with areas of necrosis and infiltration of polymorphonuclear leukocytes. We also studied 22 healthy controls, 15 men, 7 women, mean age 32 ± 2 years. Patients with severe AAH were treated with prednisolone for 4 weeks. Blood samples for determination of TNFRs and IL-10 were collected within 2 days of admission and in the last 29 patients with cirrhosis and severe AAH, on day 1 before starting prednisolone treatment, and on day 8 and day 15. Plasma concentrations of TNFRs and IL-10 were performed using ELISA assays according to the manufacturer’s recommendations (Amersham, les Ulis, France).

In the last 46 patients recruited hepatocyte proliferative activity was assessed using the proliferating cell nuclear antigen labeling index (PCNA-UL); this was confined to these patients as previously liver biopsies were not formalin fixed. PCNA is a 36 Kda nuclear protein present throughout the cell cycle, especially during the S phase. It acts as a cofactor for polymerase. PCNA was detected with the monoclonal antibody PC 10 (Novocastra Laboratories Ltd uk) and the avidin-biotin peroxidase system (SABC Duet Dako) according to the manufacturer’s protocol. Liver biopsy was performed within three days after admission and abstinence from alcohol.

**Statistical analysis**

All results are given as mean ± SEM. Differences between the various groups were analyzed with the Kruskal-Wallis test. Friedman’s two-way analysis of variance was used when more than paired variables were compared. The correlation between variables was evaluated with the Spearman test.

**Results**

**Circulating IL-10 and TNFsRp55/IL-10 ratio in the 83 patients with alcoholic liver disease**

Biological characteristics of patients are reported in table I. In patients with steatosis, cirrhosis with mild AAH and cirrhosis with severe AAH, the plasma levels of IL-10 were significantly higher (P < 0.05) than in healthy controls (table I). Plasma levels of IL-10 increased progressively with the severity of liver disease, reaching a maximum in cirrhotic patients with severe AAH (table II).

The plasma levels of TNFsRp55 were significantly higher in patients with cirrhosis and mild AAH and in patients with cirrhosis and severe AAH than in healthy controls (P < 0.05 and P < 0.05, respectively).

The TNFsRp55/IL-10 ratios were significantly lower in patients with pure steatosis and in cirrhotic patients with mild AAH than in healthy controls (P < 0.05 and P < 0.05, respectively).

In cirrhotic patients with severe AAH, the TNFsRp55/IL-10 ratio was not significantly different when compared to healthy controls (table II).

**Time course of plasma IL-10 and TNFsRp55 and TNFsRp55/IL-10 ratio in cirrhotic patients with severe AAH treated with prednisolone**

From day 1, just before starting prednisolone administration, to day 15 of treatment, the plasma levels of IL-10, and the value
of the TNFsRp55/IL-10 ratio did not change significantly. This contrasted with plasma levels of TNFsRp55: in spite of a transient increase on day 8, plasma levels of TNFsRp55 on day 15 were significantly lower than on day 1 (P < 0.05) (table III).

Ten of 29 patients with cirrhosis and severe AAH treated with prednisolone and with TNFsRp and IL-10 levels determined on day 1 (before starting treatment), on day 8 and on day 15, died within 2 months. The time course of the TNFsRp55/IL-10 ratio in the 10 patients who died exhibited a different pattern during the first week of treatment compared to the 19 others. Between day 1 and day 8, the TNFsRp55/IL-10 ratio (△ TNFsRp55/IL-10) increased by 137 ± 28 patients with severe AAH (5.2 ± 0.8%) than in the 11 patients still alive at two months (2.4 ± 0.8%) (P < 0.03) but was not significantly different when compared to the 19 patients who died within two months (2.4 ± 0.7%) than in the 19 patients still alive at two months (6.3 ± 1%) (P < 0.05) (figure 1).

In contrast, IL-10 plasma levels and TNFsRp55 plasma levels on day 1, 8 were not different between the two groups (table IV).

Hepatocyte proliferative activity and balance between pro and anti-inflammatory cytokines

PCNA-LI was lower in the 8 patients with pure steatosis (2.2 ± 1.8%) than in the 10 patients with mild AAH (9.7 ± 1.6%) (P < 0.03) but was not significantly different when compared to 28 patients with severe AAH (5.2 ± 0.8%). In patients with severe AAH, PCNA-LI was significantly lower in the 9 patients who died within two months (2.4 ± 0.7%) than in the 19 patients still alive at two months (6.3 ± 1%) (P < 0.05) (figure 1).

In only 17 of the 28 patients with severe AAH, IL-10 and TNFsRp55 plasma levels were measured at the time of liver biopsy just before prednisolone treatment. In these patients PCNA-LI was negatively correlated with the TNFsRp55/IL-10 ratio increase from day 1 to day 8 (r = -0.42; P = 0.11) but the correlation was not significant. On the other hand, PCNA-LI was positively correlated with TNFsRp75/TNFsRp55 ratio increase from day 1 to day 15 (r = 0.52; P < 0.05). Six of the 9 patients with a TNFsRp75/TNFsRp55 ratio decrease died within two months versus 0 of the 8 patients with a ratio increase (P < 0.01). In the 6 patients who died within two months, PCNA-LI was lower (2.5 ± 0.8%) than in the 11 patients still alive at two months (5 ± 0.7%) (P < 0.05) (figure 2).

Discussion

Though there was no difference between the three groups, our data suggest an up-regulation of the anti-inflammatory system in patients with alcoholic liver disease. Such variations occur as early as the pure steatosis stage. On the other hand, up-regulation of the pro-inflammatory system was not significant in patients with pure steatosis. Thus contrary to patients with severe AAH, the TNFsRp55/IL-10 ratio was significantly lower in patients with pure steatosis and in cirrhotic patients with mild AAH than in healthy controls. These results suggest that in these two groups of patients up-regulation of pro-inflammatory cytokines was counteracted by higher plasma levels of anti-inflammatory cytokines and that IL-10 production was

<table>
<thead>
<tr>
<th>Pure steatosis</th>
<th>Cirrhosis with AAH and DF &lt; 32</th>
<th>Cirrhosis with AAH and DF ≥ 32</th>
<th>Healthy controls</th>
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<tbody>
<tr>
<td></td>
<td>(N = 25)</td>
<td>(N = 17)</td>
<td>(N = 41)</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>67 ± 33*</td>
<td>75 ± 39*</td>
<td>92 ± 25*</td>
</tr>
<tr>
<td>TNFsRp55 (pg/mL)</td>
<td>1183 ± 265</td>
<td>2335 ± 335*</td>
<td>4091 ± 198*</td>
</tr>
<tr>
<td>TNFsRp55/IL-10</td>
<td>39 ± 14*</td>
<td>42 ± 18*</td>
<td>128 ± 11</td>
</tr>
</tbody>
</table>

* P < 0.05 compared with healthy controls.
probably sufficient to regulate the inflammatory response and to limit tissue damage. On the other hand, since in patients with severe AAH plasma levels of TNFsRp55 were higher than in healthy controls, the value of the TNFsRp55/IL-10 ratio, which was not different from healthy controls, suggests that plasma levels of IL-10 were unable to control inflammation in liver injury.

These results are not in contradiction with previous experimental studies. In fact, in the liver of rats, which were fed ethanol and a diet containing medium-chain triglycerides, palm oil, corn oil, or fish oil by intragastric infusion, Nanji et al. [1] reported that the levels of TNF-α mRNA were higher and the levels of IL-10 mRNA were lower in fish oil fed rats who exhibited the most severe pathological changes.

In the same way in cirrhotic patients without AAH, Le Moine et al. [14] observed diminished ex vivo IL-10 production in isolated monocytes and suggested that this phenomenon was responsible for increased ex vivo TNF-α production by isolated monocytes. Furthermore, Taieb et al. [15] observed that both unstimulated and LPS-induced IL-10 production by monocytes from patients with severe AAH seemed to be lower than in the patients with cirrhosis and healthy controls, but the difference was not significant.

According to our results, the efficacy of corticosteroid therapy could probably be partially explained by decreased activation of the TNF-α system and the prognostic value of TNFsRp55/IL-10 ratio on day 8 which was predictive of death at 2 months; this reflects the absence of IL-10 over-expression to counteract the absence of decreased TNF-α system activation. Apart confirming the role of IL-10 in the pathophysiology of AAH, it is noteworthy that TNFsRp55/IL-10 ratio had earlier prognostic value than the TNFsRp75/TNFsRp55 ratio. We previously reported the prognostic value of the TNFsRp75/TNFsRp55 ratio decrease from day 1 to day 15 [5]. In the present study, we observed the prognostic value of the TNFsRp55/IL-10 ratio on day 8 and of its change between day 1 and 8. These results cannot be used to determine the best prognostic ratio, but they do contribute to our understanding of the effects of changes in cytokine levels. These results suggest that up-regulation of the anti-inflammatory system to control inflammation is insufficient to day 8. On the other hand, the direct hepatotoxicity of TNF-α probably persists longer.

We also investigated the role of pro- and anti-inflammatory cytokines in modulating the proliferation of hepatocytes in patients with severe AAH.

In adults, hepatocytes are quiescent with a very slow renewal rate and cell proliferation is an essential component in any

### Table III

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 8</th>
<th>Day 15</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10 (pg/mL)</td>
<td>109 ± 56</td>
<td>69 ± 31</td>
<td>78 ± 31</td>
<td>NS</td>
</tr>
<tr>
<td>TNFsRp55 (pg/mL)</td>
<td>3818 ± 268</td>
<td>4358 ± 325</td>
<td>3510 ± 286</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>TNFsRp55/IL-10</td>
<td>107 ± 14*</td>
<td>161 ± 20</td>
<td>123 ± 18</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = not significant. * P values correspond to comparison between day 1 and day 15

### Table IV

<table>
<thead>
<tr>
<th></th>
<th>Patients who died within 2 months (N = 10)</th>
<th>Patients still alive at 2 months (N = 19)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10 (pg/mL) Day 1</td>
<td>52 ± 16</td>
<td>139 ± 83</td>
<td>NS</td>
</tr>
<tr>
<td>Day 8</td>
<td>28 ± 7</td>
<td>91 ± 47</td>
<td>NS</td>
</tr>
<tr>
<td>Day 15</td>
<td>54 ± 10</td>
<td>91 ± 48</td>
<td>NS</td>
</tr>
<tr>
<td>TNFsRp55 (pg/mL) Day 1</td>
<td>3566 ± 412</td>
<td>3951 ± 352</td>
<td>NS</td>
</tr>
<tr>
<td>Day 8</td>
<td>4787 ± 468</td>
<td>4133 ± 432</td>
<td>NS</td>
</tr>
<tr>
<td>Day 15</td>
<td>4152 ± 584</td>
<td>3174 ± 296</td>
<td>NS</td>
</tr>
<tr>
<td>TNFsRp55/IL-10 Day 1</td>
<td>99 ± 16</td>
<td>112 ± 18</td>
<td>NS</td>
</tr>
<tr>
<td>Day 8</td>
<td>236 ± 37</td>
<td>121 ± 18</td>
<td>0.012</td>
</tr>
<tr>
<td>Day 15</td>
<td>129 ± 34</td>
<td>119 ± 20</td>
<td>NS</td>
</tr>
<tr>
<td>∆ TNFsRp55/IL-10 between Day 1 and Day 8</td>
<td>137 ± 47</td>
<td>9.3 ± 14</td>
<td>0.031</td>
</tr>
</tbody>
</table>

NS = not significant
The lack of a significant correlation can probably be explained by the small number of patients. Another important finding in our study is that hepatic regenerative processes are insufficient. In conclusion, our data suggest up-regulation of the anti-inflammatory system in patients with alcoholic liver disease. In patients with severe AAH, the balance between pro-inflammatory and anti-inflammatory cytokines was shifted towards the pro-inflammatory axis. In patients with severe AAH our results suggest IL-10 production was insufficient to modulate TNF-α cytotoxicity mediated by TNF-Rp55. This forms a rationale for targeting therapeutic agents against TNF-α in this disorder.

**REFERENCES**


