RESEARCH ARTICLE

Similar inflammatory response in alcoholic and non-alcoholic sepsis patients

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ABSTRACT. It is well known that alcoholics are prone to severe infections and that the immune system is impaired by chronic ethanol abuse. The aim of this study is to compare serum inflammatory mediators in response to sepsis in chronic alcoholic with sepsis, non-alcoholics with sepsis and non-infected alcoholics. *Method.* We included 25 alcoholics with sepsis, 34 non-alcoholics with sepsis, 34 non-infected alcoholics admitted for programmed withdrawal, and 27 healthy control subjects. After initial evaluation, blood samples were taken for determination of serum cytokine levels. *Results.* We found similar responses for the inflammatory mediators analyzed among our sepsis patients, regardless of alcohol abuse. The only difference was that alcoholics with sepsis showed lower CRP and G-CSF than non-alcoholic sepsis patients. There were no differences regarding leukocyte count. Alcoholics admitted for programmed withdrawal showed higher IL-6, IFN-γ, IL-10, Il-4 and ICAM-1 serum levels than healthy controls. Serum IL-5 levels were decreased in both alcoholic groups. *Conclusion.* The inflammatory response of alcoholics with sepsis is similar to that of non-alcoholic sepsis patients. However, the low G-CSF levels in alcoholic sepsis patients might suggest a predisposition to infections in alcohol abusers.

Key words: inflammatory response, cytokine, sepsis, alcoholism

Alcoholic patients are prone to severe infections [1-3]. The immune system is impaired by chronic ethanol abuse, which displaces the TH1 response of pro-inflammatory cytokine secretion, decreasing TNF and IL-6, to a TH2 anti-inflammatory response, increasing IL-10 and IL-4 [4-6]. T-cell-mediated immunity suppressed by ethanol is associated with altered cytokine production [7-10].

In a model of chronic alcoholism, a suppression of tumour necrosis factor (TNF)-α, as well as IL-6 and IL-10, has been reported [11, 12]. Nevertheless, most studies deal with animal experimental models or *in vitro* human investigations exploring the ability of macrophages and lymphocytes to secrete certain cytokines in response to infection. Clinical studies are scarce. Dossow (2004) reports decreased serum levels of IL-1β, Il-6 and IL-8 in the early phases of septic shock in ethanol abusers [13].

On the other hand, increased serum levels of TNF, IL-1, IL-6, IL-8, ICAM1 have been reported in alcoholic patients without liver disease [14-16]. This effect of ethanol should be considered when other factors are able to increase cytokine levels, such as sepsis, are analyzed in alcoholics.

The aim of this study was to investigate serum levels of certain cytokines and other inflammatory mediators in response to sepsis in chronic alcoholic patients, and to compare these parameters with those of non-alcoholic sepsis patients, non-infected alcoholic patients admitted for voluntary withdrawal, and non-alcoholic, non-infected control subjects.

DONORS AND METHODS

We included 25 alcoholic patients admitted for sepsis, average age 56 (50-64) [percentile 50th (25th-75th)] years, 23 males and two females; 34 non-alcoholics also admitted for sepsis, average age 69 (50-77) years, 19 males and 15 females; 34 non-infected alcoholics admitted for voluntary, programmed withdrawal in a hospital rehabilitation unit, average age 42 (39-51) years, 27 males and seven females; and 27 non-infected and non-excessive ethanol consumer control subjects, average age 44 (36-43) years, 18 males and nine females.

All of the alcoholic patients had a consumption of ethanol of more than 80 g/day, whereas none of the non-alcohol abuser septic patients and controls subjects exceeded the amount of 10 g/day. Alcoholic sepsis patients consumed 150 (95-165) g of ethanol/day, over a median period of 33 years; patients admitted for ethanol withdrawal consumed a median of 200 (147-300) g of ethanol/day over a median period of 24 years. Liver function was assessed

Table 1
Inflammatory markers in alcohol and non-alcohol abuser patients with and without sepsis.

	Sepsis-OH (n) 50th (25th-75th)	Sepsis-no OH (n) 50th (25th-75th)	Withdrawal (n) 50th (25th-75th)	Control (n) 50th (25th-75th)	p (KW)
TNF-alpha	(25) 14.6 (11.4-23.7)	(34) 14.4 (10.4-25.9)	(34) 7.2 (6.53-9.94)	(27) 7.34 (6.43-8.34)	< 0.001
IL-6	(25) 69.7 (18.5-122)	(34) 66.5 (34.3-266)	(34) 1.88 (1.5-6.9)	(26) 1.5 (1.5-2.02)	< 0.001
CRP	(24) 70.6 (22.3-94.1)	(34) 101.8 (90-183.4)	(33) 2.4 (1.55-5.7)	(27) 2.8 (1.8-6.2)	< 0.001
PCT	(25) 0.36 (0.15-2.72)	(34) 0.63 (0.14-3.97)	(34) 0.05 (0.02-0.10)	(27) 0.03 (0.02-0.05)	< 0.001
IFN-gamma	(25) 9.8 (7.75-14)	(34) 8.87 (6.95-15.5)	(34) 4.43 (3.2-7)	(27) 3.62 (2.42-5.08)	< 0.001
IL-10	(25) 5 (5-11.4)	(34) 5.57 (5-19.7)	(34) 5 (5-5)	(27) 5 (5-5)	< 0.001
IL-4	(25) 7.06 (5.38-9.09)	(34) 8.18 (5.18-10.38)	(34) 6.26 (4.31-10.7)	(27) 3.64 (2.12-5.18)	< 0.001
IL-5	(25) 4.64 (3.51-6.4)	(34) 5.98 (4.49-7.84)	(34) 3.86 (3.1-7.98)	(27) 7.14 (6.12-9.36)	= 0.002
G-CSF	(25) 8.86 (4.28-23.4)	(33) 16.9 (9.14-44.5)	(34) 3.38 (0.8-10.3)	(25) 1.82 (1.49-5.12)	< 0.001
VCAM-1	(25) 3244 (1243-5441)	(34) 2568 (1046-4503)	(34) 832 (507-1518)	(27) 774 (525-1516)	< 0.001
ICAM-1	(25) 465 (370-904)	(34) 680 (399-1119)	(34) 421 (290-750)	(27) 284 (195-400)	< 0.001
Leukocyte	(25) 15.2 (10.9-19.6)	(34) 16.1 (11.3-20.5)	(34) 7.2 (6.2-8.7)	(27) 7.1 (5.6-8.1)	< 0.001
Granulocyte	(25) 13.2 (9-17.3)	(34) 14 (10.4-18.4)	(34) 4.6 (3.5-5.4)	(27) 4 (3.4-4.7)	< 0.001

by ASAT, ALAT, LDH, GGT, alkaline phosphatase, pro-thrombin activity and bilirubin.

The definition of sepsis and its severity were assessed using the Bone criteria. Sepsis was defined as SIRS plus clinical evidence of infection [17]. In the alcoholic group, sepsis was non-complicated in 11, severe in nine, and septic shock or multi-organ failure in five cases, whereas sepsis in the non-alcoholic group was not complicated in 19, severe in eight, with septic shock or multi-organ failure in seven cases.

Blood samples were taken on admission and frozen at -40°C for further determination of serum cytokine levels. We determined TNF-α, IL-6, IL-10 and high sensitivity CRP (DPC, Los Angeles, USA) using a che-miluminiscent enzyme immunometric assay (IMMULITE analyzer). PCT was determined using a quantitative immuno-luminometric assay (LIAISON BRAHMS PCT, Brahms Diagnostics, Berlin, Germany). Using enzyme-linked immunosorbent assay (ELISA), we determined IFN gamma, IL-4, IL-5, G-CSF, VCAM and ICAM-1 (IBL International GMBH, Hamburg, Germany). We also deter-mined the total leukocyte and differential cell count, C3 and C4 and serum immunoglobulin IgG, IgA, IgM and IgE levels.

Statistical analysis was performed using SPSS15.0. As serum cytokines and other inflammatory markers were not normally distributed (Kolmogorov-Smirnov test), we used the non-parametric tests, Kruskal-Wallis (KW) and the Mann Whitney's U test (UMW), Spearman correlation, Chi-2 and Fisher's exact test when necessary. Data are presented as median, 25th and 75th percentiles. The study was approved by the institutional review board of the hos-pital; informed consent was obtained from all patients and none of them refused to participate in the study.

RESULTS

Median values and 25th and 75th percentiles of the inflam-matory mediators of the four groups are shown in *(table 1)*.

When we compared alcoholics admitted for programmed withdrawal with healthy control subjects (the two groups of non-infected patients), we found that non-infected alcoho-lics admitted for programmed ethanol withdrawal showed higher serum levels of IL-6 (p = 0.043), IFN-γ (p = 0.035), IL-10 (p = 0.039), Il-4 (p = 0.001) and ICAM-1 (p = 0.002) than the healthy controls. In the programmed withdrawal patients, cytokine assessment was repeated after a week of abstinence, but only IL-10 had decreased in a slight but significant fashion (p = 0.043).

The increase in inflammatory markers was very much more pronounced in both groups of sepsis patients than in the non-infected alcoholics. Sepsis patients, both ethanol abusers and non-abusers, showed a marked increase in serum pro-inflammatory cytokines (TNF-α, IL-6 and IFN-γ) and acute phase proteins (CRP and PCT) levels when compared both with controls, and patients admitted for programmed ethanol withdrawal (p < 0.001 for all the comparisons). Also, serum IL-10 levels were increased in both groups of patients with sepsis when compared with controls (p < 0.001 in both cases), and when compared with the programmed withdrawal alcoholics (p = 0.006 for sepsis alcoholics and p < 0.001 for non-alcoholic sepsis patients). Serum IL-4 levels were raised in both sepsis (alcoholics and non-alcoholics) groups when compared with control subjects (p < 0.001 in both cases), but not with the programmed withdrawal patients. Serum levels of G-CSF and adhesion molecules, VCAM-1 and ICAM-1, were also increased in both groups of sepsis patients (alcoholic and non-alcoholic) when compared with control subjects (p < 0.001 for all the comparisons), and also when compared with the programmed withdrawal alcoholics.

However, differences among the sepsis patients, according to alcohol abuse, were scarce; we found that alcoholics with sepsis showed only lower serum CRP (p = 0.014) and lower G-CSF (p = 0.016) levels than non-alcoholics with sepsis.

Serum IL-5 behaves differently from other inflammatory markers. It is decreased in alcoholics with sepsis when compared with non-alcoholic sepsis patients (p < 0.001),

and in programmed withdrawal alcoholics when compared with healthy controls (p = 0.002). Moreover, there are no differences between the non-alcoholic sepsis and control groups.

We found no relationship between the daily intake of ethanol, years of consumption and serum cytokine levels in any of the alcoholic patient groups. However, in alcoholics admitted for programmed withdrawal, we found a significant correlation between serum TNF-α, IL-6 and PCT and serum, ASAT, GGT, bilirubin and alkaline phosphatase levels.

We found no significant differences regarding IgG serum levels. However, IgA was increased in both alcoholic groups, alcoholics with sepsis (p = 0.010), and programmed withdrawal alcoholics (p = 0.004) when compared with healthy controls. IgE was raised in alcoholic patients, differences being significant between patients admitted for programmed withdrawal and control subjects (p = 0.019). In this last group of patients, we found no significant correlation between IgE and IL-4, IL-5 or the eosinophil count.

DISCUSSION

The most interesting feature of our study is the similarity between the inflammatory mediators among the sepsis patients, regardless alcohol abuse. The only difference observed was a less marked increase in serum CRP and G-CSF levels in alcoholic patients with sepsis compared with non-alcoholic sepsis patients.

Most of the studies about the effect of ethanol on cytokines in response to infection have been performed *in vitro*, analyzing the capacity of macrophages to secrete cytokines after diverse stimuli. Ethanol administered to rats, half an hour before intravenous LPS, blunts – in normal and in chronic alcoholic rats – the expected increase of serum TNF-α, whereas chronic alcoholic rats, which did not received ethanol before LPS, showed the expected increase in TNF-α [13, 18]. *In vitro* LPS stimulation of alveolar macrophages from chronic alcoholics resulted in a smaller increase in TNF-α when compared with healthy control subjects [19].

In our study, we observed similar serum TNF-α, IL-6, IFN-γ and Il-10 levels in both alcoholic and non-alcoholic sepsis patients. All our alcoholic sepsis patients had been heavy drinkers over a median period of 33 years. However, all had stopped drinking on admssion to the hospital and therefore many hours before cytokine levels were determined. In alcoholic-sepsis animal models, the effect of ethanol on serum cytokines depends on the delay between alcohol and LPS (or micro-organism) challenge. In striated muscle of rats, ethanol intoxication, two hours before LPS administration acutely suppressed the increase in mRNA for TNF-α, IL-1ß and IL-6, and antagonized the increase in IL-6 concentrations. In contrast, when LPS was given 24 hours after ethanol, the increase in IL-6 was selectively enhanced [20]. So, the absence of recent ethanol consumption may explain the unaffected response of the pro-inflammatory cytokines in our sepsis patients.

In the sole clinical study on alcoholic patients with sepsis analyzing serum cytokines, Dossow V *et al.* (2004) report that in septic shock due to pneumonia or peritonitis, there was decreased serum TNF-α, IL-6, IL-1β and IL-8, and increased CRP in chronic alcoholics when compared with non-alcoholic patients. There were no differences as regards IL-10. These results do not agree with those of our study, in which alcoholics with sepsis showed increased levels of pro- and anti-inflammatory cytokines, but they are similar to those found for non-alcoholic sepsis patients of similar age and severity of sepsis.

G-CSF plays an essential role in defensive systems. We observed decreased serum G-CSF levels in alcoholic sepsis patients when compared with non-alcoholic sepsis patients. Alcoholics show leucopenia in response to infection [21] more frequently, which could be explained by low G-CSF levels. However, leucopenia was infrequent in our study (only one case in each sepsis group and none among the alcoholics admitted for programmed withdrawal). Moreover, we did not find any difference regarding leukocyte or granulocyte count between the alcoholics with sepsis and the non-alcoholic patients, and we found no correlation between G-CSF and the leukocyte count in any group of patients. However, G-CSF, in addition to increasing the leukocyte number, improves their activity. In this way, G-CSF administration has been used experimentally to improve the host response to LPS in the presence of acute ethanol intoxication [18, 22], However, it has been only occasionally used as therapy in alcoholics [23].

Patients admitted for alcohol withdrawal showed a significant increase in some cytokines, such as IL-6, IFNγ, IL-4, IL-10 and ICAM-1, but this increase, as shown in *table 1*, was very slight when compared with the increase observed in both groups of sepsis patients. The slight increase in the alcohol-withdrawal patients can be interpreted differently from that observed in sepsis patients. In programmed ethanol withdrawal patients, the inflammatory mediators closely correlated with ethanol abuse and liver damage markers. So, it was thought that the increase in serum inflammatory cytokines in chronic alcoholic, non-infected patients was probably related to liver damage in the form of acute alcoholic hepatitis with more or less intense clinical expression [14, 16]. This form of low degree inflammation in alcoholics may be considered to be similar to other forms of smoldering inflammation such as atherosclerosis. IgE is frequently raised in alcoholics [7, 24]. The shift from a TH1 to a TH2 immune response, and the increase in some anti-inflammatory cytokines, such as IL-4, may help to explain the increase in IgE. However in our patients, IgE did not show any relationship with IL-4. IL-5 is a T cell-derived cytokine involved in the pathogenesis of atopic diseases. It specifically controls the production and activation of eosinophils. The decrease in IL-5 in both groups of alcoholic patients is an outstanding feature, although it was not related to IgE or the eosinophil count. The significance of the decrease in IL-5 in alcoholics is unclear and needs confirmation. IL-5 has been studied occasionally in alcoholics, but without having demonstrated any differences from healthy control subjects [7, 25].

To conclude, we highlight the intense and comparable inflammatory response among sepsis patients regardless of alcohol abuse, apart from the smaller increase in G-CSF in alcoholics with sepsis, which might perhaps account for the frequent infections seen in alcohol abusers.

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