CHRONIC ALCOHOLISM CAUSES DELETERIOUS CONDITIONING OF INNATE IMMUNITY

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Abstract — Aims: To examine the immune consequences of chronic alcoholism in man, in relation to the known association between alcoholism and raised incidence and severity of infections. Methods: In 36 alcoholics without liver disease, at the point of commencing withdrawal from alcohol, the following measures of immune competence were assessed: the immunophenotypes of cells, acute phase proteins, the endotoxin-neutralizing capacity (ENC) of the serum, titers of anti-lipopolysaccharide (LPS) antibodies, and ex vivo cytokine inducibility in T cells and monocytes (TNFα, IL-1β, IL-1RA, IL-4, IL-6, IL-8, IL10 and IL-12). The results were compared to those from healthy volunteers (day controls). Measures were repeated after 8–13 days of abstinence. Results: LPS-binding protein (LBP) and soluble CD14 (sCD14) were significantly increased in patients’ sera at the outset of withdrawal, whereas reduced titers of anti-LPS IgG (P = 0.012) and a reduced ENC (P = 0.001) were measured. Only ENC rapidly returned to normal values after withdrawal therapy. Cytokine induction with phorbol ester showed no significant alterations in patients’ T cells. Patients’ monocytes, however, responded to LPS stimulation with enhanced IL-1β-, but reduced TNFα- and IL-12-production (P = 0.004, P = 0.0042 and P = 0.001, respectively). While IL-1 and TNFα-responses normalized after the withdrawal period, impairment of the IL12 response persisted throughout the observation period of 2 weeks. Conclusions: Alcoholism results in a prolonged LPS-mediated hypoinflammatory conditioning of the innate but not the adaptive immune system, which is not reversed immediately after withdrawal. This alcohol-induced status of the immune system predisposes to infections and sepsis by blunting initial response to the pathogens.

INTRODUCTION

Large efforts have been devoted to the prediction of mortality during infection, bacteremia or sepsis. The initial hyperinflammatory response to bacteria can lead to septic shock. The risk factors for this include advanced age, diminished physical performance, presence of pathogens, low platelet count, leukocytosis or leukopenia, low albumin concentrations, increased levels of liver enzymes and high plasma creatinine concentrations (Bone et al., 1989; Grandsen et al., 1990; Aube et al., 1992; Pittet et al., 1995; Leibovici et al., 1997; Slotman and Quinn, 1997) which are very common in alcoholics.

Septic shock is characterized by overproduction of cytokines such as tumor necrosis factor (TNFα) or IL1 and numerous clinical trials were conducted with agents that block the inflammatory cascade (Fisher et al., 1994, 1996; Abraham et al., 1995). Although proinflammatory cytokines are considered to be dangerous by causing organ failure or immune cell apoptosis (Hotchkiss et al., 2001, 2002), they also have essential beneficial effects. Blocking TNF worsens survival from peritonitis in animal models (Jack et al., 1997; Echternacher et al., 2001; Heinrich et al., 2001) and clinical studies (Fisher et al., 1996; Reinhart and Karzai, 2001).

However, death from sepsis is not always attributable to an overstimulated immune system. Advances in our understanding demonstrated that as sepsis persists, there is a shift towards an anti-inflammatory immunosuppressive state (Lederer et al., 1999; Oberholzer et al., 2001), first described by Döcke et al. (1997). This situation is characterized by increasing levels of the anti-inflammatory cytokine IL10 in septic patients, the degree of which predicts mortality (Gogos et al., 2000; Opal and DePalo, 2000). Immune cells from patients with burns or polytrauma also produce reduced levels of proinflammatory cytokines but increased levels of IL10 and IL4 (Ertel et al., 1995; O’Sullivan et al., 1995; Lederer et al., 1999). Nevertheless, a secondary switch to a hyperimmune state can occur during secondary infection. In some cases, however, the immune response is blunted from the start. This occurs in patients with chronic diseases such as diabetes or others (Hotchkiss and Karl, 2003) where there is an initial failure of antibacterial defense culminating in higher mortality.

Alcohol is the third leading cause of preventable mortality in the United States. This prompted the question of whether or not alcohol abuse leads to detectable immune conditioning towards proinflammatory TH1- or anti-inflammatory TH2-type cytokine profiles. Many direct mechanisms of alcohol-induced organ toxicity are well known, but the effects on the immune system are among the least appreciated medical complications of alcohol abuse (National Institute of Health, 2000).

Several years ago elevated levels of systemic endotoxin (lipopolysaccharide, LPS) have been reported in alcoholics (Bode et al., 1987; Adachi et al., 1995) suggesting translocation of gut-derived endotoxin due to the ethanol (EtOH)-induced damage of the gut mucosal wall (Parlesak et al., 2000) and to the reduced hepatic LPS clearance. If EtOH increases the gut permeability, LPS, a component of the Gram-negative bacterial cell wall, is a prime candidate for inducing immune deviation.

LPS is a pathogen-associated molecular pattern, which is recognized by pattern recognition receptors like membrane bound CD14 (mCD14), soluble CD14 (sCD14) and liver-derived LPS binding protein (LBP). Minute amounts of LPS sensed by these molecules contribute to the very rapid cellular activation of Kupffer cells, monocytes or macrophages,
resulting in proinflammatory cytokine release (Schumann et al., 1990). This enables the host to establish a rapid antibacterial defense (Jack et al., 1997). An exaggerated response to LPS can be harmful, leading to multiorgan failure and death. In this context TNFα plays an important role in the development of liver injury. LPS toxicity depends on its capacity to bind to the major high-affinity cellular receptor CD14. This is low unless catalyzed by LBP. The signal transduction across the membrane then involves the Toll-like receptor 4 (TLR4). Important factors in the blood limit the interaction of LPS with monocytes. LBP and sCD14 can transfer LPS to lipoproteins, which neutralize its biological activity (Kitchens et al., 2001; Vreugdenhil et al., 2001). Thus, both LBP and sCD14 have the paradoxical dual function of sensitizing the immune system to endotoxin and, on the other hand, contributing to its detoxification. Pre-existing anti-LPS antibodies also interfere with LPS toxicity (Reid et al., 1997). Which other serum proteins enhance or attenuate LPS toxicity and how they act in concert is not yet fully understood.

Recently, the so-called endotoxin-neutralizing capacity (ENC) of patients’ sera was analyzed using a modified Limulus amoeocyte lysate (LAL) assay (Urbaschek et al., 2001). ENC was reduced in alcoholic and non-alcoholic liver disease, but the serum factors mediating ENC remain to be identified. In a pilot study in which patients at high risk of infection and sepsis were monitored, we also noted a striking reduction of ENC, particularly in patients with alcoholic liver cirrhosis. Surprisingly, ENC was reduced even in alcoholics without infections or liver cirrhosis (Schilling et al., 1995). This low ENC might increase the susceptibility to small concentrations of LPS in the serum, which have been reported in alcohol abuse.

Exposure to LPS, even at very low doses, alters the cytokine responses to a second high-dose LPS challenge both in vitro and in vivo, e.g. it reduces TNF secretion (Randow et al., 1995). This phenomenon, referred to as LPS tolerance or LPS desensitization, has been observed in many patients who survived the acute phase of a septic shock but who died from persisting infections weeks later in such a hypoinflammatory phase (Volk et al., 1993).

We therefore undertook this study based on the hypothesis that there is an immune deviation in alcoholics and with the aim to define the status of immune conditioning under EtOH abuse.

**METHODS**

The study sample was 36 male alcoholics without liver disease (AWLD) newly admitted for in-patient alcohol withdrawal. Healthy male volunteers were used as day controls. All patients underwent detailed, questionnaire-based anamnesis, a physical examination and abdominal sonography to exclude liver cirrhosis. Furthermore basic laboratory tests (blood cell counts, liver and kidney parameters, urine status) and where appropriate ECG, EEG, X-ray, blood glucose, pancreatic enzymes and lipid parameters accompanied the in-patient withdrawal therapy. The selection for the study was made according to the following criteria: (i) regular alcohol consumption during the last 6 months corresponding to at least 120 g EtOH/day; (ii) first withdrawal therapy or no more than two prior alcohol-related therapies; (iii) no manifest organ disease, especially no active alcoholic hepatitis or alcoholic liver cirrhosis; (iv) no infection; (v) motivation for and compliance with withdrawal therapy. Selected patients were informed about the additional investigations and written consent obtained. The ethics committee of the University of Greifswald approved the study design. In 30 patients a second investigation was performed after 8–13 days of withdrawal therapy. The remaining six patients either did not wish to continue the study or started drinking alcohol within the withdrawal period. Patients were free of ethanol at the point when the first tests were done, which was between 7:00 and 8:00 a.m. on the second day of admission (‘before withdrawal’). Samples were taken into LPS-free sodium heparin tubes (Endotube, Chromogenex/Haemochrom Diagnostica, Essen, Germany) for measurement of endotoxin, endotoxin-neutralizing capacity and for ex vivo whole blood cytokine induction. For hematological and serological parameters EDTA-containing Serum-Vactuator tubes (Becton Dickinson, Heidelberg, Germany) were used. For each patient sample we collected in parallel a control probe from a healthy volunteer who had abstained from alcohol for at least 24 h. Patient and control samples were processed side by side under exactly the same conditions. Endotoxin and endotoxin-neutralizing capacity were measured by a modified turbidimetric-kinetic Limulus amoeocyte lysate (LAL) assay (Urbaschek et al., 2001) using a microplate reader (ThermoMax, Molecular Devices, Munich, Germany). For the determination of endotoxin, plasma was diluted 1:5 in endotoxin-tested water (Acilia, Pyroquant Diagnostik, Waldorf, Germany) and heated (80°C, 10 min). Endotoxin measurements and calculations with the software program Pyrokin (kindly provided by Klaus-Peter Becker, Institute for Medical Microbiology and Hygiene, Klinikum Mannheim, Germany) were done as described in detail earlier (Urbaschek et al., 2001). For the determination of the endotoxin-neutralizing capacity, unheated plasma was diluted 1:10 and 50 µl aliquots were spiked with 25 µL LPS at final concentrations of 5000, 2500, 1250, 625, 312, 156 and 78 pg/ml. The addition of 25 µl lysate and measurement were analogous and performed in parallel to the endotoxin measurements (Urbaschek et al., 2001). In unheated plasma the added LPS spikes are detectable only above a certain threshold concentration. This threshold concentration is defined as endotoxin-neutralizing capacity (ENC) and is expressed in endotoxin neutralizing units (ENU). The calculation was also done with the Pyrokin software which determines ENC by interpolation between the last non-detectable and the first detectable LPS spike concentrations. To minimize measurement fluctuations due to the biological nature of the Limulus lysate a single lysate batch was used throughout. sCD14 and LBP were measured in serum by ELISA as described earlier (Grunwald et al., 1992; Schroedl et al., 2001). Anti-LPS-IgG and anti-LPS-IgM were determined in the plasma by an indirect enzyme-immunoassay using immobilized Re-LPS from *Escherichia coli* mutant J5 (Sigma, Deisenhofen, Germany), and peroxidase labeled goat anti-human IgG or goat anti-human-IgM antibodies, respectively (Dianova, Hamburg, Germany and Immunotech, Marseille, France). The results were normalized by comparison with a serum pool from healthy volunteers which
was defined to contain 100 arbitrary Units (aU)/ml of each anti-LPS-IgG and anti-LPS-IgM. Clinical chemistry was done in the routine laboratory according to common standards. For ex vivo whole blood stimulation and intracellular cytokine detection by flow cytometry we used heparinized whole blood, which was diluted 1:2 with RPMI-1640 medium. For stimulating monocytes, LPS from E. coli O55:B5 (100 ng/ml) was added; for stimulating lymphocytes, phorbol-12-myristate-13-acetate (PMA, 20 ng/ml) and calcium ionophore A23187 (1 µg/ml) were used. After 8 h of incubation, monocytes were stained with anti-CD14-Tricolor (Medac, Hamburg, Germany). Lymphocytes were stained with anti-CD4 and anti-CD8 simultaneously; both antibodies were coupled with CyChrome (PharMingen, San Diego, CA). Subsequently, the cells were fixed, permeabilized and erythrocytes were lysed using IntraPrep reagents (Immunotech, Marseille, France). For staining of intracellular cytokines the following fluorochrome-labeled antibodies were used in pair-wise FITC/PE combinations (three-colour immunofluorescence staining): anti-TNFα–FITC, anti-IL1α–FITC, anti-IL6–FITC, anti-IL8–FITC, anti-IL-10–PE, anti-IL12(p40/p70)–PE, anti-IFNγ–FITC, anti-IL4–PE (PharMingen, San Diego, CA), anti-IL1β–PE, anti-IL-1RA–PE (Becton Dickinson, Heidelberg, Germany). After 1 h intracellular staining and washing, 50 000–100 000 cells were measured on a FACScan flow cytometer (Becton Dickinson, Heidelberg, Germany). Listmode data analysis was performed with the PC program WinList 4.0 (Verity Software House, Topsham, ME) to evaluate the percentage of cytokine-positive monocytes (CD14 high+), cytotoxic T cells (CD8 high+) and T helper cells (CD4+).

All clinical and laboratory data were recorded or imported in a SQL-database system (Interbase, Inprise Corp., Scotts Valley, CA) with specially adapted or developed interface programs. Final statistical analysis was performed with the software package Systat 9.0 (SPSS Inc., Chicago, IL). Main data analysis was done by the distribution-free Wilcoxon sign-rank test suitable for the pair-wise study design (sample of alcoholics/control donor in the same laboratory run). Furthermore, Spearman rank correlation analysis was done between the LPS-related parameters (ENC, sCD14, LBP, anti-LPS-IgG, anti-LPS-IgM) and all other parameters. In all test statistics \( P < 0.05 \) was taken as significant, for the Spearman rank correlation the corresponding critical correlation coefficient \( |r| > 0.3059 \) for \( n = 30 \) was used.

RESULTS

Patients’ characteristics

All alcoholics had a long history of alcohol abuse (median 12.5 years; P25–P75: 3.9–24.3), a high EtOH uptake (median 250 g/day; P25–P75: 160–490) and a high alcohol dependency score (median 0.94; P25–P75: 0.61–1.00). The percentage of consumed volume was for beer 85% and for schnapps 15%. Wine did not play a role in this group of patients. Patients reported common physical and withdrawal symptoms like tremor, blood pressure–pulse-reactions, hyperhidrosis and paresthesia. The biochemical characteristics of these patients were similar to those previously documented for alcoholics: e.g. granulocytosis (\( P = 2.2E-04 \)), elevated MCV and MCH indices (\( P = 6.9E-07 \) and \( P = 2.3E-06 \), respectively), elevated red cell distribution width (\( P = 2.6E-04 \)), thrombopenia (\( P = 0.007 \)), reduced albumin (\( P = 4.3E-04 \)), elevated \( \alpha \)-antitrypsin (\( P = 1.5E-04 \)), \( \alpha \)-macroglobulin (\( P = 0.003 \)), haptoglobin (\( P = 0.003 \)), \( \gamma \)-GT (\( P = 2.7E-07 \)) and CD transferrin (\( P = 4.1E-06 \)). Total cholesterol (\( P = 0.003 \)), HDL–cholesterol (\( P = 1.3E-04 \)) and lipoproteins Apo A1, Apo A2, Apo B and Apo E (\( P = 0.011 \), \( P = 3.6E-04 \), \( P = 0.019 \) and \( P = 0.002 \), respectively) were also elevated.

Immunological results

Alcoholics had a reduced capacity of their plasma components to neutralize endotoxin compared to healthy controls (\( P = 0.001 \)), which normalized within 1–2 weeks of withdrawal. In contrast, no significant rise in plasma endotoxin concentrations could be detected in AWLD (Table 1). The serum levels of LPS binding proteins sCD14 and LBP were highly elevated before and also after (\( P = 2.3E-06 \) and \( P = 8.0E-05 \), respectively) the withdrawal period. On the other hand, anti-LPS-IgG concentrations were decreased in AWLD compared to controls (\( P = 0.012 \)) and did not return to normal during the observation period (Table 1). There was no significant correlation of the endotoxin-neutralizing capacity with any of the other parameters measured (data not shown).

At the cellular level we analyzed the capacity of lymphocytes and monocytes to produce cytokines upon activation ex vivo. Whole blood cell cultures were stimulated either with PMA/Ca-ionophore for an overall T cell activation or with endotoxin to activate monocytes, and intracellular cytokine production at single cell level was recorded by flow cytometry. Both CD4+ T helper cells and CD8+ cytotoxic T cells from alcoholics did not differ in their spontaneous or inducible production of INFγ, TNF, IL4 and IL10 when compared to cells of daily matched controls (data not shown). In contrast, monocytes from alcoholics demonstrated differences in terms of proinflammatory cytokine inducibility (Fig. 1). The percentage of TNFα synthesizing monocytes was significantly lower than that of healthy controls, 35.7% vs 51.5% (\( P = 0.042 \)), whereas the percentage of IL1β synthesizing monocytes was elevated, 84.8% vs 76.4% (\( P = 0.004 \)). The most intriguing result was the strongly impaired capacity of patients’ monocytes to produce IL12 upon LPS activation (27.8% vs 46.2%; \( P = 0.001 \)). The inducibility of IL1α, IL1RA, IL6, IL8 and IL10 was not significantly altered in patients’ monocytes (data not shown). Noteworthy, only the reduced inducibility of IL12 persisted over the withdrawal period (Fig. 1).

DISCUSSION

There is strong evidence that LPS at high concentrations is toxic and that it modulates the immune response at low concentrations.

The intestine contains large amounts of Gram-negative bacteria and hence endotoxin. Vascular occlusion or reduction in splanchic blood flow, hypoxia and elevated core temperature (Cohen, 2000) or EtOH damage of the mucosal
barrier (Parlesak et al., 2000) lead to leakage of LPS to the portal circulation, overwhelming the ability of the liver reticulo-endothelial system to remove LPS from the circulation. The cellular responses to endotoxin are the net result of the interaction of LPS with various plasma components, such as LBP, sCD14, lipoproteins, antibodies and cellular receptors such as mCD14 and TLR4.

In these 36 alcoholic patients without liver disease we did not observe significant endotoxemia prior to withdrawal (Table 1). The difficulty in interpreting this result lies in the inherent variability of the supposed endotoxemia (Cohen, 2000) and the restriction to a single time point measurement. However, the strong increase of sCD14 and LBP and changes in serum lipoproteins in conjunction with the reduced anti-LPS-IgG concentrations in the group of alcoholics prior to withdrawal argue in favor of repeated episodes of endotoxemia in AWLD. Previous studies have also linked increased sCD14 and LBP with endotoxemia (Randow et al., 1995; Kitchens et al., 2001; Vreugdenhil et al., 2001). A fall in anti-endotoxin antibody titers is discussed as consumption during sepsis (Reid et al., 1997) or endotoxemia (Brock-Utne et al., 1988). As shown here, alcoholism even in the absence of liver disease was accompanied by a drastic reduction of ENC of patients’ sera (Table 1), which returned to normal values during the short withdrawal period. In our study ENC did not show any strong correlation with other serum parameters, suggesting that either ENC is mediated by a plasma component that we did not measure, or that ENC represents a more complex phenomenon. However, the reduced ENC could allow the repeated exposure of immune cells to low concentrations of bioactive LPS, which could then lead to LPS tolerance. LPS tolerance has been well

<table>
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<tr>
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<th>AWLD before withdrawal</th>
<th>Control (n = 36)</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>(n = 36)</td>
<td>Median (P25–P75)</td>
<td></td>
</tr>
<tr>
<td>Endotoxin (pg/ml)</td>
<td>2.3 (1.7–2.5)</td>
<td>1.8 (1.4–2.5)</td>
<td>0.080</td>
</tr>
<tr>
<td>ENC (ENU/ml)</td>
<td>4317 (2167–9992)</td>
<td>11 500 (5500–12 750)</td>
<td>0.001*</td>
</tr>
<tr>
<td>sCD14 (ng/ml)</td>
<td>6220 (5064–8195)</td>
<td>3460 (3163–4484)</td>
<td>2.3E-06*</td>
</tr>
<tr>
<td>LBP (ng/ml)</td>
<td>5836 (3936–9657)</td>
<td>3459 (2353.8–6373)</td>
<td>8.0E-05*</td>
</tr>
<tr>
<td>Anti-LPS-IgG (aU/ml)</td>
<td>77.0 (47–122)</td>
<td>130.3 (84.8–198)</td>
<td>0.012*</td>
</tr>
<tr>
<td>Anti-LPS-IgM (aU/ml)</td>
<td>83.0 (43–136)</td>
<td>88.7 (58.9–203)</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td>AWLD after withdrawal</td>
<td>Control (n = 30)</td>
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<tr>
<td></td>
<td>(n = 30)</td>
<td>Median (P25–P75)</td>
<td></td>
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<tr>
<td>Endotoxin (pg/ml)</td>
<td>2.0 (1.1–2.5)</td>
<td>1.5 (1.1–2.5)</td>
<td>0.419</td>
</tr>
<tr>
<td>ENC (ENU/ml)</td>
<td>11 000 (7158–21 833)</td>
<td>12 000 (7142–15 917)</td>
<td>0.957</td>
</tr>
<tr>
<td>sCD14 (ng/ml)</td>
<td>4481 (3910–6013)</td>
<td>3457 (3206–4240)</td>
<td>2.8E-04*</td>
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<tr>
<td>LBP (ng/ml)</td>
<td>5956 (3742–10 596)</td>
<td>3656.5 (2006–4977)</td>
<td>1.6E-05*</td>
</tr>
<tr>
<td>Anti-LPS-IgG (aU/ml)</td>
<td>56.5 (44–113)</td>
<td>106.5 (76.2–190.8)</td>
<td>0.011*</td>
</tr>
<tr>
<td>Anti-LPS-IgM (aU/ml)</td>
<td>82.5 (50–121)</td>
<td>123.8 (46.0–226.4)</td>
<td>0.096</td>
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</table>

Fig. 1. ALTERED CYTOKINE INDUCIBILITY IN MONOCYTES OF AWLD (WHITE BARS) IN COMPARISON TO HEALTHY VOLUNTEERS (GRAY BARS). Whole blood cell cultures were stimulated with LPS (E. coli O55:B5, 100 ng/ml) for 8 h and the intracellular (ic) cytokines were measured at the single cell level using flow cytometry. Monocytes were defined by CD14 and the percentages of IL1b-, TNFα- and IL12-producing CD14-positive cells are shown. The inducibility of IL1α, IL1RA, IL6, IL8 and IL10 was not significantly altered in AWLD. The significance of the results is shown for the comparison of alcoholics vs. healthy controls and the alcoholics before vs. after withdrawal.
characterized in vitro, in vivo and ex vivo as a state of relative non-reactivity (Munoz et al., 1991; Randow et al., 1995; Ertel et al., 1997). Therefore we examined the ex vivo cytokine inducibility in whole blood cell cultures to evaluate pro- vs anti-inflammatory cytokine profiles. T cell activation was measured after the commonly used stimulation by PMA and calcium ionophore, which induces the maximal response in T cells. Using this type of stimulation we had no evidence for an altered T cell response in alcoholics. Recently, however, the protein kinase C activator, phorbol ester, has been shown to be able to overcome a refractory state in LPS-tolerant cells (West et al., 1997). Thus, this stimulus might not adequately mirror the in vivo situation. In contrast, patients’ monocytes were defective in TNF- and IL12-synthesis in response to an ex vivo LPS challenge. The reduced inducibility of IL12 was a robust, long lasting functional status (Fig. 1). This is in agreement with a characteristic feature of LPS tolerance, which, once established, persists for up to 30 days or even weeks after discontinuing the endotoxin application (Beeson, 1947; Morgan, 1948) depending on the parameters used to measure the presence of tolerance (Cross, 2002). Few studies address the molecular events involved in the recovery from established LPS tolerance.

Whereas TNF is believed to be crucially involved in the development of liver injury (Enomoto et al., 1998; Yin et al., 1999), IL12 is essential for protective immunity, as demonstrated by numerous animal models. IL12, a heterodimeric cytokine, consists of two covalently linked subunits, p40 and p35. When p40 is associated with p35 subunit, the heterodimer acts as an IL12 receptor agonist mediating biological activity. It is required for the production of IFNγ and supports the development of the TH1 phenotype. However, when IL12p40 associates with another p40 the homodimer behaves as an antagonist in vitro (Ling et al., 1995). And, to confuse matters further, the p40 subunit is a component of both IL12 and the newly discovered IL23. The latter protein is produced by macrophages, and has functions that are similar to those of IL12, e.g. it also induces the production of INFγ by T cells (Trinchieri, 2003). We have measured IL12 inducibility in monocytes with the anti-IL12 mAb C11.5, which binds to the bioactive p70 heterodimer as well as to p40 dimers. Thus, whether or not suppressive IL12p40 homodimers or IL23 play an additional role in AWLD, remains to be determined. The regular INFγ inducibility by PMA and Ca-ionophore in T cells of AWLD does not conflict with the reduced IL12 production by monocytes, because it was tested in an LPS- and IL12-independent manner. Moreover, the results presented here fit with the recent observation that LPS tolerant macrophages are unable to induce INFγ production by T cells, whereas T cells from tolerant animals do make INFγ when co-cultured with control macrophages. Importantly, even during LPS tolerance some cellular responses remain unaffected: IL1β and IL8 have shown a variable response to LPS in previous studies (Ziegler-Heitbrock et al., 1992; Erroi et al., 1993; Astiz et al., 1995), which fits our results (Fig. 1).

IL12 appears to play a critical role in human sepsis. In a prospective study of 184 patients the monocyte IL12 production was severely and selectively impaired in patients developing post-operative sepsis compared to patients showing uneventful recovery from major elective surgery (Hensler et al., 1998). This study revealed that monocyte IL12p70 heterodimer secretion was suppressed even before surgery and remained low until the onset of sepsis. Therefore, the IL12 hyporesponsiveness was not the consequence of the major surgery or the sepsis. With respect to inducible IL12 secretion, alcoholics resemble the high-risk patients before surgery. The impairment of IL12 secretion could thus be (partly) responsible for the high risk of severe infection in alcohol abuse.

It has been shown by others that LPS tolerance can induce suppression of IL12 production in vitro (Karp et al., 1998; Wysocka et al., 2001). Another recent study showed that the cells of alcohol-fed mice tend to shift toward a TH2 response with a decrease of the TH1 response, which could be restored by administration of IL12 (Petersen et al., 1998). In vitro, IL12 is able to reverse LPS desensitization of monocytes by inducing INFγ production by non-monocytic cells (O’Suilleabhaín et al., 1996; Randow et al., 1997). LPS desensitization could also be prevented by neutralizing the endogenous IL10 and TGFβ, suggesting that the tolerant state is mediated by these cytokines (Randow et al., 1995). In fact, IL-10 and TGFβ can replace LPS in tolerance induction (Randow et al., 1995).

Taken together, our data and the reports from the literature support the concept that LPS desensitization due to gut-derived endotoxin does play a role in chronic alcoholism. While it has been described that EtOH can directly enhance the TGFβ production by rat macrophages in vitro (Singhai et al., 1999), the increased TGFβ mRNA levels observed after chronic alcohol feeding in mice were dependent on the presence of CD14 (Yin et al., 2001). Moreover, CD14-deficient mice did not develop an alcohol-induced liver injury (Yin et al., 2001). This confirms the key role of LPS and endotoxin receptor (CD14)-positive cells, e.g. Kupffer cells (Enomoto et al., 1998), for the innate immune alterations in chronic EtOH uptake.

In summary, the results of this study support the hypothesis that endotoxin, possibly in conjunction with direct effects of EtOH on monocytes, significantly contributes to the increased risk of infection in AWLD. Thus, alcoholics are a group of patients with an immune conditioning of their innate immune system, which carries a high risk for infection and sepsis.

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REFERENCES


Parlesak, A., Schäfer, C., Schütz, T., Bode, J. C. and Bode, C. (2000) Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different...


