

# Concomitant Endocrine and Immune Alterations during Alcohol Intoxication and Acute Withdrawal in Alcohol-Dependent Subjects

Sven Kutscher<sup>a</sup> Dirk J. Heise<sup>b</sup> Markus Banger<sup>a</sup> Bernhard Saller<sup>c</sup>  
Martin C. Michel<sup>c</sup> Markus Gastpar<sup>a</sup> Manfred Schedlowski<sup>b</sup> Michael S. Exton<sup>b</sup>

<sup>a</sup>Clinic for Psychiatry and Psychotherapy, <sup>b</sup>Department of Medical Psychology, and <sup>c</sup>Department of Endocrinology, University of Essen, Essen, Germany

## Key Words

Alcohol withdrawal · Cortisol · Leukocyte subsets · Monocyte · Addiction

## Abstract

Although both alcohol intoxication and withdrawal have been demonstrated to produce significant endocrine alterations, no data exist on the effects of acute withdrawal on immune functions. Therefore, the current study investigated the effect of alcohol intoxication and acute withdrawal on plasma cortisol, prolactin and catecholamines, and blood leukocyte subset distribution in alcohol-dependent subjects. Nine male alcoholics admitted to the university clinic for alcohol dependence and 9 age-matched controls participated in the study. Blood was drawn from the alcohol-dependent subjects at 10:30 a.m. on day 0 (chronic alcohol intoxication), at the same time during acute alcohol withdrawal (day 1) and following the resolution of acute withdrawal (day 7). Blood was drawn from age- and gender-matched healthy control subjects at the corresponding time points. Plasma was then analyzed for hormone concentrations and blood examined for leukocyte subsets by flow cytometry. Alcohol-dependent patients displayed significantly elevated

plasma cortisol during intoxication and withdrawal, which decreased to control levels following resolution of acute withdrawal. Small elevations of plasma prolactin and catecholamines were also observed during intoxication. Furthermore, alcohol-dependent subjects showed reduced absolute numbers of CD4<sup>+</sup> and CD8<sup>+</sup> T cells and natural killer cells compared with healthy controls across all time points. In contrast, although monocyte numbers were lower in alcohol-dependent patients during intoxication, acute alcohol withdrawal increased the number of monocytes in patients. Thus, alcohol dependence produces a general suppression of leukocyte subset populations in blood. However, resolution of acute alcohol withdrawal is associated with a return of plasma cortisol to control levels, and a concomitant increase in peripheral blood monocyte numbers.

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## Introduction

Alcohol dependence is a chronic disorder affecting 5–10% of western populations. Significantly, alcoholism produces considerable morbidity and mortality from a range of chronic hepatic, cardiovascular, pulmonary, and

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Dr. Mike Exton  
Department for Medical Psychology, University of Essen  
Hufelandstrasse 55  
D-45122 Essen (Germany)  
Tel. +49 201 723 4282, Fax +49 201 723 5948, E-Mail [michael.exton@uni-essen.de](mailto:michael.exton@uni-essen.de)

metabolic diseases [1]. Of these, endocrine dysregulation and alteration in immune function may significantly contribute to deaths caused by cancer and infection in alcohol-dependent people.

Chronic alcohol consumption alters leukocyte distribution and cellular immune function in humans [2–4]. Although the nature of the effect and the mechanisms of chronic alcohol-induced immunomodulation in humans are equivocal [5], animal models have clearly demonstrated that alcohol consumption particularly suppresses components of the innate immune system [6, 7], resulting in increased incidence of infection [7, 8].

Although *in vitro* studies have demonstrated that ethanol directly suppresses cellular immune function [9, 10], the immunomodulatory effects of alcoholism *in vivo* may be regulated by endocrine alterations. Chronic alcohol abuse, alcohol intoxication, and withdrawal all induce activation of the hypothalamus-pituitary-adrenal (HPA) axis [11–14], and increased peripheral prolactin and catecholamine concentrations [13–15]. Thus, both alcohol intoxication and withdrawal may potentially alter immune function via neuroendocrine mechanisms, as acute and chronic activation of the HPA axis and sympathetic nervous system as well as elevations of other pituitary hormones produce marked changes in the distribution and function of immune cells [16].

Therefore, the current study extended previous data by examining whether alcohol intoxication and withdrawal produce differential endocrine activation, and whether these changes are associated with an altered immune status. Specifically, we examined cortisol, prolactin and catecholamine secretion during alcohol intoxication, acute alcohol withdrawal, and following the resolution of acute withdrawal in alcohol-dependent patients compared with healthy controls. Furthermore, we examined whether HPA alterations were associated with changes in leukocyte subset numbers in peripheral blood at the corresponding time points.

## Materials and Methods

### *Participants*

Nine male alcohol-dependent subjects (mean age  $47.1 \pm 2.5$  years), admitted to the Unit for Alcohol Dependence of the University of Essen, Germany, participated in the study. Participation in the detoxification program was voluntary. Nine age-matched ( $44.1 \pm 2.8$  years) male healthy control subjects, without a history or current symptoms of alcohol abuse, drug dependence, or any psychiatric disease, were investigated in parallel. All subjects were briefed on the study design prior to participation, and provided their written informed consent. The study was approved by the local Ethics Com-

mittee for Human Research. Alcohol-dependent patients were included in the study following a diagnosis of chronic alcoholism of at least 2 years' duration (ICD 10 F10.25). Subjects were excluded when demonstrating chronic disease, infection (confirmed by leukocyte counts), hypertension, renal insufficiency, severe liver disease (hepatitis, cirrhosis), illicit drug or benzodiazepine use (confirmed by urine drug screening), depression, acute suicidal tendencies, psychoses, alcohol-induced psychotic disturbances, previous seizures or delirium, current intake of  $\beta$ -blockers or neuroleptic, antidepressive or anticonvulsive medication, and previous pharmacotherapy of alcohol dependence (disulfiram, naltrexone, or acamprosate regimens). All patients demonstrated normal liver function, including blood coagulation, and levels of both cholinesterase and albumin. However, patients displayed elevated  $\gamma$ -glutamyl transferase levels, which is the most sensitive marker of liver damage and/or elevated transaminases. Because patients were rated clinically by 3 independent psychiatrists as being currently alcohol-dependent, and moreover, most patients were well known to our detoxification service, we did not measure carbohydrate-deficient transferrin to operationalize abstinence from alcohol.

### *Procedure*

Alcohol-dependent patients voluntarily reported to the clinic for a period of inpatient alcohol detoxification on the morning of day 0 (alcohol intoxication) prior to 8:00 a.m. Subjects underwent a physical examination, spirometric analysis of alcohol intoxication, and examination according to inclusion/exclusion criteria of the study at 9:00 a.m. For each participant, inclusion/exclusion criteria were rated by 3 independent psychiatrists (professional experience 5–15 years) using a semi-structured interview. Once a subject met all study criteria, 20 ml blood was drawn in EDTA tubes at 10:30 a.m. for examination of plasma cortisol concentrations and flow cytometric analysis of blood leukocyte subsets. All subjects who met the strict initial inclusion criteria also met the laboratory standards expected for the study, and thus no subject was excluded post hoc on the basis of laboratory assessment. The alcohol-dependent subjects were subsequently stationed in the clinic for an average of  $12.5 \pm 0.6$  days, during which time patients were unable to leave. Thus, they were unable to consume alcohol throughout the duration of the study. In addition to the abstinence from drug intake, the patients were treated in single and group psychotherapy, and therapies designed to keep them physically and psychologically active. When participating in therapies outside the clinic (e.g. taking a walk), the patients were examined for possible alcohol use via spirometer. Further blood samples were drawn at the same time on days 1 (acute alcohol withdrawal) and 7 (resolution of acute withdrawal) for examination of endocrine and immune parameters. Throughout the study, the patients were administered 200 mg thiamine orally, and potassium and magnesium supplementation when peripheral levels fell below 4.0 mmol/l and 0.65 mmol/l, respectively. Control subjects underwent the physical and psychological examinations without being stationed in the clinic. However, the control subjects were instructed to refrain from alcohol intake over the course of the study.

### *Endocrine Analysis*

The subjects were placed in a seated position, and 10 ml of blood were then collected in an EDTA tube (Sarstedt, Nürnberg, Germany). Blood was immediately centrifuged at  $4^\circ\text{C}$  for 15 min at 2,000 g, with plasma collected and stored in glass aliquots at  $-20^\circ\text{C}$  until the time of the hormone assays. All samples from the same participant

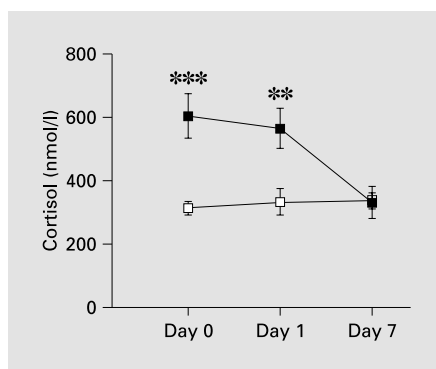


Fig. 1. Plasma concentrations of cortisol (nmol/l) in alcoholics (■) during alcohol intoxication (day 0), acute withdrawal (day 1), and following withdrawal resolution (day 7) compared with healthy control subjects (□). (\*\*\*)  $p < 0.001$ , (\*\*)  $p < 0.01$ , in post-hoc analysis of group  $\times$  time interaction effects).

Table 1. Plasma prolactin and catecholamine concentrations during alcohol intoxication, acute withdrawal and resolution of withdrawal in alcohol-dependent and control subjects

	Day 0 (intoxication)	Day 1 (withdrawal)	Day 7 (recovery)
<i>Prolactin, ng/ml</i>			
Alcohol-dependent	8.3 $\pm$ 1.8	6.0 $\pm$ 1.1	5.4 $\pm$ 0.8
Control	6.1 $\pm$ 0.6	6.6 $\pm$ 0.6	5.9 $\pm$ 0.4
<i>Adrenaline, pg/ml</i>			
Alcohol dependent	60.4 $\pm$ 13.7	59.3 $\pm$ 19.1	33.9 $\pm$ 4.3
Control	38.3 $\pm$ 6.8	43.9 $\pm$ 11.4	36.7 $\pm$ 9.2
<i>Noradrenaline, pg/ml</i>			
Alcohol-dependent	606.0 $\pm$ 135.2	504.0 $\pm$ 101.1	325.8 $\pm$ 52.2
Control	442.1 $\pm$ 60.3	464.0 $\pm$ 52.5	436.3 $\pm$ 42.0

were assayed in duplicate, with all samples measured within the same assay. Plasma concentrations of catecholamines were analyzed by high pressure liquid chromatography using electrochemical detection (Gynkoteck, Germany). The inter- and intra-assay variability for noradrenaline was 8.0 and 6.2%, respectively, and 5.1 and 4.0%, respectively, for adrenaline. Both prolactin and cortisol plasma concentrations were assayed using commercial assay kits. Plasma prolactin was evaluated by an immunoradiometric assay (MAIAclone, Biodata Diagnostics, Rome, Italy) and cortisol by a radioimmunoassay (Coat-A-Count RIA, DPC Diagnostics, Los Angeles, Calif., USA). Inter- and intra-assay variability were 7.1 and 5.0%, respectively, for prolactin, and 4.3 and 2.8%, respectively, for cortisol.

#### Flow Cytometry

Leukocytes were analyzed by flow cytometry using standard double-staining with monoclonal antibodies (DAKO, Hamburg, Germa-

ny). Antibody combinations allowed the assessment of T helper cells (CD3 PE/CD4 FITC), cytotoxic T cells (CD3 PE/CD8 FITC), monocytes (CD14 PE/CD45 FITC), and natural killer (NK) cells (CD56 PE/CD16 FITC). Information on at least 10,000 events was stored. The absolute number of each leukocyte subset was calculated by multiplying the percentage with the total leukocyte count obtained by means of an automated cell counter (Coulter Corporation, Miami, Fla., USA).

#### Statistical Analysis

Two-way (group  $\times$  time) repeated analyses of variance were used to analyze endocrine and immune parameters. Alterations were considered significant when  $p < 0.05$ , with post-hoc analyses conducted using Student's *t* test, and with Bonferroni correction for multiple comparisons. Data are presented graphically as mean  $\pm$  SEM, with significant interaction (group  $\times$  time) effects reported unless otherwise stated.

## Results

### General Patient Characteristics

The alcohol-dependent patients had a history of 10.9  $\pm$  2.2 years of dependence, consuming on average 287  $\pm$  23.9 g alcohol per day. Patients commenced the study in a state of chronic intoxication, with an average blood alcohol concentration of 1.72  $\pm$  0.16‰. Patients showed a mean corpuscular volume of 98.0  $\pm$  1.4  $\mu\text{m}^3$  and  $\gamma$ -glutamyl transferase levels of 79  $\pm$  31 U/l (range 12–283 U/l).

### Endocrine Response to Acute Alcohol Withdrawal

Alcoholics displayed significantly higher plasma cortisol concentrations than normal controls at the start of abstinence, lowering over the course of withdrawal [ $F(2, 30) = 10.521$ ,  $p < 0.0001$ ; group  $\times$  time interaction]. Post-hoc analysis revealed that plasma cortisol was significantly raised in alcoholics on days 0 and 1 of withdrawal. However, plasma cortisol decreased to control levels by day 7 of abstinence (fig. 1).

In contrast, levels of prolactin, adrenaline, and noradrenaline showed no significant difference between the alcohol-dependent patients and healthy controls over the course of the study (table 1) (all  $p > 0.05$ ).

### Blood Leukocyte Subsets

The alcohol-dependent patients displayed lower absolute numbers of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, and CD16<sup>+</sup>/CD56<sup>+</sup> NK cells across the whole period of abstinence (fig. 2). Statistically significant group differences were revealed for NK cells [ $F(1, 15) = 8.030$ ,  $p = 0.013$ ; group effect] and CD4<sup>+</sup> T cells [ $F(1, 15) = 4.629$ ,  $p = 0.046$ ; group effect]. Likewise, the number of monocytes (CD4<sup>+</sup>/CD14<sup>+</sup>) in peripheral blood were lower in the alcohol-

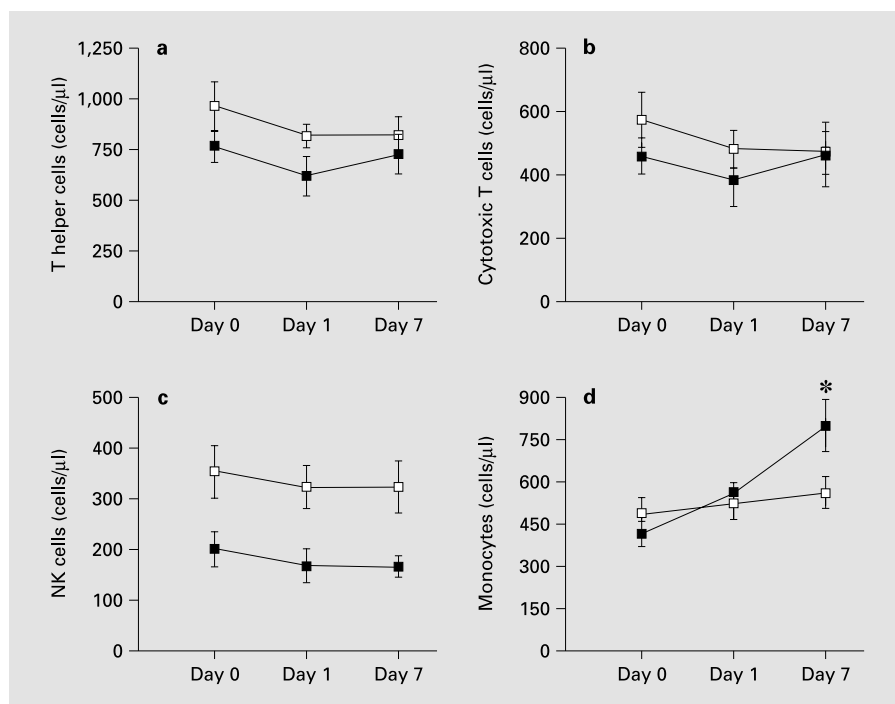


Fig. 2. Absolute number of T helper cells (CD3<sup>+</sup>/CD4<sup>+</sup>) (a), cytotoxic T cells (CD3<sup>+</sup>/CD8<sup>+</sup>) (b), NK cells (CD16<sup>+</sup>/CD56<sup>+</sup>) (c) and monocytes (CD4<sup>+</sup>/CD14<sup>+</sup>) (d) in peripheral blood of alcoholics (■) during alcohol intoxication (day 0), acute withdrawal (day 1), and following withdrawal resolution (day 7) compared with healthy control subjects (□) (\*  $p = 0.012$ , in post-hoc analysis of group  $\times$  time interaction effects).

dependent subjects compared with the healthy controls during intoxication (fig. 2). However, monocyte numbers increased across the detoxification period in the alcohol-dependent patients, showing significantly higher numbers than the controls on day 7 [ $F(2, 30) = 5.847$ ,  $p = 0.007$ ; group  $\times$  time interaction].

Further investigation of the neuroendocrine basis for altered monocyte numbers during withdrawal was performed by correlation analysis. However, no significant correlations were observed at any of the three time points between monocyte numbers and cortisol levels (day 0:  $r = -0.24$ ; day 1:  $r = -0.25$ ; day 7:  $r = -0.27$ ; all  $p > 0.05$ ), nor between the percentage increase (over the 3 time points) of monocytes and percentage decrease of cortisol (day 0:  $r = 0.29$ ; day 1:  $r = 0.36$ ; day 7:  $r = 0.35$ ; all  $p > 0.05$ ). Additionally, no significant correlation was observed between the level of alcohol intoxication on day 0 and cortisol levels (day 0:  $r = 0.11$ ; day 1:  $r = 0.27$ ; day 7:  $r = -0.19$ ; all  $p > 0.05$ ).

## Discussion

The present data revealed three major findings. First, the alcohol-dependent patients demonstrated lower numbers of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, NK cells, and monocytes

in peripheral blood compared with the healthy matched controls. Second, the alcoholics presented to the clinic with significantly elevated levels of plasma cortisol during intoxication, which fell slightly during acute withdrawal, and returned to control levels following acute withdrawal resolution. Third, although T cell and NK cell numbers remained decreased in the patients over the course of acute withdrawal, detoxification increased monocyte numbers in peripheral blood of the patients, reaching control numbers during acute withdrawal, and further increasing to numbers significantly greater than the control by the end of acute abstinence.

The alcohol-dependent subjects displayed prominent suppression of NK cells, T helper cells and cytotoxic T cells. Lowered cell numbers of these populations in patients was unaltered by acute withdrawal. These data support the position that chronic alcohol abuse in humans produces a suppression of blood T cell subpopulations, as well as suppressed cellular immune function [2–5]. Nevertheless, the nature of the effect of chronic alcohol-induced immunomodulation is equivocal, as in contrast to the suppression of NK cell numbers across all measurements, previous studies have revealed an increase of blood NK cells in chronic alcohol abuse [2, 3]. These dissimilarities are not surprising, as defining alcohol-induced alterations of immune functions in alcohol-dependen-

dent patients is complicated by immune changes due to concurrent disease/illness [17], nutrition, as well as comorbid psychiatric conditions [18]. The current patients were selected according to strict health criteria, excluding immune changes that may have been due to common alcohol-induced diseases, such as cirrhosis, hepatitis, or pancreatitis [2, 3]. Thus, although it has been shown that disease-free alcoholics display a relatively robust immune status [17], the current data suggest that chronic alcohol abuse alone is sufficient to induce a general suppression of leukocyte subpopulations in blood. Further, as these changes were not due to comorbid depression in alcohol-dependent patients, chronic alcohol abuse, independent of concurrent psychological disorder, suppresses leukocyte subpopulations in peripheral blood.

Although most leukocyte subsets remained suppressed over the entire duration of alcohol withdrawal, a significant elevation of blood monocyte numbers was observed following acute alcohol withdrawal. As chronic alcohol dependence is associated with increased rates of infection [19], upregulation of blood monocytes may have significant implications for disease in people with alcohol dependence. However, we must note that the current changes in status of the immune system represent enumerative parameters that do not necessarily reflect the functional status of immunocompetent cells. Nevertheless, the recognition that detoxification may improve resistance to infection in alcohol-dependent patients via improving the immune system status [20] warrants further investigation of the impact of withdrawal on components of the innate immune system.

Confirming previous data [11], the alcohol-dependent patients displayed markedly higher levels of plasma cortisol compared with the healthy controls during alcohol intoxication. However, in line with more recent data [12], cortisol hypersecretion was also observed during acute alcohol withdrawal, with attenuation following 1 week of detoxification. These data demonstrate that paradoxically, hypercortisolemia is a feature of both alcohol intoxication and acute withdrawal. However, it is unclear whether increased HPA activity observed at day 1 is a cortisol response to withdrawal, or whether this is a carry-over effect of hypercortisolemia during intoxication. Furthermore, it is unclear whether these changes may have been due to disturbed liver function, which is known to alter cortisol metabolism [21]. Additionally, as the circadian rhythm of cortisol secretion is altered in alcoholics [22], a single sampling of cortisol is not a precise method of determining HPA axis activity during abstinence. Rather, a challenge test (e.g. dexamethasone) might have

been a more appropriate tool for the examination of the HPA axis status.

Suppression of blood leukocyte subpopulations in alcohol-dependent patients may have been induced by alcohol-induced lymphocyte recirculation or cellular apoptosis [23]. Alternatively, the effects may have been due to chronic alcohol-induced endocrine alterations. As chronic hypercortisolemia suppresses leukocyte subpopulation numbers in peripheral blood [24, 25], HPA overactivity may drive the suppression of leukocyte subpopulation numbers observed in the alcohol-dependent patients in the present study. Removal of cortisol hypersecretion via acute alcohol withdrawal may have produced elevated numbers of monocytes. Indeed, this position is supported by data demonstrating that glucocorticoid therapy reduces monocyte numbers and function in human peripheral blood, while cessation of therapy produces monocytosis [26]. Nevertheless, as the small sample size did not produce statistically significant associations between cortisol levels and monocyte numbers, further research is required to test this position.

Despite the data suggesting a link between cortisol and changes in blood leukocyte distribution, the mechanism driving the current changes remains unclear. Altered HPA axis activation, liver disease, malnutrition or comorbid somatic or psychiatric disease may all contribute to the observed data. Therefore, further work is required to identify contributing mechanism(s) involved in the observed changes in immune status.

In summary, the current data demonstrate that although chronic alcohol abuse reduces peripheral blood leukocyte subpopulation numbers, acute detoxification produces a significant rebound of monocytes to levels in excess of levels measured 7 days after withdrawal. The alteration of monocyte numbers may be regulated by decreases in cortisol levels by the end of acute withdrawal, and may furthermore have the potential to improve disease resistance in alcohol-dependent patients.

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