

Altered Cell-mediated Immunity and Increased Postoperative Infection Rate in Long-term Alcoholic Patients

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Background: **Preoperative alteration of T cell–mediated immunity as well as an altered immune response to surgical stress were found in long-term alcoholic patients. The aim of this study was to evaluate perioperative T cell–mediated immune parameters as well as cytokine release from whole blood cells after lipopolysaccharide stimulation and its association with postoperative infections.**

Methods: **Fifty-four patients undergoing elective surgery of the aerodigestive tract were included in this prospective observational study. Long-term alcoholic patients (n = 31) were defined as having a daily ethanol consumption of at least 60 g and fulfilling the *Diagnostic and Statistical Manual of Mental Disorders* for either alcohol abuse or alcohol dependence. The nonalcoholic patients (n = 23) were defined as drinking less than 60 g ethanol/day. Blood samples to analyze the immune status were obtained on morning before surgery and on the morning of days 1, 3, and 5 after surgery.**

Results: **Basic patient characteristics did not differ between groups. Before surgery, the T helper 1:T helper 2 ratio (Th1:Th2) was significantly lower ($P < 0.01$), whereas plasma interleukin 1β and lipopolysaccharide-stimulated interleukin 1ra from whole blood cells were increased in long-term alcoholic patients. After surgery, a significant suppression of the cytotoxic lymphocyte ratio (Tc1:Tc2), the interferon γ:interleukin 10 ratio from lipopolysaccharide-stimulated whole blood cells, and a significant increase of plasma interleukin 10 was observed. Long-term alcoholics had more frequent postoperative infections compared with nonalcoholic patients (54% *vs.* 26%; $P = 0.03$).**

Conclusions: **T helper cell–mediated immunity was significantly suppressed before surgery and possibly led to inadequate cytotoxic lymphocyte and whole blood cell response in long-term alcoholic patients after surgery. This altered cell-mediated immunity might have accounted for the increased infection rate in long-term alcoholic patients after surgery.**

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

EVERY fifth patient admitted to a general hospital abuses alcohol.[1] In patients undergoing surgery of the aerodigestive tract, the rate of alcohol abuse even exceeds 50%.[2–8] Long-term alcoholic patients have a twofold to fivefold increased risk of postoperative morbidity after surgery.[9–11] Because of this increased postoperative morbidity, intensive care unit (ICU) treatment and overall hospital stay are prolonged.[2,3,9,11,12] Among all complications, infections are most relevant and are associated with a worse outcome.[2,3,9,11]

The mammalian immune system responds to any injury, *i.e.*, alcohol-related injury,[13–18] cancer,[19] surgical trauma,[20–24] by rapidly producing proinflammatory cytokines and other mediators of acute inflammation. After this initial inflammatory response, a compensatory anti-inflammatory response ensues. Although this response scenario may have evolved as a means to protect the injured host from the harmful effects of injury-induced inflammation, many of the mediators of this type of counterinflammatory response also have strong immunosuppressive activity. Consequently, clinical observations along with numerous studies in animal models suggest that injury often leads to a transient state of immune suppression that predisposes the injured host to infections caused by opportunistic pathogens. Advances in our understanding of how injury influences host immune responses suggest that injury causes a phenotypic imbalance in the regulation of T helper 1 (Th1)– and T helper 2 (Th2)–type immune responses. *In vivo* studies strongly suggest that injury skews T-cell responses toward increased Th2-type reactivity.[25] Therefore, the effect of injury on host immunity remains a significant clinical problem.

In general, T cell–mediated immunity of cancer patients is thought to be impaired, and the T-cell balance

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can be expected to shift from Th1 to Th2.[19] Appropriate induction of a Th1 response is required for effective eradication of intracellular pathogens and involves macrophage activation and production of complement fixing and opsonizing antibodies.[16,17] In alcoholic patients, controversial results were found. An increase in Th1 responses and a decrease in Th2 responses were reported,[18] whereas delayed-type hypersensitivity (DTH), reflecting the Th1:Th2 ratio, was decreased in long-term alcoholic patients before surgery.[9]

Surgery *per se* decreased the Th1:Th2 ratio.[20–23] In a previously published study, a depressed interleukin (IL) 12–producing activity by monocytes and a shift toward Th2-type lymphocyte pattern was seen on postinjury day 2, and this was associated with an increased infection and complication rate after injury.[26]

It remains unclear whether this altered Th1:Th2 response to surgery and in long-term alcoholics is associated with the cytotoxic lymphocyte responses (Tc1:Tc2 ratio) known to induce similar cytokine responses[23,26–29] as well as cytokine release from whole blood cells. In a previous study, decreased proinflammatory tumor necrosis factor (TNF) α and increased antiinflammatory IL-10 production was seen in long-term alcoholic patients during surgery.[30] A similar reaction of an exaggerated IL-10 response to surgery was found by our group in long-term alcoholic patients during surgery.[31]

No study has investigated the perioperative T cell–mediated immunity in long-term alcoholics undergoing surgery for cancer of the aerodigestive tract and its association with surgical stress and clinical outcome. Therefore, the primary aim of the study was to evaluate the T cell–mediated immune parameters as well as the cytokine release of whole blood cells in the perioperative period. The secondary aim was to determine whether any of these parameters might be relevant for postoperative infections in long-term alcoholic patients.

Materials and Methods

Patients

Fifty-four white patients undergoing surgery for aerodigestive tract tumor were included in this prospective observational study after receiving the approval of the institutional ethical committee and written informed consent from the patients. Basic patient characteristics and current smoking status were documented. Patients were not included in the study if they had any diagnosed infection in the previous 14 days before surgery, were HIV positive, had liver cirrhosis (Child B or C) because this is well known to change immune modulation toward a more proinflammatory state in long-term alcoholic patients,[32] were on corticosteroids, were mentally ill, were not admitted to a surgical ICU after surgery, had an unclear alcohol history, had abused multiple substances, or had a body mass index less than 20 kg/m^2. Ninety consecutive patients were screened for this study. Eleven patients did not give their informed consent to participate in this study. In total, 79 patients gave their written informed consent to participate in this study: 25 patients were excluded after inclusion because they were not admitted to the ICU (n = 19) or did not permit blood drawing during the whole study period (n = 6). The remaining 54 patients with tumors of the upper digestive tract were stratified into two groups: a long-term alcoholic group (n = 31) and a nonalcoholic group (n = 23).

Diagnosis of Long-term Alcohol Abuse and Group Assignment

Preoperatively, the patients' histories were obtained and an alcoholism-related questionnaire, the CAGE Questionnaire, was given.[33] The patients' daily ethanol intakes were documented. The *Diagnostic and Statistical Manual of Mental Disorders* (fourth edition)[34] criteria for alcohol dependence or alcohol abuse were obtained. Long-term alcoholics had a daily intake of at least 60 g ethanol/day for at least 1 yr preoperatively. Because of the fact that in previous studies postoperative outcome did not differ between social drinkers drinking 20–59 g/day and those patients with an ethanol intake of less than 20 g/day, in this study, all patients drinking less than 60 g/day were referred to the nonalcoholic group.[2]

Investigational Protocol and Measurements

All patients were included in this study at least 48 h before surgery. They underwent surgery of the aerodigestive tract and standardized anesthesia with isoflurane, fentanyl, and cisatracurium. Blood samples were drawn the morning before surgery and on days 1, 3, 5, and 7 between 7:00 AM and 10:00 AM after surgery. DTH tests (Multitest immignost®; Biosyn Arzneimittel GmbH, Fellbach, Germany) were performed twice, immediately after patients were included in the study, *i.e.*, 48 h before surgery and on the first postoperative day. Skin reactions to seven antigens (*Proteus*, *Trichophyton*, *Candida*, tetanus, diphtheria, *Streptococcus*, tuberculin) plus glycerin as control were taken. Results were taken after 48 h, looking for both the number of positive reactions and the sum of induration diameters. Hemodynamic measurements, including mean arterial pressure as well as heart rate and temperature, were also taken. Oxygenation index, defined as the ratio of arterial oxygen tension to fraction of inspired oxygen (Pa_{O_2}:F_{IO_2}) was determined and documented before surgery, and at each measurement point if the patient was still in the ICU. All alcohol-dependent patients received prophylactic treatment with midazolam and clonidine.[35] All patients were treated with piritramide to achieve a visual analog score of less than 3. The perioperative antibiotic prophy-

laxis was standardized.[36] All patients were treated perioperatively with cefuroxime–metronidazole. They received a single dose of antibiotics intravenously immediately before surgery started; if surgery proceedings lasted longer than 4 h, patients received a second dose.[36,37]

Laboratory Markers

Th1:Th2, and Tc1:Tc2 Ratios. Th1:Th2 and Tc1:Tc2 cytokine ratios from peripheral blood T cells were analyzed by flow-cytometric measurement of intracellular cytokine production after *in vitro* whole blood stimulation with phobol-12-myristate-13-acetate and ionomycin (fig. 1). The working procedure was based on the protocol for flow-cytometric measurement of intracellular cytokine production (Fa. Becton Dickinson, Heidelberg, Germany).

Cell Preparation and Cell Culture. Peripheral blood, 4.5 ml, was obtained in sterile heparinized tubes (4.5 ml S-Monovette® ammonium heparin, 15 immunizing units of heparin/ml blood; Fa. Sarstedt, Nümbrecht, Germany). For analysis including the control analysis (A, B, and C), four samples were necessary: Sample 1 with 50 μl was used to determine the T-lymphocyte subpopulation, in particular $CD4^+$ and $CD8^+$ T lymphocytes. The lyse-no-wash method was used, adding immune fluorescent antibodies of 5 μl CD3 peridinin chlorophyll (PerCP), phycoerythrin (PE)-conjugated antibody CD4, and fluorescein isothiocyanate–conjugated (FITC) CD8, vortexing 3 s with 1,500 U/min, and followed by a 15-min incubation (20–25°C) in the dark. Then, 500 μl FACS® lysing solution (Fa. Becton Dickinson) was applied to sample 1. After vortexing at 1,500 U/min for 3 s to avoid cell clumping, this sample was stored again for 15 min at room temperature (20–25°C) in the dark.

Stimulation. Samples A, B, and C with 1 ml heparinized blood were applied each in 5-ml sterile tubes (Falcon®; Becton Dickinson). For stimulation, phorbol-12-myristate-13-acetate at an end concentration of 1 μg/ml and ionomycin at an end concentration of 50 μg/ml were added to sample A (stimulation control). To sample B (native control to determine basal cytokine production, unstimulated), only the secretion inhibitor Brefeldin-A at an end concentration of 500 μg/ml (Fa. Sigma, Deisenhofen, Germany) was added. Phorbol-12-myristate-13-acetate and ionomycin as well as Brefeldin-A were applied to sample C (stimulation). All three samples (A, B, and C) were filled with RMPI-1640 medium (Fa. Sigma; total volume, 2 ml) and vortexed for 3 s at 1,500 U/min followed by a 4-h incubation at 37°C with 5% CO_2.

Staining. CD3 PerCP, 10 μl; 10 μl CD8 FITC; and 10 μl CD69 PE were added to sample A, followed by the lyse-and-wash method. Samples B and C were stained

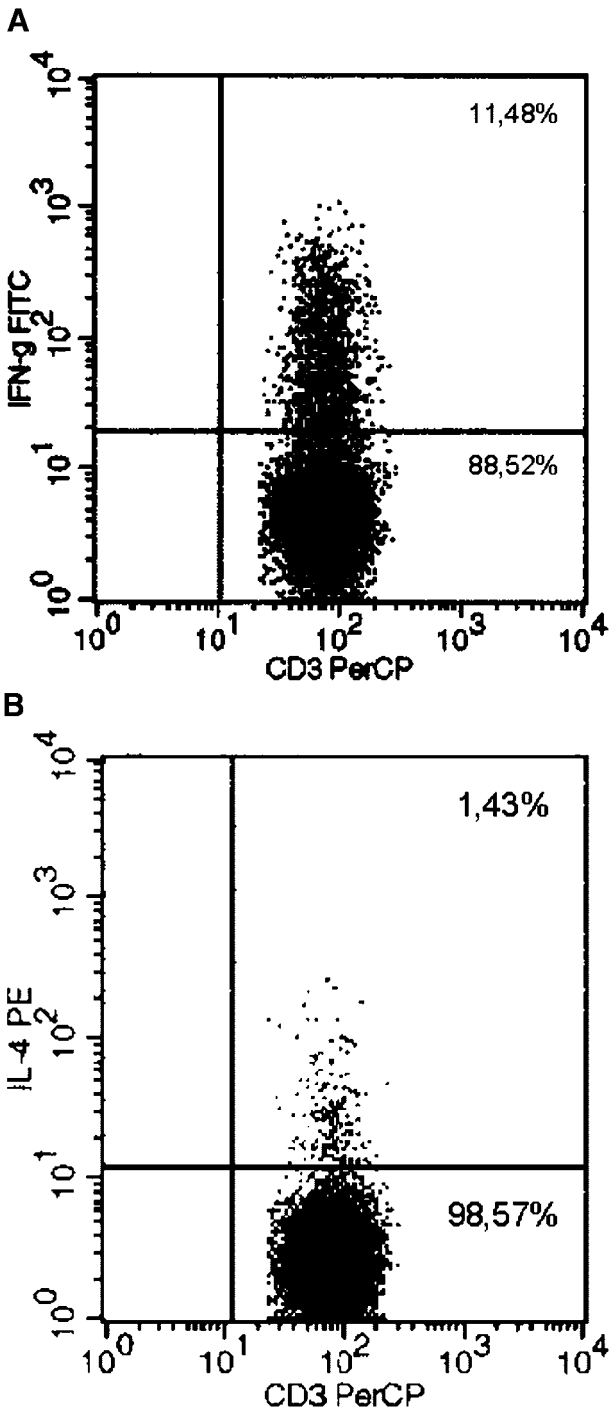

Fig. 1. Flow-cytometric dot plots of Th1 (interferon [IFN] γ) (*A*) and Th2 (interleukin [IL] 4 PE) (*B*) cells. FITC = fluorescein isothiocyanate conjugated; *horizontal line* = isotype control.

first. Sample B included seven subdivisions with 100 μl in 5-ml sterile tubes (Falcon®):

- B1–3: staining with 10 μl CD3 PerCP and 10 μl CD8 FITC
- B4 and 5: staining with 10 μl CD3 PerCP and 10 μl CD8 PE
- B6 and 7: staining with 10 μl CD3 PerCP

Sample C included seven subdivisions with 100 μl in 5-ml sterile tubes (Falcon®):

- C1–3: staining with 10 μl CD3 PerCP and 10 μl CD8 FITC
- C4 and 5: staining with 10 μl CD3 PerCP and 10 μl CD8 PE
- C6 and 7: staining with 10 μl CD3 PerCP

All samples were vortexed for 3 s at 1,500 U/min and incubated at room temperature in the dark for 20 min. This was followed by centrifugation (5 min at 500*g*). The pellet was mixed with 500 μl FACS® Permeabilizing Solution (Fa. Becton Dickinson), followed by vortexing (3 s) and a 15-min incubation in the dark for permeabilizing. After subsequent washing in a solution of 2 ml phosphate-buffered saline, 0.1% natrium-acetate solution, and 0.5% bovine serum albumin and centrifugation at 500*g*, the second staining of the now surface-marked permeabilized cells with fluorescent antibodies started, using samples of B1–7 and C1–7 (Falcon®):

- B1 and C1: staining with 10 μl CD69 PE
- B2 and C2: staining with 10 μl FastImmune anti-Hu IL4 PE
- B3 and C3: staining with 10 μl FastImmune γ_1PE IL4 isotype control
- B4 and C4: staining with 10 μl FastImmune anti-Hu interferon (IFN) γ FITC
- B5 and C5: staining with 10 μl FastImmune γ_{2a} IFN-γ FITC isotype control
- B6 and C6: staining with 20 μl FastImmune anti-Hu IFN-γ FITC–anti-Hu IL4 PE
- B7 and C7: staining with 20 μl FastImmune γ_{2a} FITC–γ_1 PE isotype control

All samples were vortexed (3 s) at 1,500 U/min and were incubated for 20 min in the dark at room temperature. After subsequent washing in a solution of 1 ml phosphate-buffered saline, 0.1% natrium-acetate solution, and 0.5% bovine serum albumin and centrifugation at 500*g*, all cells were fixed with 500 μl CellFix (Fa. Becton Dickinson). All samples were stained and analyzed on the same day because there was fading of the FITC fluorescence signal with overnight storage.

Multiparameter Flow Cytometric Analysis. A FACScan Cytometer (Fa. Becton Dickinson) fitted with a 15-mW air-cooled 488-nm argon ion laser and filter settings for FITC (530 nm), PE (585 nm), and PE-Cy5 emitting in the deep red (> 650 nm) was used. Data acquisition on the flow cytometer was performed with FACScan Research Software (Fa. Becton Dickinson). After appropriate instrument settings and spectral compensations, the settings were not changed, and stability was regularly checked with fluorescent beads (Calibrite; Fa. Becton Dickinson). A minimum of 15,000 events was computed using log-amplified fluorescence signals and linearly amplified side-scatter and forward-scatter signals. The data were analyzed using CellQuest Software (Fa. Becton Dickinson), and results are shown as percentage of positive cells (fig. 1). A gate was set around the lymphocyte cluster on forward-scatter *versus* side-scatter dot plots to exclude monocytes, neutrophils, and debris from data analysis. Negative control reagents were used to verify the staining specificity of experimental antibodies and as a guide for setting markers to delineate positive and negative populations. According to the literature, the ratios were given as percentages of: Th1:Th2 ratio: %IFN-γ $CD3^+$ $CD4^+$/%IL-4 $CD3^+$ $CD8^-$; and Tc1:Tc2 ratio: %IFN-γ $CD3^+$ $CD8^+$/%IL-4 $CD3^+$ $CD8^+$. To exclude natural killer cells, monocytes, and dendritic cells, it was a prerequisite that $CD3^+$ $CD8^-$ and $CD3^+$ $CD4^+$ were 5% or less, and $CD3^+$ $CD8^+$ and $CD3^+$ $CD4^-$ were 5% or less.

Lipopolysaccharide-stimulated Whole Blood Cells. Blood samples to determine lipopolysaccharide-stimulated whole blood cells were drawn at 8:00 AM the morning before surgery and postoperative days 1, 3, 5, and 7. Heparinized whole blood, 50 μl, was incubated at 37°C for 4 h with the stimulation solution containing culture medium with 500 pg/ml lyophilized lipopolysaccharide. Whole blood cells were stimulated to produce cytokines, in particular IL-1ra, TNF-α, IFN-γ, IL-10, and IL-12, which were measured in the supernatant after centrifugation for 5 min at 1,000*g* using commercially available kits (Quantikine® Immunoassay Kit; R&D Systems, Minneapolis, MN, for IL-1-ra and TNF-α; Enzyme Immunoassay Kit; Immunotech, Beckman Coulter Company, Marseille, France, for IFN-γ, IL-12, and IL-10). Detection limits were: Il-1ra, 14 pg/ml (intraassay and interassay coefficients, 4.8 and 5.3%, respectively); TNF-α, 4.4 pg/ml (4.6 and 5.8%); IFN-γ, 0.08 U/ml (6.0 and 8.3%); IL-10, 5 pg/ml (3.0 and 7.0%); IL-12, 5 pg/ml (2.8 and 6.7%)

Cytokines. Blood samples were collected in iced sterile tubes (EDTA–serum), and after centrifugation, the supernatants were stored in liquid nitrogen at −70°C. All mediators were analyzed at 23°C. The cytokines IL-1β, IL-6, IL-8, and IL-10 were analyzed by a sandwich enzyme-linked immunosorbent assay, using commercially available kits (Quantikine® Immunoassay Kit for IL-1β; Enzyme Immunoassay Kit for IL-6, IL-8, and IL-10). Detection limits (EDTA–plasma) were as follows: IL-1β, 0.1 pg/ml (intraassay and interassay variation coefficients, 3.0% and 12.5%, respectively); IL-6, 3 pg/ml (4.6 and 12.1%); IL-8, 8 pg/ml (5.0 and 11.1%); IL-10, 5 pg/ml (3.0 and 7.0%).

Cortisol, ACTH, and β Endorphin. Blood samples were collected in iced sterile tubes (EDTA–serum), and after centrifugation, the supernatants were stored in liquid nitrogen at −70°C. Plasma adrenocorticotrophic hormone (ACTH) concentrations were determined by commercially available immunoassay kits (Immulite® ACTH; Diagnostic Products Corporation, Los Angeles,

CA). The assay sensitivity was 9 pg/ml, and the intraassay and interassay variation coefficients were 3.1 and 5.9%, respectively. Plasma cortisol concentrations were analyzed using commercially available kits (Calibrator kit; Fa. Bayer Corporation, BGD, Tarrytown, NY). The assay sensitivity was 5.5×10^{-6} M and the intraassay and interassay coefficients of variation were 3.1 and 9.1%, respectively. In addition, β-endorphin blood samples were collected using a chilled syringe and transferred into a polypropylene tube containing EDTA (1 mg/ml) and aprotinin (500 KU/ml blood) at 0°C, were centrifuged at 0°C, and were stored at −70°C for maximum stability. β-endorphin serum concentrations were analyzed using a standardized commercial kit (β-endorphin human RIA Kit 125-I; Peninsula Laboratories Europe, Merseyside, England).

Epinephrine and Norepinephrine. Blood samples were collected in iced sterile tubes (prepared with EGTA–GSH solution), and after centrifugation, the supernatants were stored in liquid nitrogen at −70°C. Epinephrine and norepinephrine plasma concentrations were analyzed using a standardized high-performance liquid chromatography method of our department of laboratory medicine. Detection limits were 10–1,000 pg/ml. The intraassay and interassay variation coefficients were, respectively, 5.4 and 4.3% for epinephrine and 5.8 and 4.0% for norepinephrine.

Conventional Laboratory Markers. Alcohol-related laboratory data, including mean corpuscular volume, γ-glutamyl-transferase, and carbohydrate-deficient transferrin, were obtained on admission to the hospital.[12] Routine laboratory parameters, including hemoglobin, hematocrit, leukocyte count, C-reactive protein, bilirubin, creatinine, thrombocyte count, and plasmatic coagulation, were determined at each measurement point plus two times a day after surgery in the ICU. Arterial blood gases (acid–base balance, electrolytes, lactate, and glucose) were obtained before surgery *via* a radial artery catheter 1 h before surgery and after admission to the ICU.

Postoperative Period

Postoperatively, all patients were admitted to the ICU. Diagnosis, surgery, the Acute Physiology and Chronic Health Evaluation Score III,[38] the Multiple Organ Failure Score,[39] ventilatory needs, and the duration of ICU and hospital stay were documented. In addition, infection criteria and other intercurrent complications, such as cardiac complications, bleeding disorders, and alcohol withdrawal syndrome, were recorded on a daily basis. The researchers who performed the laboratory analysis were blinded to data collection and ICU outcome.

All infections were diagnosed according to the criteria recommended by the Center for Disease Control and Prevention,[40] and the frequency of patients who had any infection was documented. *Tracheobronchitis* was diagnosed if the patient had at least three of the following five signs or symptoms: cough, rhonchi, wheezing, or other auscultatory findings without evidence of pulmonary consolidation; purulent sputum production; body temperature greater than 38°C; leucocytosis; or organisms isolated from culture obtained by tracheal aspirate or bronchoscopy. In case of *pneumonia*, the diagnosis was made if systemic signs of infection were present, new or worsening infiltrates were seen on the chest x-ray, and new onset of purulent sputum or a change of sputum with bacteriologic evidence was found.[41] A *surgical site infection* (superficial/deep) wound infection was diagnosed if the infection occurred within 30 days after the operative procedure and if the patient had at least one of the following criteria: purulent draining from the superficial/deep incision or organisms isolated from an aseptic obtained culture from the superficial/deep incision; and at least one of the following signs or symptoms: pain, tenderness, swelling, redness, or heat. *Symptomatic urinary tract infection* was diagnosed if the patient had at least one of the following criteria: fever (> 38°C), urgency, frequency, dysuria, suprapubic tenderness, and a positive urine culture ($> 10^5$ microorganisms/cm^3).

The microbiologic screening was started on admission to the ICU and was performed according to clinical routine in case of clinical signs of infections.[37] It included routine nose, throat, and wound swabs as well as cultures from tracheal aspirate or bronchoalveolar lavage. The antimicrobiologic therapy was according to the specific sensitivity of the strains in these microbiologic screenings.

Cardiac complications included arrhythmias, left ventricular failure, and myocardial ischemia.[2,42] Each of these cardiac complications were diagnosed according to the internationally accepted criteria.[42–44] *Bleeding incidences* were defined as requiring either blood transfusion or surgical revision.[2] The differential diagnosis of *alcohol withdrawal syndrome* (AWS) was made according to the Clinical Institute Withdrawal Assessment for Alcohol-Revised scale.[45] The diagnosis was confirmed by a psychiatric consultation. The onset of AWS was documented in the study protocol. All patients were treated with flunitrazepam if AWS occurred. Haloperidol was added if the patient developed productive-psychotic signs, and the α_2-agonist clonidine was added if the patient had autonomic signs. In addition, propofol infusion was applied for the night if necessary in a dose to achieve a Ramsay Sedation Scale score of 2 or 3. Treatment of AWS was guided to achieve a Clinical Institute Withdrawal Assessment for Alcohol-Revised scale score of less than 20.[12,45,46]

Statistics

All data were expressed as median and interquartiles. All parameters of neuroendocrine immune axis with respect to time were analyzed using nonparametric mul-

Table 1. Demographic Characteristics and Alcoholism-relevant Data of Long-term Alcoholics and Nonalcoholics

	Long-term Alcoholics (n = 31)	Nonalcoholics (n = 23)	*P* Value
Age, yr	56 (49–61)	55 (51–60)	0.871
BMI, kg/m^2	24 (22–29)	28 (24–30)	0.174
Sex (M/F)	27/4	20/3	0.988
CAGE	3 (2–3)	0 (0)	< 0.001*
Ethanol consumption, g/day	75 (60–120)	15 (0–30)	< 0.001*
CDT, %	7 (5–11)	4 (3–5)	< 0.001*
GGT, U/l	45 (19–81)	18 (10–26)	< 0.001*
MCV, fl	96 (92–100)	91 (89–93)	< 0.001*
ASAT maximum, U/l	8 (8–21)	11 (9–18)	0.759
ALAT maximum, U/l	12 (6–24)	17 (13–21)	0.558
Current smoking, No.	25/31 (81%)	12/23 (52%)	0.009*
Preoperative oxygenation index, mmHg	350 (307–393)	378 (319–393)	0.420

Data are presented as median (quartiles 25–75) or No. (= frequency). All alcoholism-related parameters were taken on admission to the hospital.

* $P < 0.05$.

ALAT = alanine aminotransferase (5–22 U/l); ASAT = aspartate aminotransferase (5–18 U/l); BMI = body mass index; CAGE = alcoholism-associated questionnaire (normal range, 0–1); CDT = carbohydrate-deficient transferrin (0–5%); fl = femtoliters; GGT = γ-glutamyl-transferase (5–28 U/l); MCV = mean corpuscular volume (normal range, 80–96 fl).

tivariate analysis of variance for repeated measurements in a two-factorial design (first factor (group): long-term alcoholics *vs.* nonalcoholics; second factor (time).[47] Therefore, we compared all the four time points simultaneously on the corresponding response curves. Differences in chosen clinical parameters (such as patient characteristics, conventional laboratory data, and others) between long-term alcoholics and nonalcoholics were proven by means of the Mann–Whitney U test and the Fisher exact test, respectively. Diagnostic test performance was evaluated by receiver operating characteristic analysis. The receiver operating characteristic analysis was performed as described previously.[48] $P < 0.05$ was considered significant. The numerical calculations were performed with SAS for WINDOWS (release 8.02, copyright 1999–2001; SAS Institute Inc., Cary, NC).

Results

Basic patient characteristics differed in alcohol-related history and laboratory markers, as well as current smoking (table 1). Seventeen patients were alcohol dependent; the remaining 14 patients were alcohol abusers. No patient had signs of any infection on the day of surgery.

Preoperatively, Th1:Th2 ratios were significantly lower in long-term alcoholic patients compared with nonalcoholic patients (fig. 2). During surgery, Th1:Th2 ratios decreased in nonalcoholic patients and remained low in both groups after surgery (fig. 2). Tc1:Tc2 ratios decreased in long-term alcoholic patients during surgery and remained significantly suppressed after surgery, whereas Tc1:Tc2 ratios increased in nonalcoholic patients during surgery and remained increased after surgery (fig. 2). In contrast, the IFN-γ:IL-10 ratio from lipopolysaccharide-stimulated immune cells increased in nonalcoholic patients but decreased in long-term alcoholic patients during surgery and remained different between groups until day 5 after surgery (fig. 2). The DTH skin response was preoperatively and postoperatively significantly impaired in long-term alcoholic patients compared with nondrinkers (fig. 3).

Interleukin 1β plasma concentrations were increased in alcoholic patients preoperatively and decreased during surgery, whereas they remained unchanged in nonalcoholic patients until day 5 after surgery (fig. 4). IL-10 plasma concentrations increased during surgery in long-term alcoholic patients and remained increased until day 5 after surgery (fig. 4). IL-1ra from lipopolysaccharide-stimulated whole blood cells was increased in long-term alcoholic patients preoperatively and remained increased during surgery, whereas it remained unchanged in nonalcoholic patients (fig. 5). No other immune parameters differed between groups after surgery (tables 2 and 3).

Parameters of the stress axis as cortisol, ACTH, and β endorphin as well as catecholamines, *i.e.*, epinephrine and norepinephrine, did not differ between groups (table 4). Conventional laboratory markers indicating an infection such as C-reactive protein, leukocytes, lactate, and thrombocytes did not differ between groups (C-reactive protein maximal values: long-term alcoholics, 11.3 [9–16] mg/dl; nonalcoholics, 8.8 [1–17] mg/dl; lactate maximal values: long-term alcoholics, 1.0 [0.7–1.9] mM; nonalcoholics, 0.8 [0.6–1.1] mM; leukocyte maximal values: long-term alcoholics, 11 [7–16] nl; nonalcoholics, 13 [10–17] nl; thrombocyte minimal values: long-term alcoholics, 189 [141–239] nl; nonalcoholics, 161 [138–214] nl).

The Acute Physiology and Chronic Health Evaluation scores and Multiple Organ Failure Score on admission to the ICU did not differ between long-term alcoholic pa-

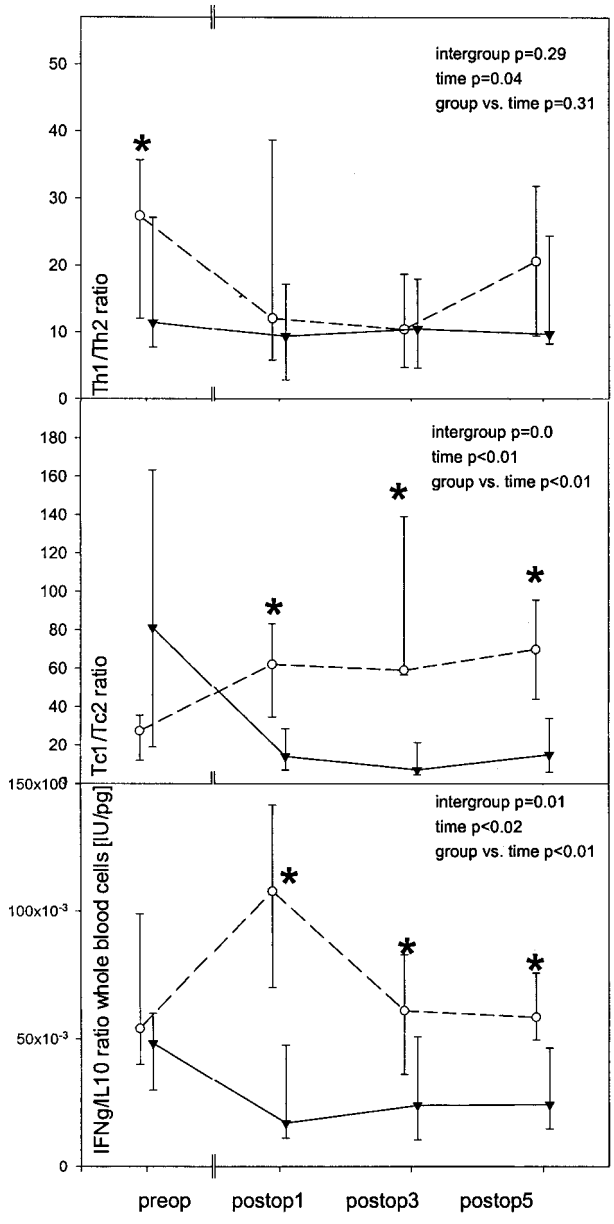

Fig. 2. Perioperative T-helper cell ratio (non-CD8$^+$) (Th1/Th2), cytotoxic T-lymphocyte ratio (CD8$^+$) (Tc1/Tc2), and lipopolysaccharide-stimulated whole blood cell interferon (IFN) γ:interleukin (IL) 10 ratio in long-term alcoholics (*filled triangles*) and nonalcoholics (*open circles*). Postop = days after surgery; preop = 7:00 AM the day before surgery. * $P < 0.01$.

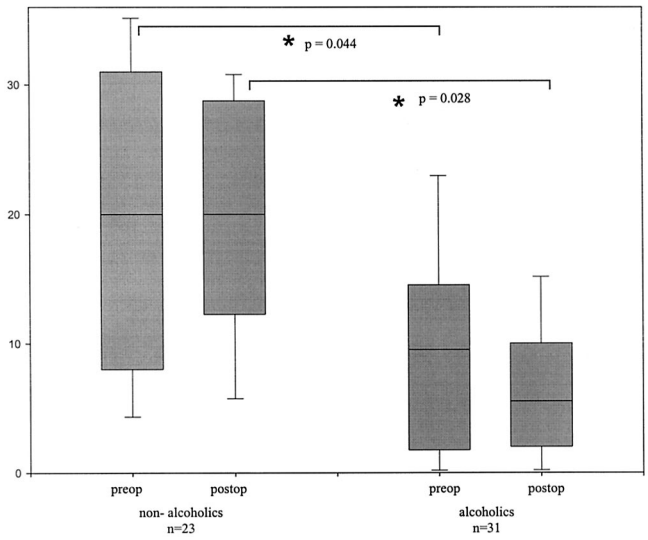

Fig. 3. Delayed-type hypersensitivity skin test. Delayed-type hypersensitivity is presented as the median (quartiles 25–75) of the skin test area of delayed-type hypersensitivity responses in square millimeters. * $P < 0.05$; number of positive reactions in long-term alcoholics (before surgery [preop], 1.5 [1–2]; 48 h after surgery [postop], 1.0 [1–2]) and nonalcoholics (preop, 2 [2–4]; postop, 3 [2–4]); $P < 0.05$.

tients and nonalcoholics; ICU and hospital stay were prolonged in long-term alcoholic patients (table 5). The overall postoperative infection rate and the incidence of pneumonia were significantly increased in long-term alcoholic patients (table 5). Multivariate logistic regression revealed that the preoperative Th1:Th2 ratio, the postoperative Tc1:Tc2 ratio, and the IFN-γ:IL-10 ratio from lipopolysaccharide-stimulated whole blood cells 1 day after surgery were predictive of postoperative infections ($P = 0.03$). Considering a possible marker function by receiver-operating characteristics, however, only the immediate postoperative IFN-γ:IL-10 ratio from lipopolysaccharide-stimulated whole blood cells showed an area under the curve of 0.83 (lower 95% confidence limit, 0.53).

Dual addiction (alcoholism and smoking) further increased the postoperative infectious complication rate (table 6). Despite the fact that long-term alcoholics and current smokers did not significantly differ in their TNF-α concentrations, the combination of both addictions significantly increased TNF-α (long-term alcoholics and smokers [n = 25], median, 23.9 pg/ml [quartiles, 11.8–164.0 pg/ml]; long-term alcoholics and nonsmokers [n = 6], 19.7 [4.6–32.4] pg/ml; nonalcoholics and smokers [n = 12], 11.3 [4.6–33.6] pg/ml; nonalcoholics and nonsmokers [n = 11], 9.5 [5.6–30.6] pg/ml; $P_{\text{ANOVA-type}} = 0.04$).

In addition to the infectious complications, cardiac and bleeding complications did not significantly differ between groups (frequency in long-term alcoholic patients *vs.* nonalcoholic patients: cardiac complications, 6 of 31 [20%] *vs.* 2 of 23 [9%]; $P = 0.280$; bleeding complications, 4 of 31 [13%] *vs.* 2 of 23 [9%]; $P = 0.288$). Catecholamines were given in 3 patients (dopamine plus norepinephrine) in the long-term alcoholic group *versus* 1 patient in the nonalcoholic group; hemodynamics such as mean arterial pressure and heart rate did not differ between groups. AWS was only seen in long-term alcoholic patients (frequency, 6 in 31 [20%]; $P = 0.026$). The beginning of AWS was in median on day 1 (range, 0–3 days) after surgery. The maximal Clinical Institute Withdrawal Assessment for Alcohol-Revised scale score was 29.

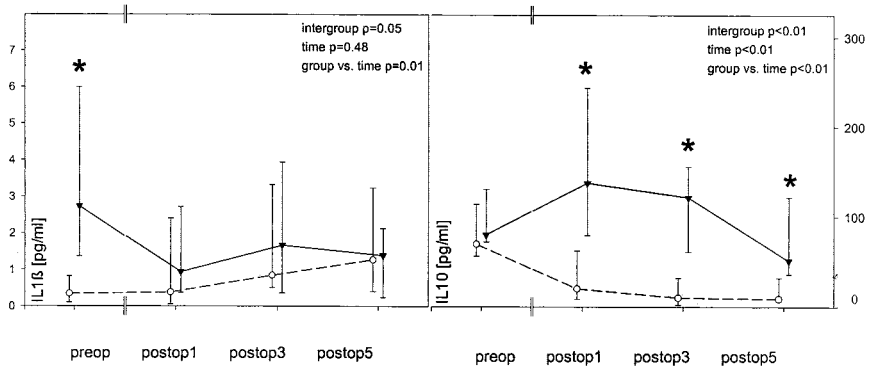

Fig. 4. Perioperative plasma interleukin (IL) 1β and IL-10 concentrations in long-term alcoholics (*filled triangles*) and nonalcoholics (*open circles*). Postop = days after surgery; preop. = 7:00 AM the day before surgery. $P < 0.01$.

Discussion

The most important result of this study was that the Th1:Th2 ratio was significantly lower in long-term alcoholic patients before and after surgery. This was associated with a significantly lower Tc1:Tc2 ratio as well as a decreased IFN-γ:IL-10 ratio after surgery in long-term alcoholics. In addition, DTH skin response was impaired in long-term alcoholic patients before and after surgery. This altered cell-mediated immunity might be a relevant pathomechanism for the increased postoperative infection rate in these long-term alcoholic patients.

T Cell–mediated Immunity

Long-term alcoholic patients in our study had lower Th1:Th2 ratios before surgery. This is in accord with the significantly impaired DTH skin response. There is only one other surgical study, by Tonnesen *et al.*,[9] in which it was also found that the DTH skin response of patients undergoing colorectal surgery was impaired among alcoholic patients preoperatively compared with nonalcoholics. In experimental settings, there is an increase in Th1 and a decrease in Th2 response seen in ethanol-consuming mice.[14–16] Antibody responses, regulated by Th2 lymphocytes, are either unimpaired or enhanced.[13,16] This preferential induction of Th2 *versus* Th1 immune response suggested in long-term alcoholics is in accord with the reduced DTH reaction.[49,50] Th1 unresponsiveness can be infectious for unrelated antigens.[51] Appropriate induction of a Th1 response is required for effective eradication of intracellular pathogens and involves macrophage activation and production of complement fixing and opsonizing antibodies.[13,14]

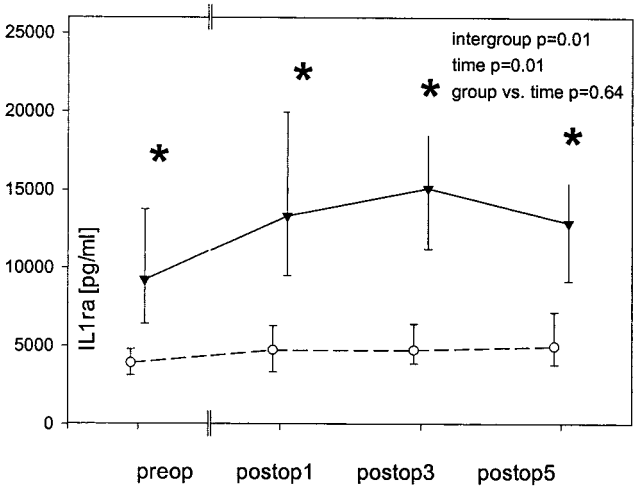

Fig. 5. Perioperative cytokines of lipopolysaccharide-stimulated whole blood cells interleukin (IL) 1ra supernatant in long-term alcoholics (*filled triangles*) and nonalcoholics (*open circles*). Postop = days after surgery; preop = 7:00 AM the day before surgery. $P < 0.01$.

During surgery, Tc1:Tc2 ratios decreased in long-term alcoholic patients and remained significantly low in long-term alcoholic patients after surgery, whereas Tc1:Tc2 ratios increased in nonalcoholic patients during surgery and remained increased after surgery. In contrast, Th1:Th2 ratios decreased in nonalcoholic patients during surgery and remained low in both groups after surgery. It is well known that surgery and major injury induces a shift toward a suppressed Th1:Th2 ratio.[20–23,52,53] Therefore, surgery can add to the ethanol-induced altered immune response and alter Th1:Th2 ratios in the same manner.[54] In a study by Tonnesen *et al.*,[55] the DTH skin response of patients undergoing colorectal surgery was reduced after surgery in all patients, but to a significantly larger extent in long-term alcoholic patients. In our study, both the number of positive reactions and the sum of induration diameters were significantly lower in long-term alcoholic patients postoperatively, whereas they were unchanged in nonalcoholic patients. $CD4^+$ T cells are required for the development of cytotoxic $CD8^+$ T cells.[24,27] The increase of Tc1:Tc2 ratio in nonalcoholic patients after surgery may be considered an adaptation to maintain effective immunity, and progression of infection such as this is observed in many other settings, such as viral, in particular HIV, and *Mycobacterium tuberculosis* infections.[24,27,28] Postburn changes in T-cell reactivity in $CD8^+$ rather than in $CD4^+$ cells can be considered in accord with our study in nonalcoholic patients after surgery.[56] Because long-term alcoholic patients cannot regulate their cell-mediated pathways, in particular cytotoxic lymphocytes, in the same way as

Table 2. Plasma Cytokines in Long-term Alcoholics and Nonalcoholics

	Long-term Alcoholics (n = 31)	Nonalcoholics (n = 23)	*P* Value
TNF-α, pg/ml			
Preop	10.9 (5.3–24.8)	6.4 (3.9–17.2)	0.201
Postop 1–3	39.3 (9.9–112.7)	16.6 (5.2–25.6)	0.193
Postop > 3	23.6 (9.4–78.5)	8.8 (3.3–34.1)	0.065
IL-6, pg/ml			
Preop	20.0 (12.8–42.9)	13.3 (6.3–27.2)	0.126
Postop 1–3	216.4 (108.6–374.8)	115.4 (55.4–201.4)	0.069
Postop > 3	37.2 (16.0–93.0)	15.1 (9.4–37.7)	0.018*
IL-8, pg/ml			
Preop	25.5 (11.2–58.4)	10.6 (0–80.3)	0.263
Postop 1–3	37.5 (24.1–96.1)	21.0 (0–149.8)	0.371
Postop > 3	46.8 (19.4–163.8)	12.2 (0.3–139.9)	0.147

Data are presented as median (quartiles 25–75).

* $P < 0.05$.

IL = interleukin; postop 1–3 = intensive care unit days 1–3 after surgery; postop > 3 = bedside days 5–7 after surgery; preop = bedside days 1–7 before surgery; TNF = tumor necrosis factor.

nonalcoholic patients after surgery, as was shown for the first time in the current study, this may be another hint of a severe postoperative immune suppression. However, it remains unclear in this study what the exact factors and mechanisms for these different cellular immune responses are, *i.e.*, antigen presentation, down-regulation of human leukocyte antigen or up-regulation of FAS ligand, or different recognition pathways.[26–28,56]

Lipopolysaccharide-stimulated Whole Blood Cells and Plasma Cytokine Concentrations

The IFN-γ:IL-10 ratio from lipopolysaccharide-stimulated whole blood cells increased in nonalcoholic patients but decreased in long-term alcoholic patients during surgery and remained different between groups until day 5 after surgery. IFN-γ encourages the differentiation of precursors into Th1 or Tc1 cells, whereas IL-10 induces the generation of Th2 or Tc2 cells. Th1 cells and Tc1 cells can produce IFN-γ, and IFN-γ from whole blood cells can inhibit Th2 and Tc2 generation. Th2 and Tc2 can produce IL-10, and IL-10 from whole blood cells can inhibit Th1 and Tc1.[57]

Table 3. Lipopolysaccharide-induced Stimulation of Whole Blood Cells in Long-term Alcoholics and Nonalcoholics

Whole Blood Cells	Long-term Alcoholics (n = 31)	Nonalcoholics (n = 23)	*P* Value
IL-12, pg/ml			
Preop	5.0 (5.0–7.0)	5.1 (5.0–13.6)	0.798
Postop 1–3	9.2 (5.0–21.8)	9.9 (5.1–85.9)	0.772
Postop > 3	5.4 (5.0–23.8)	6.7 (5.0–17.1)	0.718
TNF-α, pg/ml			
Preop	4.5 (3.1–9.1)	6.0 (2.8–7.6)	0.873
Postop 1–3	3.3 (2.5–5.0)	9.2 (4.0–12.8)	0.078
Postop > 3	6.2 (4.6–8.1)	6.9 (5.4–9.7)	0.423

Data are presented as median (quartiles 25–75).

IL = interleukin; postop 1–3 = intensive care unit days 1–3 after surgery; postop > 3 = bedside days 5–7 after surgery; preop = bedside days 1–7 before surgery; TNF = tumor necrosis factor.

Interleukin 1β plasma concentrations were increased in long-term alcoholics before and decreased during surgery, whereas they remained unchanged in nonalcoholic patients. In contrast, IL-10 plasma concentrations increased during surgery in long-term alcoholic patients and remained increased until day 5 after surgery. In addition, IL-1ra from lipopolysaccharide-stimulated whole blood cells was increased in long-term alcoholic patients before surgery and further increased during surgery but remained unchanged in nonalcoholic controls until day 5 after surgery.

The different immune modulation after surgery and its association with a worse outcome in long-term alcoholic patients was seen in previous studies conducted by our group.[31,58] An immediate postoperative suppressed IL-6:IL-10 ratio of plasma concentrations was predictive of later onset of postoperative infections.[31] This may be considered a different immune response to IL-6 release in long-term alcoholic patients. IL-6 may trigger a proinflammatory response in patients without acute or chronic diseases, whereas an antiinflammatory surgical response may be triggered in long-term alcoholic patients.[31,32] Significantly decreased concentrations of proinflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8 were found in early septic shock patients with a history of alcohol abuse.[58] In experimental settings, the presence or absence of IFN-γ was critical in determining the effect of short-term alcohol use on monocyte IL-12 *versus* IL-10 induction.[59]

With respect to outcome, an increased susceptibility to postoperative sepsis was already seen in patients with impaired monocyte IL-12 production.[60] Anti–IL-10 antibody restored burn-induced defects in T-cell function.[61] In experimental settings, IL-12 therapy restored cell-mediated immunity in ethanol-consuming mice, and adenoviral-mediated IFN-γ gene therapy augmented pulmonary host defense of ethanol-treated rats.[62,63] Therefore,

Table 4. ACTH, β-Endorphin, Cortisol, and Catecholamines in Long-term Alcoholics and Nonalcoholics

	Long-term Alcoholics (n = 31)	Nonalcoholics (n = 23)	*P* Value
ACTH, pg/ml			
Preop	6.5 (4.2–13.1)	4.9 (3.3–6.6)	0.131
Postop 1–3	5.1 (2.4–16.5)	5.2 (2.7–7.3)	0.419
Postop > 3	4.7 (3.1–12.4)	5.7 (3.3–6.1)	0.609
β-Endorphin, pg/ml			
Preop	19.4 (12.4–24.6)	16.9 (13.3–21.4)	0.666
Postop 1–3	25.8 (14.7–31.2)	17.3 (13.4–20.8)	0.039*
Postop > 3	26.7 (15.8–32.9)	18.4 (15.9–25.1)	0.179
Cortisol, μg/dl			
Preop	381.3 (310.0–470.6)	411.1 (307.8–506.9)	0.591
Postop 1–3	525.7 (335.8–1168.1)	368.9 (292.1–641.3)	0.162
Postop > 3	417.4 (268.9–781.6)	414.3 (309.1–499.7)	0.658
Epinephrine, pg/ml			
Preop	217.5 (177.8–427.0)	200.0 (110.8–380.8)	0.471
Postop 1–3	317.5 (106.0–1040.0)	253.0 (67.5–575.8)	0.524
Postop > 3	183.3 (56.7–438.3)	167.5 (86.0–419.8)	0.763
Norepinephrine, pg/ml			
Preop	2,470.0 (1,496.0–2,883.1)	2,810.0 (1,852.5–3,966.7)	0.209
Postop 1–3	1,922.5 (583.8–4,167.8)	1,884.5 (766.3–2,395.3)	0.671
Postop > 3	2,875.0 (1,518.8–4,307.5)	3,707.0 (2,238.3–5,183.0)	0.554

Data are presented as median (quartiles 25–75).

$P < 0.05$.

ACTH = adrenocorticotrophic hormone; postop 1–3 = intensive care unit days 1–3 after surgery; postop > 3 = bedside days 5–7 after surgery; preop = bedside days 1–7 before surgery.

the suppressed proinflammatory and increased antiinflammatory response in long-term alcoholic patients after surgery may predispose to infections.

Immune Functions and Postoperative Infections

In our study, the rate of postoperative infections was significantly increased in long-term alcoholic patients after surgery. This is in accord with previous publications.[2,11] Wound infections, urinary tract infections, tracheobronchitis, and pneumonia are reported to be more frequent among alcoholic patients compared with controls in either medical or surgical settings.[11,64–67] In the ICU, the most frequent infection is pneumonia, which was reported to occur in 38% of the alcoholic patients compared with 7% of the nonalcoholic patients after gastrointestinal surgery.[2,11,66] These results are in accord with our findings.

Because of the limited number of patients and the primary aim of the study, the relevance of the altered immune parameters on postoperative infections cannot be answered. The preoperative Th1:Th2 ratio was associated with a postoperative decrease of the Tc1:Tc2 ratio and the IFN-γ:IL-10 ratio from lipopolysaccharide-stimulated whole blood cells. However, only the immediate

Table 5. Postoperative Course and Infectious Complications in Long-term Alcoholics and Nonalcoholics

	Long-term Alcoholics (n = 31)	Nonalcoholics (n = 23)	*P* Value
APACHE III score on admission to ICU	23 (19–26)	19 (16–24)	0.175
APACHE III score maximum during ICU stay	51 (40–61)	30 (19–36)	0.018
MOF score on admission to ICU	1 (0–3)	1 (0–1)	0.182
MOF score maximum during ICU stay	3 (0–5)	4 (0–8)	0.219
Ventilation during ICU stay, days	2 (1–10)	1 (1–2)	0.012*
ICU stay, days	4 (2–12)	2 (1–3)	< 0.001*
Hospital stay, days	40 (29–54)	18 (14–24)	< 0.001*
Infections, No.	17 (55%)	6 (26%)	0.036*
Gram positive	17	2	
Gram negative	10	1	
Mycotic	5	0	
Pneumonia	11 (35%)	1 (4%)	0.007*
Tracheobronchitis	12 (39%)	4 (17%)	0.092
Urinary tract infections, No.	3 (10%)	1 (4%)	0.463
Wound infections, No.	7 (23%)	2 (9%)	0.069

Data are presented as median (quartiles 25–75), No. (= frequency) or days.

APACHE = Acute Physiology and Chronic Health Evaluation; ICU = intensive care unit; MOF = multiple organ failure.

Table 6. Postoperative Infections with Respect to Alcoholism and Smoking

	Long-term Alcoholics (n = 31)	Nonalcoholics (n = 23)	*P* Value
Infection			
Smokers	14/25 (56%)	3/12 (25%)	0.070
Nonsmokers	3/6 (50%)	3/11 (27%)	0.350
Pneumonia			
Smokers	9/25 (36%)	1/12 (8%)	0.017*
Nonsmokers	2/6 (33%)	0/11 (0%)	0.210
Tracheobronchitis			
Smokers	11/25 (44%)	3/12 (25%)	0.265
Nonsmokers	1/6 (17%)	1/11 (9%)	0.640
Urinary tract infections			
Smokers	1/25 (4%)	1/12 (8%)	0.580
Nonsmokers	2/6 (33%)	0/11 (0%)	0.041*
Wound infections			
Smokers	8/25 (32%)	0/12 (0%)	0.027*
Nonsmokers	1/6 (17%)	2/11 (18%)	0.940

Data are presented as No. (= frequency).

* $P < 0.05$.

postoperative IFN-γ:IL-10 ratio from lipopolysaccharide-stimulated whole blood cells showed marker function for postoperative infections in this limited patient population. Because postoperative infections may be related to preoperative immune suppression,[68] our findings might be an immunologic hint to higher postoperative infection rates, but because of the limited number of patients and therefore potential bias, this requires larger outcome studies.

Dual Addiction and Immune Function

In our study, the criterion for smoking was different between groups. Therefore, we cannot exclude the criterion for smoking as a confounder of the postoperative infection data in our patients. Cigarette smoking has been implicated as a risk factor for postoperative pulmonary complications.[69,70] Smokers have an increased frequency of pulmonary, circulatory, and infectious complications as well as impaired wound healing.[70] Bluman *et al.*[70] demonstrated that smokers were approximately six times more likely then never-smokers to experience postoperative pulmonary complications. In contrast, Møller *et al.*[71] studied the effects of preoperative smoking intervention on postoperative complications in 120 patients undergoing surgery. The overall complication rate was 18% in the smoking intervention and 52% in controls ($P < 0.0003$). The most significant effects of intervention were seen for wound-related and cardiovascular complications. Interestingly, a very low frequency of postoperative pulmonary complications (2%) was seen in both groups in the study of Møller *et al.*[71] Therefore, these controversial results might be a result of different kinds of surgery. In our patients, dual addiction had the strongest effect on postoperative infections. Considering the overall infection rate and, in particular, pneumonia, alcohol seemed to have a major impact, whereas for tracheobronchitis and wound infection, smoking seemed to be more relevant in our patients.

Long-term cigarette smoking affects T-cell responses in humans.[72] The molecular mechanism through which smoking affects the lymphocyte function is largely unknown. Nouri-Shirazi *et al.*[73] provided evidence that dendritic cells exposed to nicotine produce lower concentrations of IL-1β, IL-10, TNF-α, and IL-12. Also, ethanol affects antigen-presenting cell function by decreasing IL-12.[59,74] APC-produced IL-12 is important for the development of Th1[75] and the inhibition of Th2,[76] and it plays a key role in the ethanol-induced alteration of immune responses.[15,16,59] Therefore, the effects of ethanol on T-cell interaction might have been influenced by nicotine in the same manner in our study, although only for TNF-α was a significant difference found for the combination of both addictions.

Intercurrent Complications

In this study, AWS developed in 6 of 17 alcohol-dependent patients despite prophylactic treatment. Fourteen patients were alcohol abusers and did not require prophylaxis. The relation between alcohol-dependent patients and alcohol abusers, and the incidence of AWS in alcohol-dependent patients is in accord with previous studies.[2,12,69] Which pharmacologic intervention is used has a minor impact, but that any intervention is used has a major impact on outcome.[12,32,77] Prophylactic treatment decreases the risk of postoperative infections and improves outcome.[2]

The primary task in terms of perioperative assessment is to determine the level of alcohol consumption.[2,11,12] In accord with other studies and our own studies, we considered a level of 60 g/day as relevant for postoperative complications.[11,12] Besides the accepted laboratory markers for long-term abuse, such as γ-glutamyl-transferase, carbohydrate-deficient transferrin, and mean corpuscular volume,[12] the use of short-term consumption markers is clinically extremely relevant because continued preoperative abuse is associated with an increased rate of postoperative complications.[77] In our patients, we did not find any positive blood alcohol levels immediately before surgery.

In conclusion, the lower preoperative Th1:Th2 ratio was relevant for the immediate postoperative suppression of the Tc1:Tc2 ratio in long-term alcoholic patients as well as a decreased IFN-γ:IL-10 ratio from lipopolysaccharide-stimulated whole blood cells. Despite the fact that preoperative IL-1β plasma concentrations were increased, this was accompanied by increased IL-1ra concentrations from lipopolysaccharide-stimulated whole blood cells. After surgery, IL-1β was not different between groups, but IL-1ra from lipopolysaccharide-stimulated whole blood cells was still increased. Therefore, a preoperative and immediate postoperative alteration of the immune function was evident in long-term alcoholic

patients undergoing aerodigestive tract surgery. Because infections occurred twice as often in long-term alcoholic patients as in nonalcoholic patients, interventional strategies directed to increase the immune competence of long-term alcoholic patients might help to improve outcome.

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