Research Paper

Innate immunity recovers earlier than acquired immunity during severe postoperative immunosuppression

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Abstract

Background: Postoperative immune suppression, particularly a loss of cell-mediated immunity, is commonly seen after surgery and is associated with worse outcome, i.e. delayed wound healing, infections, sepsis, multiple-organ failure and cancer recurrence. However, the recovery of immune cells focusing on differences between innate and acquired immunity during severe postoperative immunosuppression is not investigated. **Methods:** In this retrospective randomized controlled trial (RCT) subgroup analysis, 10 postoperatively immune suppressed patients after esophageal or pancreatic resection were analyzed. Innate and acquired immune cells, the expression of human leukocyte antigen-D related on monocytes (mHLA-DR), lipopolysaccharide (LPS)-induced monocytic TNF-α and IL-10 secretion *ex vivo,* Concanavalin A (Con A)-induced IFN-γ, TNF-α, IL-2, IL-4, IL-5 and IL-10 release were measured preoperatively (*od*) until day 5 after surgery (*pod5*). Recovery of immune cells was defined by a significant decrease respectively increase after a significant postoperative alteration. Statistical analyses were performed using nonparametric statistical procedures. **Results:** Postoperative alterations of innate immune cells recovered on *pod2* (eosinophils), *pod3* (neutrophils) and *pod5* (mHLA-DR, monocytic TNF-α and IL-10 secretion), whereas alterations of acquired immune cells (lymphocytes, T cells, T helper cells, and cytotoxic T cells) did not recover until *pod5*. Peripheral blood T cells showed an impaired production of the T helper (Th) 1 cytokine IFN-γ upon Con A stimulation on *pod1,* while Th2 specific cytokine release did not change until *pod5*. **Conclusions:** Innate immunity recovered earlier than acquired immunity during severe postoperative immunosuppression. Furthermore, we found a more anti- than pro-inflammatory T cell function on the first day after surgery, while T cell counts decreased.

Key words: immune suppression; monocytic function; T cell function; innate immunity; acquired immunity

Introduction

Postoperative immune suppression particularly a loss of cell-mediated immunity is commonly seen after surgery due to an increased release of immune suppressing hormones such as catecholamines, prostaglandins and cortisol depending on the amount of surgical stress and tissue damage [1, 2]. Blood transfusion, hypothermia, dehydration and anesthetics can further attenuate immunity [3-6]. An impaired immunity after surgery is associated with worse outcome, i.e. delayed wound healing, infections, sepsis, multiple-organ failure and cancer recurrence [1, 7-12]. In particular, postoperative immunosuppression comprises decreased numbers of natural killer (NK) cells, T lymphocytes, as well as an impaired function of T lymphocytes and monocytes including a suppressed expression of human leukocyte antigen-D related on monocytes (mHLA-DR) [1, 13-19]. B lymphocytes seem to be less effected [1, 20]. Furthermore, increasing numbers of T regulatory (Treg) cells and neutrophils often occur

after surgery [13, 21]. While major surgery may suppress cellular immunity for several days, humoral immunity remains relatively intact [8]. However, the recovery of immune cells focusing on differences between innate and acquired immunity during severe postoperative immunosuppression has not been investigated, yet.

Patients and Methods

Study Participants and Treatment

This retrospective subgroup analysis of a previously published study of our research group [22, 23] investigated innate and acquired immune cells as well as monocytic and T cell immune function during severe postoperative immune suppression (mHLA-DR ≤ 10,000 antigens per cell on *pod1)* that were measured in 10 out of 20 patients of the placebo group of the bigger cohort until *pod5* after elective esophageal or pancreatic resection (measurement had to be stopped after 10 patients for economic reasons, no selection of the patients; Figure 1). All patients received guideline-based anesthesiological and surgical treatment according to our standard operating procedures [24].

Measurement of parameters of immune function

Blood samples were drawn from *od* until *pod5*. mHLA-DR and further parameters of immune function were measured from *od* until *pod5*. Expression of mHLA-DR was determined by cytometric analysis using a highly standardized quantitative assay as described earlier [25]. For determination of soluble mediators, ethylene diamine tetraacetic acid (EDTA) and heparin plasma samples were collected and stored at -80°C until assay. All immunological parameters were analyzed in collaboration with the Institute of Medical Immunology and Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Charité – Universitätsmedizin Berlin, Berlin, Germany. White blood cell differential count was measured in a standard hematology analyzer (Sysmex). For flow cytometry analysis, lymphocyte subpopulations were identified using the following antibody combinations: CD45 for leukocytes, CD3+ for T lymphocytes, CD3+CD4+ for T helper cells (Th), CD3+CD8+ for cytotoxic T cells, CD2+CD3-CD16+ for natural killer (NK) cells and CD19+ for B lymphocytes. Cell phenotyping was performed by flow cytometry/fluorescence-activated cell sorting (FACS) on a FACSCalibur™ using CELLQuest™ Software (BD Biosciences). LPS-induced monocytic tumor necrosis factor alpha (TNF-α) and Interleukin (IL)-10 secretion *ex vivo* as well as Concanavalin A (Con A)-induced interferon gamma (IFN-γ), TNF-α, IL-2, IL-4, IL-5 and IL-10 release were determined as described earlier [23].
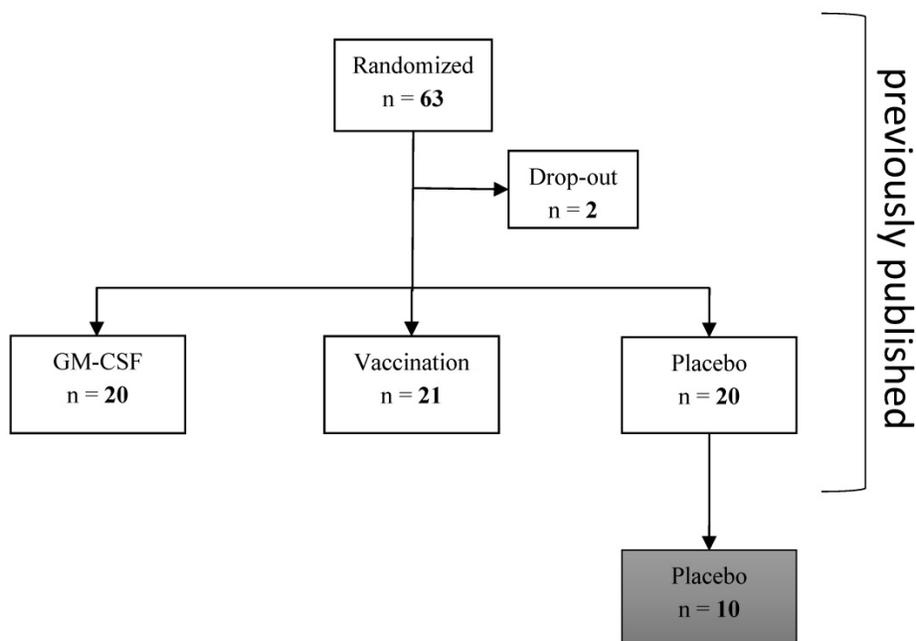

Figure 1. Consort diagram. 10 patients of the placebo group were analyzed in this subgroup analyses: immune cells and functional parameters were determined in these patients without any prior selection.

Statistical analysis

Data were expressed according to their scaling as arithmetic mean ± standard deviation (SD), median [25%, 75% quartiles], or frequencies [%]. After exploratory data analysis, all tests were accomplished by means of non-parametric exact statistical tests. Longitudinal data were analyzed using nonparametric univariate procedures. For each immune parameter, we first tested over all six time points (*od* until *pod5*). In a second step, we determined the first significant postoperative alteration by comparing postoperative and presurgical values. The specific time point was then compared with further time points to determine the recovery of immune parameters, i.e. a significant decrease respectively increase after a significant postoperative alteration. A two-tailed p-value < 0.05 was considered statistically significant. All tests should be understood as constituting exploratory data analysis, so that no adjustments for multiple testing have been made. Numerical calculations were performed using IBM© SPSS© Statistics, Version 23.

Results

Study population

Basic patient characteristics, intraoperative- and outcome parameters are shown in Table 1. Some of the results were already shown to analyze group differences after postoperative immune stimulation [23].

Innate immune cells

Neutrophils showed significant differences from *od* until *pod5* (p < 0.001; Figure 2A), increased on *pod1* (p = 0.005) and recovered on *pod3* (p = 0.037). Eosinophils differed from *od* until *pod5* (p = 0.002; Figure 2B), decreased on *pod1* (p = 0.018) and recovered on *pod2* (p = 0.043). Basophils differed from *od* until *pod5* (p = 0.035; Figure 2C) and decreased on *pod3* (p = 0.047). NK cell counts showed significant differences from *od* until *pod5* (p < 0.001; Figure 2D), decreased on *pod2* (p = 0.024) and again on *pod3* (p = 0.015). Monocytes differed from *od* until *pod5* (p < 0.001; Figure 2E), increased on *pod1* (p = 0.007) and again on *pod5* (p = 0.050). mHLA-DR showed significant differences from *od* until *pod5* (p < 0.001; Figure 2F), decreased on *pod1* (p = 0.005) and recovered on *pod5* (p = 0.008).

Function of innate immune cells

TNF-α release of LPS-stimulated monocytes significantly decreased on *pod2* (p = 0.038; Figure 3A) and recovered on *pod5* (p = 0.050). IL-10 release of LPS-stimulated monocytes decreased on *pod3* (p = 0.038; Figure 3B) and recovered on *pod5* (p = 0.012).

Table 1. Basic patient characteristics, intraoperative and outcome parameters.

	Placebo group (n = 10)
Age [years]	62 (55-69)
Gender male/female [n]	7/3
Body Mass Index [kg/m²]	25.5 (24.2-27.5)
Pancreatic/esophageal resection [n]	6/4
ASA score II/III [n]	7/3
Smokers/non-smokers [n]	4/6
AUDIT score	3 (0-6)
Non-diabetes/diabetes [n]	9/1
Metabolic equivalent (MET) <4/4-10/>10	0/8/2
Surgical time [min]	308 (280-378)
Intraop. blood loss [mL]	600 (313-950)
Intraop. mean blood glucose [mg/dL]	127 (122-142)
Intraop. max. blood lactate [mmol/L]	1.0 (0.8-1.3)
Intraop. mean systolic blood pressure [mmHg]	113 (109-117)
APACHE II score on admission to ICU	12 (9-16)
SAPS II score on admission to ICU	22 (12-27)
SOFA score on admission to ICU	2 (1-4)
TISS 28 score on admission to ICU	32 (27-36)
ICU stay [d]	3.2 (2.4-4.9)
Hospital stay [d]	14.4 (11.5-20.6)
Survived/deceased [n]	10/0

Continuous quantities in median (25%-75% percentiles), frequencies with n (%); ASA, American Society of Anesthesiologists; AUDIT score, Alcohol Use Disorders Identification Test; APACHE, Acute Physiology and Chronic Health Evaluation; SAPS, Simplified Acute Physiology Score; SOFA, Sequential Organ Failure Assessment; TISS, Therapeutic Intervention Scoring System; ICU, Intensive Care Unit.

Acquired immune cells und subsets

Lymphocytes showed significant differences from *od* until *pod5* (p = 0.007; Figure 4A) and decreased on *pod1* (p = 0.015). B cells did not show any significant differences (Figure 4B). T cells showed significant differences from *od* until *pod5* (p = 0.027; Figure 4C) and decreased on *pod1* (p = 0.011). T helper cells decreased on *pod1* (p = 0.007). Cytotoxic T cells showed significant differences from *od* until *pod5* (p = 0.016; Figure 4E) and decreased on *pod2* (p = 0.005). The ratio of T helper and cytotoxic T cells significantly increased on *pod3* (p = 0.011; Figure 4F) compared to *pod1*.

Function of acquired immune cells

After stimulation of whole-blood cultures for 24 h with Con A, the cytokines for Th1 and Th2 responsiveness IFN-γ, TNF-α, IL-2, IL-4, IL-5 and IL-10 were measured. While the Th1 cytokine IFN-γ decreased on *pod1* (p = 0.028; Figure 5A), TNF-α and IL-2 did not change significantly after surgery (Figure 5B, C). The Th2 cytokines IL-4, IL-5 and IL-10 did not show any differences after surgery (Figure 5D, E, F).

Discussion

The major finding of this subgroup analysis is that innate immunity recovered earlier than acquired immunity during severe postoperative immune-suppression. To the best of our knowledge, no other study has investigated differences in recovery between innate and acquired immune cells during severe postoperative immunosuppression after major cancer surgery, yet.
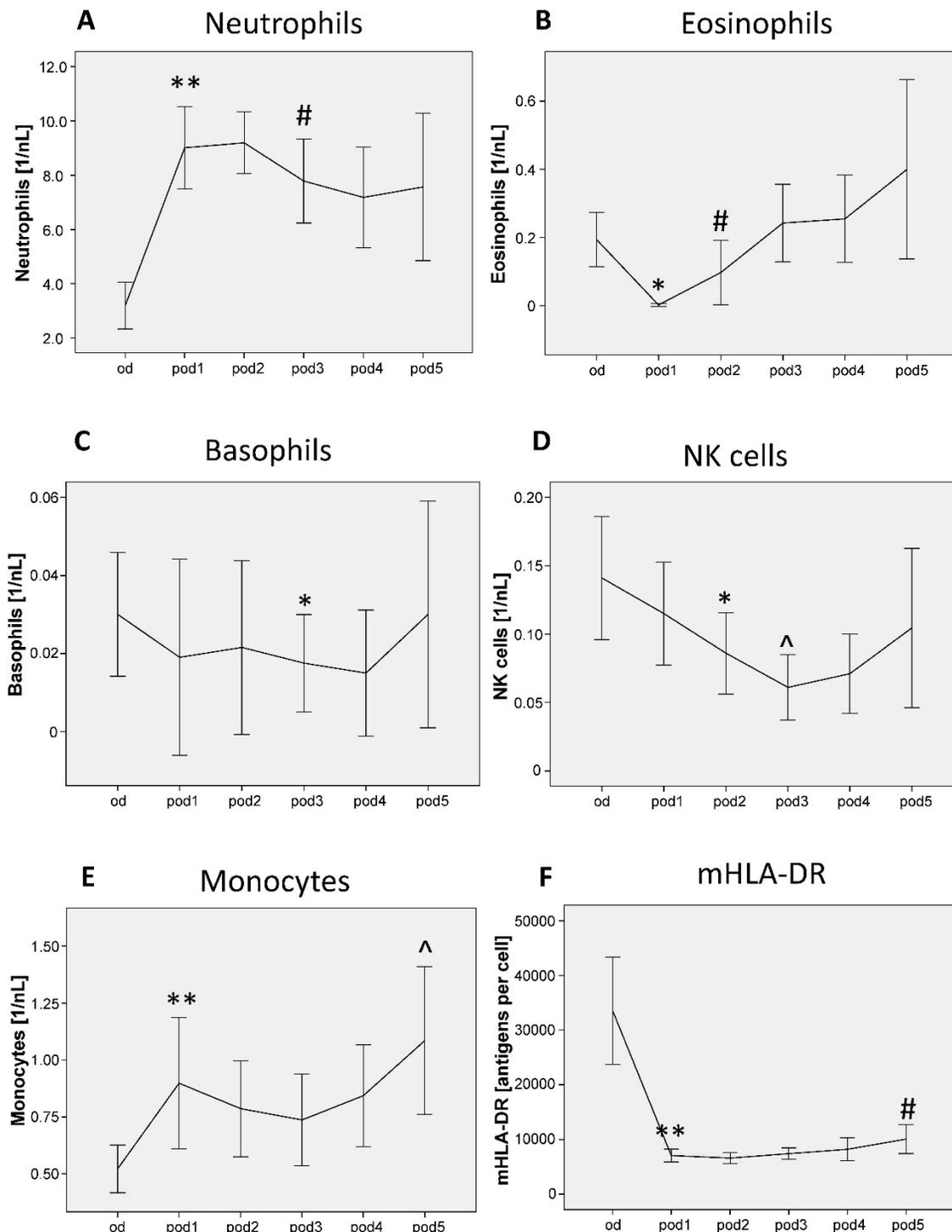

Figure 2. Neutrophils, eosinophils, basophils, natural killer (NK) cells, monocytes and mHLA-DR from day of surgery before surgery *(od)* until day 5 after surgery *(pod5)*. Neutrophils increased on *pod1* and recovered on *pod3*, eosinophils decreased on *pod1* and recovered on *pod2*, basophils decreased on *pod3*, NK cells decreased on *pod2* and again on *pod3* compared to *pod2*. Monocytes increased on *pod1* and again on *pod5* compared to *pod1*. mHLA-DR decreased on *pod1* and recovered on *pod5*. **P<0.01, *P<0.05 represent the first significant differences between pre- and postsurgical values. ^P<0.05 represents the second significant alteration compared to the prior significant alteration. #P<0.05 represents recovery, i.e. the first significant in- respectively decrease after significant postoperative alteration. Error bars with 95% confidence intervals.
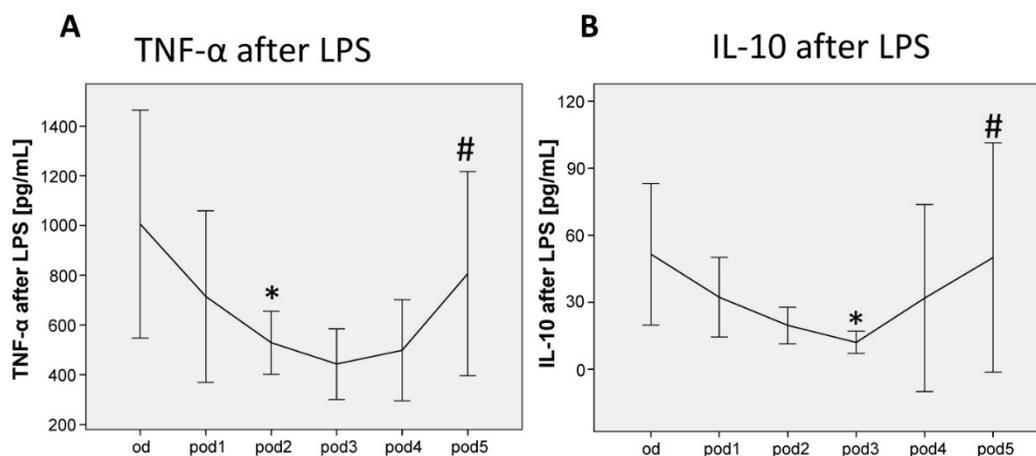
Figure 3. TNF-α and IL-10 release of LPS-stimulated monocytes from day of surgery before surgery *(od)* until day 5 after surgery *(pod5)*. TNF-α decreased on *pod2* and recovered on *pod5*, IL-10 decreased on *pod3* and recovered on *pod5*. *$P<0.05$ represents the first significant differences between pre- and postsurgical values. #$P<0.05$ represents recovery, i.e. the first significant in- respectively decrease after significant postoperative alteration. Error bars with 95% confidence intervals.

In general, innate and acquired immune defense play a key role in eliminating of infective pathogens and malignancies. When stimulated by pathogens, immune cells of the innate immune system produce cytokines and other co-stimulatory molecules, whereas the adaptive immune system is essential for immunologic memory and release of antibodies for more specific immune responses [26]. Interactions between innate and acquired immunity, i.e. monocytes and T lymphocytes with antigen presentation and consequent T cell response are essential for adequate immune function [27]. Particularly in postsurgical patients, profound immune alterations occur with a highly attenuated and restricted immunity [1], which can be measured by a decreased mHLA-DR [12].

We included immune suppressed patients with a mHLA-DR concentration not higher than 10,000 antibodies per monocyte on day one after surgery, which indicates a highly suppressed immune function. Therefore, all patients showed a postoperative immune suppressed state: counts of basophils, eosinophils, NK cells, lymphocytes except of B cells, as well as function of monocytes (mHLA-DR, TNF-α and IL-10 release of LPS-stimulated monocytes) and T cells (IFN-γ release after stimulation) decreased, whereas counts of neutrophils and monocytes increased. Immune alterations during the postoperative period are well described and in accordance with our findings [1, 13-19].

The exact pathological mechanism for an impaired postoperative immune function is still speculative. Perioperatively secreted catecholamines and prostaglandins are assumed to be a major cause of postoperative immune suppression following anesthesia [2, 6]. Latest research suggests that so-called alarmins released depending on tissue damage might lead to a pronounced pro-inflammatory response [28]. The initial pro-inflammatory response aims to activate immunity to the site of injury and induces a systemic anti-inflammatory state whose physiological effect should prevent the formation of inflammatory tissue and organ damage with the negative effect of leading to a pronounced postoperative immunosuppression [29].

We found an earlier recovery of innate immune cells compared to acquire immune cells. Concretely, innate immune cells recovered on *pod2* (eosinophils), *pod3* (neutrophils) and *pod5* (monocytic function), whereas counts of acquired immune cells (lymphocytes, T cells, T helper and cytotoxic T cells) did not recover until *pod5*. B cells did not decrease postoperatively. In the present study, we additionally investigated changes in lymphocyte subsets in patients with postoperative immunosuppression. Our findings suggest that the homeostasis of T cells is perturbed in immune suppressive patients after surgery. The decreased numbers of T cells in peripheral blood of immunosuppressed patients may reflect an increased rate of apoptosis of these cells. Clinical research showed an influence of surgical procedures on circulating blood lymphocyte apoptosis [30]. Considering functional parameters of T cells, Th1 specific cytokines were decreased and Th2 specific cytokines were unchanged on day one after surgery, which suggests an anti-inflammatory state immediately postoperatively. The imbalance of Th1 and Th2 cytokines is associated with an increased susceptibility to postoperative infections [31]. Our results therefore suggest that particularly acquired immune cells are highly vulnerable to postoperative immunosuppression, and compared to innate immune cells remain suppressed for a longer period of time.

It is of major importance to minimize postoperative immunosuppression due to its high impact on outcome regarding sepsis and cancer recurrence [8, 9, 11]. Adequate perioperative pain control particularly epidural analgesia was shown to reduce postoperative immune suppression after major abdominal surgery [32]. Furthermore, perioperative hypothermia must be avoided [5]. Another approach might be postoperative immune stimulation, which was shown to reduce infection days [22]. The impact of this stimulation on cancer metastases and recurrence is unknown and should be further investigated.
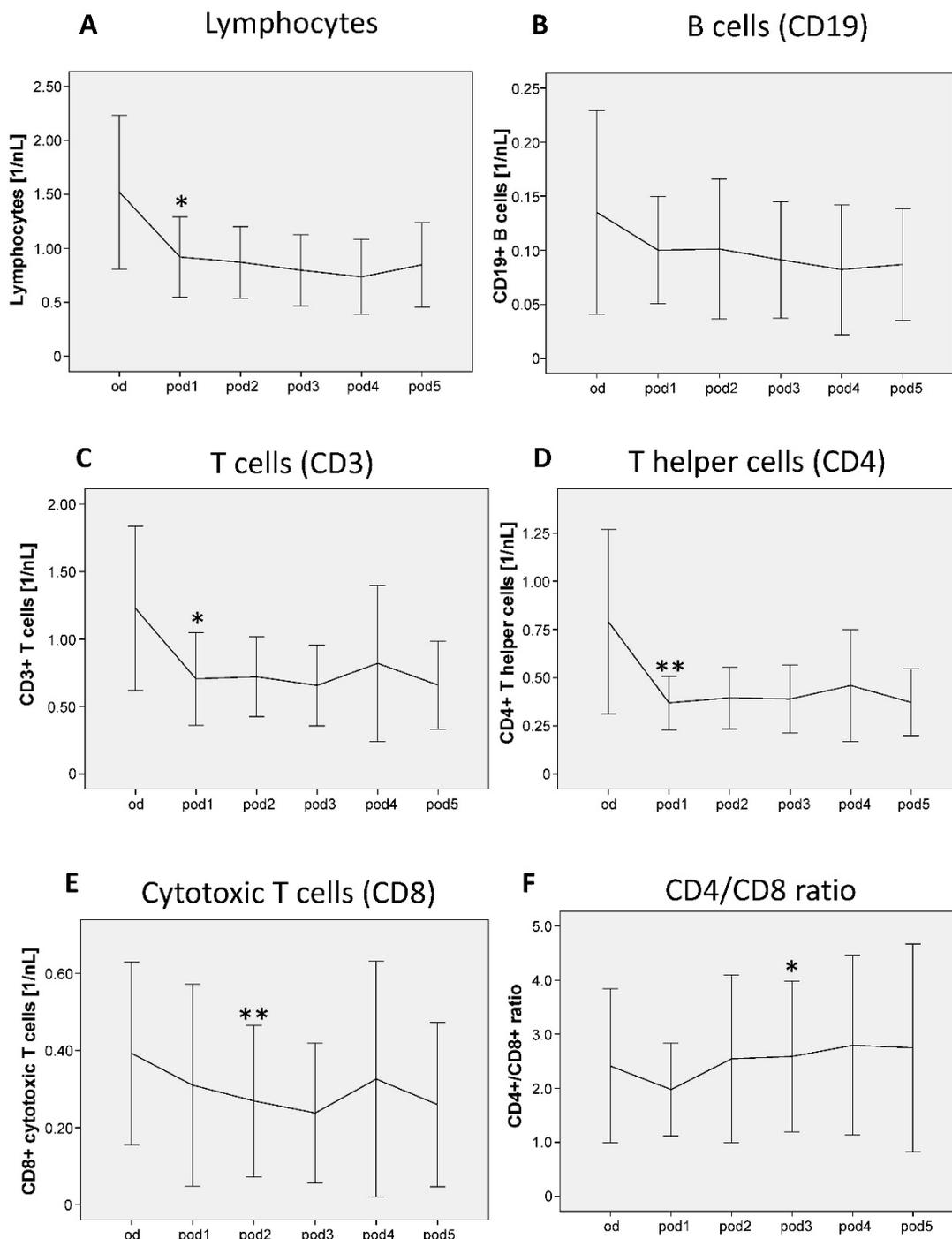

Figure 4. Lymphocytes, B cells, T cells, T helper cells and cytotoxic T cells from day of surgery before surgery *(od)* until day 5 after surgery *(pod5)*. Lymphocytes decreased on *pod1*, B cells did not show any significant differences. T cells and T helper cells decreased on *pod1* and cytotoxic T cells decreased on *pod2*. The ratio of T helper and cytotoxic T cells increased on *pod3* compared to *pod1*. **P<0.01, *P<0.05 represent the first significant differences between pre- and postsurgical values. Error bars with 95% confidence intervals.
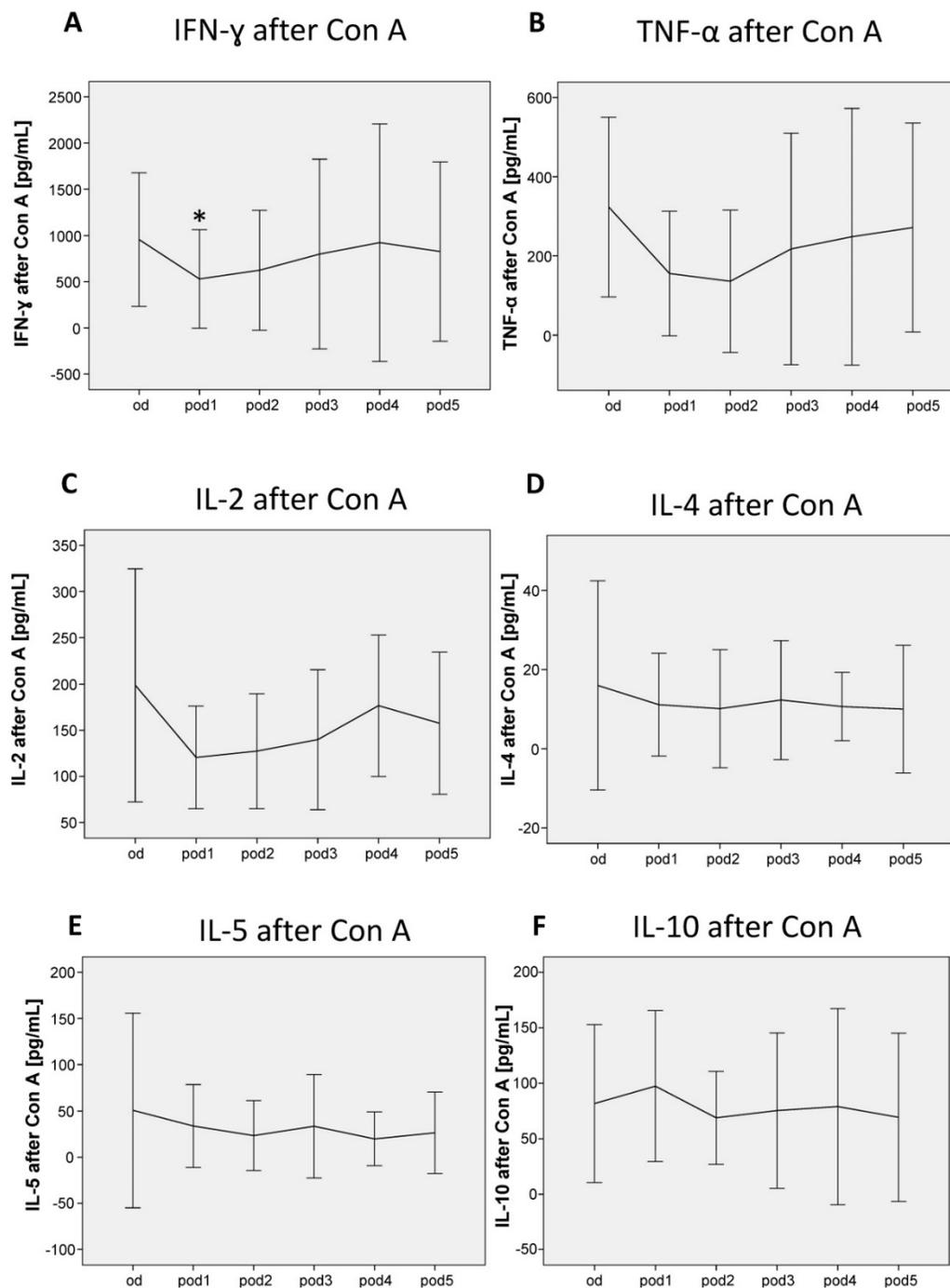
Figure 5. Con A-induced lymphocytic IFN-γ, TNF-α, IL-2, IL-4, IL-5 and IL-10 secretion from day of surgery before surgery *(od)* until day 5 after surgery *(pod5)*. The Th1 cytokine IFN-γ decreased on *pod1*, TNF-α and IL-2 did not significantly change after surgery. The Th2 cytokines IL-4, IL-5 and IL-10 did not significantly change after surgery. *$P < 0.05$ represents the first significant differences between pre- and postsurgical values. Error bars with 95% confidence intervals.

This study reveals several limitations. First of all, it is a retrospective subgroup analysis. Secondly, we analyzed only a small sample size of 10 patients, i.e. some results might possibly be not significant. Thirdly, patients were analyzed only until *pod5*. The course of immune cells and function after this period is unknown. Finally, the optimal threshold level for mHLA-DR (≤ 10,000 mAb/cell in our study) used to stratify patients with severe surgery-induced immunosuppression is unclear. Studies suggest values between 5,000 and 10,000 mAb/cell as indicator of severely impaired immune function in critically ill patients [25, 33, 34].

Conclusions

Postoperative innate immunity recovered earlier than acquired immunity during severe postoperative immunosuppression. Furthermore, we found a more

anti- than pro-inflammatory T cell function on the first day after surgery, while T cell counts decreased. Further research should focus on strategies to avoid postoperative immune suppression and improve outcome.

Abbreviations

AMG: German Drug Law
APACHE: Acute Physiology and Chronic Health Evaluation
ASA: American Society of Anesthesiologists
AUDIT: Alcohol Use Disorders Identification Test
BCRT: Berlin-Brandenburg Center for Regenerative Therapies
Con A: Concanavalin A
EDTA: ethylene diamine tetraacetic acid
FACS: fluorescence-activated cell sorting
ICU: Intensive Care Unit
IL: interleukin
IFN-γ: interferon gamma
LaGeSo: Landesamt für Gesundheit und Soziales Berlin
LPS: lipopolysaccharide
mHLA-DR: human leukocyte antigen DR on monocytes
NK: natural killer cell
od: preoperatively
pod: postoperative day
RCT: randomized controlled trial
SAPS: Simplified Acute Physiology Score
SD: standard deviation
SOFA: Sequential Organ Failure Assessment
Th: T helper cell
TISS: Therapeutic Intervention Scoring System
TNF-α: tumor necrosis factor alpha
Treg: T regulatory cells

Authors' contributions

Conceived and designed the experiments: CS. Performed the experiments: GL, CVH, JK, FY. Analyzed the data: GL, CVH, JK. Contributed materials / analysis tools: CVH, JK, FY. Wrote the paper: GL, CVH.

Ethics approval and consent to participate

This clinical trial was approved by the Ethics Committee of the Landesamt für Gesundheit und Soziales Berlin (LaGeSo), Germany (ref ZSEK15287/08) on September 01, 2008. The study further meets the requirements set out by the ICH-GCP, Declaration of Helsinki and the German Drug Law (AMG). Written informed consent was obtained from the patients.

Availability of data and materials

Due to legal restrictions imposed by the Ethics Committee of the Landesamt für Gesundheit und Soziales Berlin (LaGeSo) and the data protection commissioner of the Charité, public sharing of study data with other researchers or entities is not allowed. Requests may be sent to dai-researchdata@charite.de.

Competing Interests

The authors have declared that no competing interest exists.

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