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Complement and Neutrophil Function Changes After Liver Resection in Humans

Christoph Werner Strey · Britta Siegmund · Saskia Rosenblum · Rosa Maria Marquez-Pinilla · Elsie Oppermann · Markus Huber-Lang · John D. Lambris · Wolf O. Bechstein

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Abstract
Background Complement activation contributes to the regulation of liver regeneration after liver resection (LR) in mice.
Methods We hypothesized that complement activation and changes in C5a-receptors (C5aR, C5L2) on polymorphonuclear cells (PMN) and monocytes are important in clinical LR. Anaphylatoxin and C5b9 plasma levels were measured (bead-array, ELISA) (25 patients) and receptor expression was assessed after LR (19 patients) (FACS). In vitro PMN C5a-dependent chemotactic response (7 patients) as well as L-selectin shedding and Mac-1 expression (3 patients) was determined.
Results C3a increased after LR (31.1 ± 4 before LR vs. 41.6 ± 5 ng/ml, 30 min after LR, $P < 0.01$), as did C5b9 (12.7 ± 1 before LR vs. 26.9 ± 3 ng/ml, 60 min after LR, $P < 0.001$). C4a and C5a decreased after LR, by 25% 24 h after LR and 30% 2 h after LR, respectively ($P < 0.01$). C5L2 expression decreased at 4 h, rising at 24 h after LR (PMN: 6.3 ± 1 before LR, 3.1 ± 1, 4 h, 8.3 ± 2, 24 h; $P < 0.01$). The receptor-related changes accompanied a diminished C5a-dependent chemotactic response by PMN (42.1 ± 17 before LR vs. 2.1 ± 3 4 h after LR; $P < 0.01$) and a reduction of activation upon C5a-R stimulation as measured by L-selectin shedding and Mac-1 expression on PMN. Changes in C5L2 expression on monocytes paralleled postoperative impairment of liver function.
Conclusions These results indicate that complement components are released after clinical LR and subsequently PMN display altered C5a-dependent functional responses.

C. W. Strey (✉) · B. Siegmund · S. Rosenblum · R. M. Marquez-Pinilla · E. Oppermann · W. O. Bechstein
Department of General and Vascular Surgery, Medical School, Johann Wolfgang Goethe-University Frankfurt, Theodor-Stern-Kai 7, Frankfurt am Main 60590, Germany
e-mail: strey@gmx.de

M. Huber-Lang
Department of Traumatology, Hand and Reconstructive Surgery, University of Ulm Medical School, Ulm, Germany

J. D. Lambris
Department of Pathology and Laboratory Medicine, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Introduction

The complement system has been implicated in inflammatory processes as well as tissue regeneration and differentiation [1, 2]. Complement and the anaphylatoxins, such as C5a, play a key role in the systemic inflammatory response that occurs after liver resection (LR) and participate in regulating the subsequent regeneration of the liver [1]. Acute loss of functional liver volume requires adaptive systemic and liver-related processes to rebalance metabolic demands [3]. Thus, understanding the compensatory, inflammation regulated mechanisms that come into play after functional liver impairment is relevant for the clinical setting. Systemic inflammatory reaction and liver regeneration after functional liver loss are impaired when complement activation or signaling is inhibited [1, 2]. C3- or C5-knockout animals or blockade of C5a receptor (C5aR) signaling have been found to abrogate the physiological regenerative response in partially hepatectomized mice [2, 4] and anaphylatoxins have a cytoprotective effect during liver regeneration [5]. The anaphylatoxin C5a and its receptors have been the focus of clinical studies to investigate the influence of C5aR-dependent regulation of inflammation [6]. Systemic complement activation after

LR as shown in the animal model [5] has not been analyzed in the clinical context. Similarly, a regulatory role for complement in human liver regeneration has yet to be elucidated.

Given that aseptic surgery by itself is capable of inducing a systemic inflammatory response syndrome [7], it is likely that complement also is involved in the inflammatory response initiated after LR. Consequences of a systemic inflammatory response also can be detected in the expression levels of C5aR and C5L2 on polymorphonuclear cells (PMN), which have been analyzed in septic shock and trauma patients, where they correlated with clinical outcome [6]. Thus, we hypothesized that C5L2 and C5aR expression might be altered on PMN after LR and accompanied by functional changes of these cells. The C5a receptors also might be indicators of the systemic insult that is caused by liver surgery.

As a first approach, we have analyzed the systemic generation of anaphylatoxins and the soluble terminal complex sC5b9 after LR in a set of 25 patients. In a second set of patients we analyzed the changes in C5aR and C5L2 expression on PMN and monocytes that occur soon after LR and looked for possible correlations between our findings and the patients' clinical data. We then examined the chemotaxis and activation of neutrophils as estimated by Mac-1 expression [8] in response to C5aR stimulation to assess the functional relevance of the corresponding changes in receptor expression.

Materials and methods

Patients and clinical data analysis

A total of 53 patients (25 women; median age, 64 [range, 35–81 years]) who underwent hepatic resection at the Johann Wolfgang Goethe-University Hospital, Department of General and Vascular Surgery, between July 2004 and March 2009, were enrolled in the study. The study was approved by the University's ethics committee (reference number 186/04), and all patients gave written informed consent. In a subgroup of 25 patients, systemic complement activation was determined by anaphylatoxin and C5b9 measurement. C5aR expression on PMN and monocytes was measured in a second subgroup of 20 patients. In one patient of the second subgroup and in six additional patients (total: 7) chemotaxis experiments of neutrophils were undertaken in response to a C5aR peptide agonist. In three additional patients, Mac-1 expression and L-selectin shedding was studied. The study group consisted of 16 patients with primary liver malignancies (7 with hepatocellular carcinoma, 9 with cholangiocellular carcinoma), 35 with hepatic metastases, and 2 with bile duct cancers. The types of LR performed were: 27 major resections, including hemihepatectomies and extended resections, and 26 segmental resections (1–3 segments). Pre- and postoperative plasma samples were obtained at specific intervals as shown in Fig. 1. The perioperative parameters that were prospectively documented were: age, sex, operating time, length of hospital stay, hepatic ischemia time during the Pringle maneuver, operative procedures, and laboratory values for standard liver function tests.

Complement activation assays

C3a/C3a des-Arg, C4a/C4a des-Arg, and C5a/C5a des-Arg concentrations were detected using the Anaphylatoxin Cytometric Bead Array (BD Biosciences, San Diego, CA) according to the manufacturer's instructions, as described elsewhere [9]. In brief, 50 µl of the diluted (1:200) plasma and standard were incubated with 50 µl of capture beads for 2 h at room temperature (RT). After incubation, the mixture was washed and then incubated with 50 µl of PE-conjugated detection antibodies for 1 h at RT. The resulting sandwich complexes were analyzed (300 events per capture bead) in two-color flow cytometric analysis (FL2-H/FL3-H) using a FACSCalibur flow cytometer (Becton–Dickinson Immunocytometry Systems, San Jose, CA). Data analysis was performed using Becton–Dickinson Cytometric Bead Array CBA software. The sC5b9 in plasma was quantified using an ELISA-kit (Quidel, San Diego, CA): 100 µl of the diluted (1:4) samples, standard, and controls were dispensed into microassay wells precoated with anti-sC5b9 monoclonal antibody, and then incubated for 60 min at RT. After incubation, the wells were washed five times, and 50 µl of HRP-conjugated sC5b-9 was added and incubated for 60 min at RT. After five additional washes, 100 µl of chromogenic enzyme substrate solution was added and incubated for 30 min at RT; 50 µl stop solution was added, and the absorbance (405 nm) was measured spectrophotometrically (Bio-Tek Instruments, Inc., Winooski, VT) and sample concentrations were then calculated from the standard curve.

C5aR and C5aL2 expression on peripheral blood monocytes and neutrophils

Flow cytometric analysis was conducted after whole blood (EDTA-treated) collection. A total of 7.5 µl of rabbit anti-human C5L2 (0.1 mg/ml; Hycult Biotechnology b.v., Uden, NL) and/or 10 µl of mouse anti-human C5aR (0.1 mg/ml; clone: S5/1; Hycult Biotechnology) was incubated with 100 µl of human whole blood for 30 min at RT. Then cells were washed with phosphate-buffered saline with 0.5% bovine serum albumin and incubated with

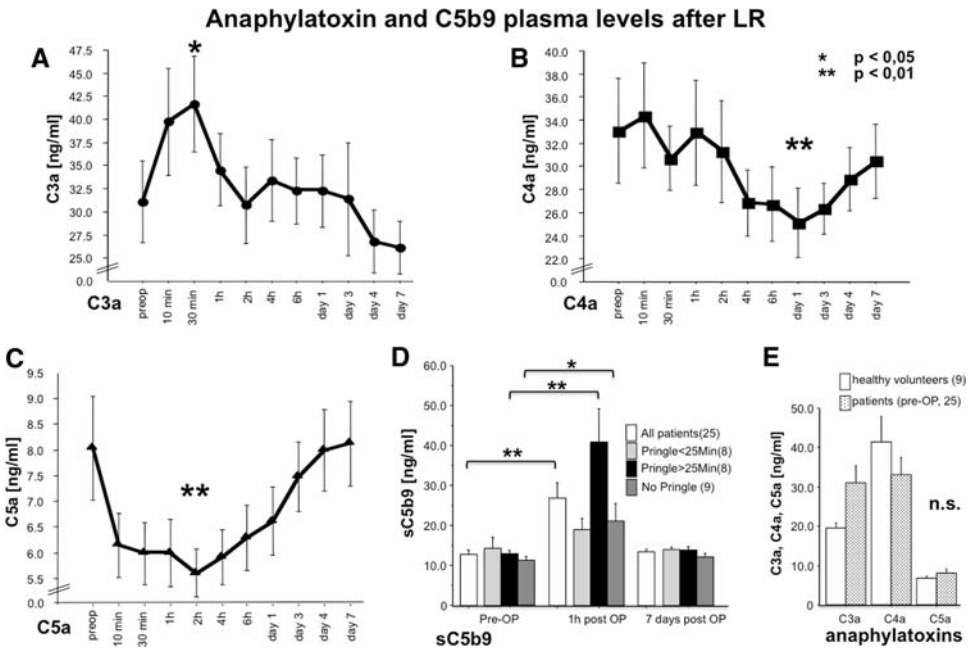

Fig. 1 Anaphylatoxin and C5b9 release. **a** Serial C3a and C3a des-Arg plasma levels over time in 25 LR patients (study group I). Six measurements were made within the first 6 h after surgery, followed by daily tests for 7 days. **b** C4a and C4a des-Arg plasma levels, and **c** C5a and C5a des-Arg plasma levels at the same intervals. **d** sC5b9 plasma levels were measured preoperatively and at 1 h and 7 days after LR. Values are separately plotted for all patients (*open bars*), patients who underwent Pringle maneuver for longer or shorter than 25 min (*black bars*, *light grey bars*), and patients without Pringle maneuver (*dark grey bars*). Most prominent differences are seen in patients who underwent Pringle maneuver beyond 25 min. **e** Preoperative patient plasma anaphylatoxin levels (*grey bars*) compared with baseline anaphylatoxin plasma levels of healthy volunteers (*open bars*) are not different. Data are means ± SEM. *LR* liver resection

1 µl of FITC-labelled anti-rabbit IgG (Serotec, Oxford, UK) and/or 1 µl of FITC-labelled anti-mouse IgG (BD Biosciences Pharmingen) for 30 min at RT. To rule out nonspecific binding of the secondary antibodies, controls were performed by incubating 100 µl whole blood with each of the secondary antibodies alone, excluding the primary antibodies or by using control IgG as control antibodies. Erythrocytes were lysed with FACS lysing solution (BD Biosciences) according to the manufacturer's instructions. The cells were then washed and analyzed in a flow cytometer FACSCalibur (Becton–Dickinson Immunocytometry Systems) using Cellquest Pro (version 4.0.2) software. Monocytes and PMN were gated using forward and side scatter profiles; for each measurement, 10,000 events were analyzed.

Neutrophil chemotaxis and activation assay

(a) Isolation of PMN: PMN were isolated from EDTA-treated whole blood using Polymorphprep™ (Axis Schield PoC AS, Oslo, Norway) according to the manufacturer's instructions. Erythrocytes were lysed using 0.83% ammonium chloride, washed, and resuspended at 5×10^5 cells/ml in Hank's buffered salt solution with Ca^{2+} Mg^{2+}. The purity of the PMN preparation, as determined by flow cytometry (Becton–Dickinson Immunocytometry Systems), was 95–99%; cell viability was determined by trypan blue dye exclusion.

(b) Chemotaxis assay: The chemotactic activity of PMN was measured in a 12-well plate fitted with a high pore density filter, PET track-etched membrane (3.0 µm pore size, Falcon, BD Biosciences). PMN (5×10^5 cells/ml) were allowed to migrate into the lower chamber with 1 or 0.1 µM of a C5a peptide agonist (peptide analog of the C-terminal domain of C5a: Phe-Lys-Pro-D-Cha-Cha-D-Arg; its synthesis is described elsewhere [10], a control peptide, or buffer alone. The plates were incubated at 37°C in a humidified chamber (5% CO_2) for 150 min and fixed with 2% glutaraldehyde. Cell migration was assayed in triplicate, and the number of migrated cells was counted in five different visual fields (original magnification, 40×).

(c) Activation assay: Stimulation of PMN was performed in Hank's buffered salt solution with Ca^{2+} Mg^{2+} at 37°C for 15 min with a cell concentration of 5×10^5/100 µl. Stimulation was terminated by adding ice cold buffer followed by centrifugation (4°C) and resuspended in ice-cold buffer for 0subsequent staining with 20 µl of anti human CD11b-PE (Mac-1, clone: D12; Becton–Dickinson, Heidelberg) and/or 20 µl of

anti human CD62L-PE (L-Selectin, clone:SK11; Becton–Dickinson, Heidelberg) and/or 20 µl of isotype mouse IgG2a-PE (Becton–Dickinson, Heidelberg). Staining procedures were performed on ice after incubation for 30 min, cells were washed twice and fixed and analyzed in a flow cytometer FACSCalibur (Becton–Dickinson Immunocytometry Systems) using Cellquest Pro (version 4.0.2) software. Neutrophils were gated using forward and side scatter profiles; for each measurement, 10,000 events were analyzed.

Statistical analysis

Statistical analysis was performed with the Mann–Whitney nonparametric test or ANOVA for longitudinal measurements. Differences were considered significant at $P < 0.05$. All values are given with standard error of the mean (SEM).

Results

Demographic and medical characteristics of patients

Table 1 summarizes the relevant perioperative characteristics of the LR patients enrolled in this study. The patient groups were divided according to the assays performed (group I: complement activation, group II: receptor expression, group III: neutrophil chemotaxis and activation). All patients underwent various degrees of LR, depending on the tumor manifestation, and the extent of the resected liver parenchyma differed accordingly. The magnitude of the resection was reflected in the operating time and in the use and length (or absence) of hepatic ligament occlusion (Pringle maneuver).

Plasma levels of the anaphylatoxins C3a, C4a, C5a, and of the soluble terminal complex component sC5b9

In the first phase of our study, we measured anaphylatoxin and complement terminal complex (C5b9) levels in plasma to detect complement activation after LR (group I; Table 1). The multiplexing assay used for C3a, C4a, C5a, and their corresponding des-Arg forms allowed serial and simultaneous quantification of the anaphylatoxins. The anaphylatoxins showed distinct and significant changes in their concentrations after LR (Fig. 1). We saw a prominent increase in C3a very early after resection, with maximal levels being reached at 30 min after LR (Fig. 1a). At 2 h after resection, the C3a levels had returned to baseline and did not change significantly thereafter. In contrast, the levels of C4a decreased slowly over 24 h and then returned to baseline within 6 days (Fig. 1b). C5a showed a similar pattern compared with C4a, but the initial reduction in plasma level could be detected as early as 10 min after surgery and reached a minimum at 2 h after surgery. Again, this initial decrease in C5a plasma levels was followed by a gradual return to baseline level within 7 days (Fig. 1c). In addition anaphylatoxin plasma levels were analyzed in healthy volunteers and thereby we were able to rule out a systematically different pre operative level of plasma anaphylatoxins in the patient group (Fig. 1e).

To further establish the activation of complement after LR, we measured the soluble terminal complex component

Table 1 Perioperative and demographic patient data

Total patients $N = 51$	Subgroup I (complement activation, $n = 25$)	Subgroup II (C5aR and C5L2 expression, $n = 19$)	Subgroup III (PMN chemotaxis and activation, $n = 9$*)
Diagnosis			
Liver metastasis	17	14	3
HCC	2	2	4
CCC	6	1	2
Bile duct cancer	0	2	0
Age [year] (±SEM)	62 (2.2)	64 (2.5)	55 (5.9)
Male/female ratio	16/9	12/12	4/5
Length of stay [days] (±SEM)	15.8 (2.1)	18.4 (2.7)	9.6 (0.5)
Resection weight [g] (±SEM)	598 (113)	621 (109)	535 (143)
Liver resection type	Hemi hepatectomies/extended resections: 12, segment resections: 13	Hemi hepatectomies/extended resections: 9, segment resections: 10	Hemi hepatectomies/extended resections: 5, segment resections: 4

*One patient was included in subgroups II and III because both assays (receptor expression and chemotaxis) were performed

sC5b9 in plasma. Because C3a and C5a showed their most prominent changes early after LR, the 1-h time point after surgery was chosen for measuring sC5b9, and at that time point, a significant increase in sC5b9 was observed (Fig. 1d, white bars). The analysis of the impact of the Pringle maneuver on sC5b9 release revealed a relevant difference for a time of hepatic inflow obstruction beyond 25 min (Fig. 1d, black bars). Patients without Pringle maneuver (Fig. 1d, dark grey bars) or with a shorter duration of its application (Fig. 1d, light grey bars) displayed a less pronounced increase of plasma sC5b9 levels.

Taken together, our data on complement activation products after liver surgery support the involvement of complement in the inflammatory response to loss of liver parenchyma. However, the anaphylatoxins and sC5b9 appeared to be of only limited diagnostic value for monitoring patients in this clinical setting. Except for the influence of the Pringle maneuver on sC5b9 levels no further correlations of intra- or postoperative parameters with the levels of C3a, C4a, and C5a could be detected. In particular, neither the presence nor absence of the Pringle maneuver nor the extent of liver resection had a statistically significant impact.

Changes in C5L2 expression on monocytes and neutrophils after LR

Generation of complement activation products during the course of liver surgery is likely to be accompanied by activation of circulating immunocompetent cells and subsequent changes in the corresponding receptors on these cells. PMN and monocytes are among the target cells that are involved in complement-dependent regulation of inflammation [10, 11]. We therefore measured C5aR and C5L2 expression on monocytes and neutrophils at various time points after LR (group II; Table 1). To determine the maximum expression rate, we conducted a pilot study with six patients. The most prominent changes in C5L2 receptor expression became obvious as early as 4 h after surgery; the values for receptor expression increased again by 24 h after LR, with no relevant alteration thereafter (Fig. 2a). All subsequent quantitation of receptor expression was therefore limited to preoperative baseline determinations and measurements at 4 and 24 h after surgery. Figure 2b depicts the reduction of C5L2 expression on monocytes and neutrophils (Fig. 2b, first panel) at 4 h after LR. A return to baseline levels was observed at 24 h after surgery.

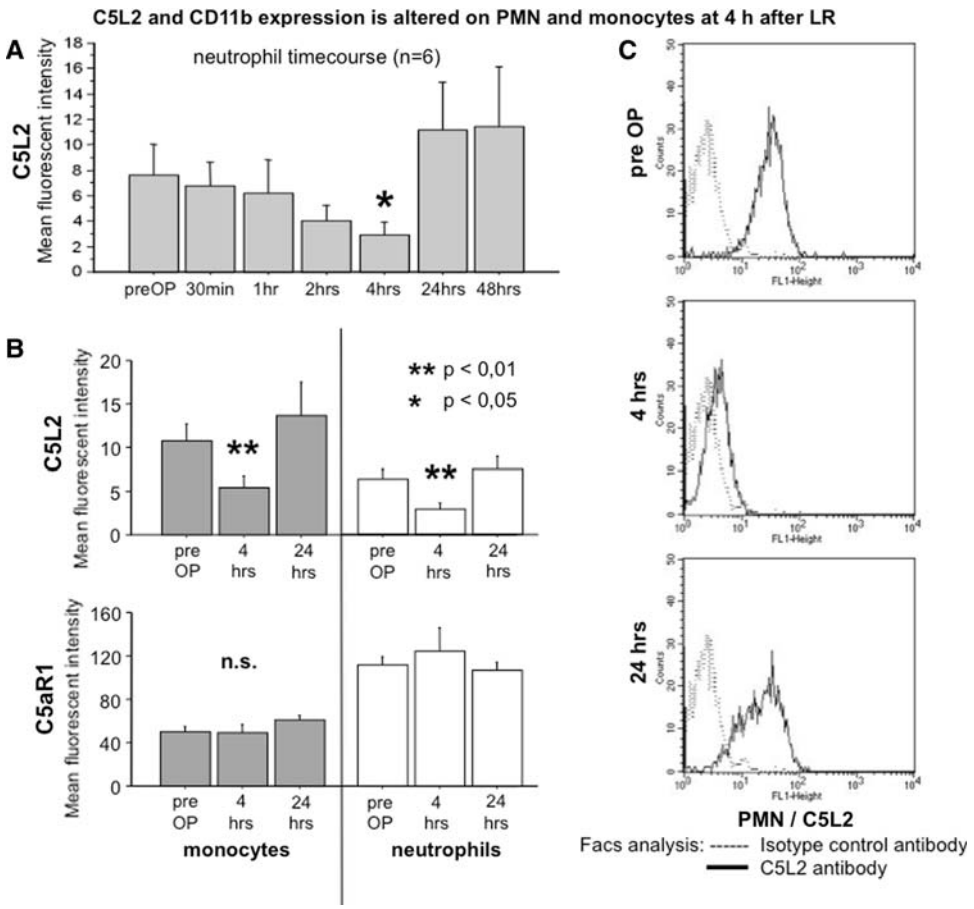

Fig. 2 C5L2 and C5aR1 expression on monocytes and PMN. Surface expression of C5L2 as determined by FACS analysis on monocytes and PMN after LR. **a** Time course of C5L2 expression on PMN of six patients revealing the most prominent changes at 4 h after LR. **b** C5L2 expression (*upper panel*) on monocytes (*left*) and PMN (*right*) displayed a significant drop at 4 h after LR, returning to baseline at 24 h after LR ($n = 19$). In contrast C5aR1 (*lower panel*) did not show significant expression differences on these cells. **c** Fluorescence histograms of C5L2 stained PMN are shown before and after surgery from a representative patient after LR. At 4 h after LR, staining for C5L2 is significantly reduced (*black line*). Data are means ± SEM. *LR* liver resection, *C5aR* C5a receptor, *PMN* polymorphonuclear cells

In contrast C5aR expression did not change after LR on monocytes and PMN (Fig. 2b, second panel).

C5a-dependent chemotactic response is decreased after LR

The changes in C5L2 expression that we observed led us to ask whether these changes paralleled the functional alterations in the cells. Therefore, we performed a chemotaxis experiment using a C5a peptide agonist and freshly isolated neutrophils obtained from seven patients preoperatively and at 4 and 24 h after LR (group III; Table 1). The results of these experiments indicated that the changes in C5L2 expression were paralleled by a diminished chemotactic response to a C5a agonist at 4 and 24 h after surgery; by 7 days after surgery, the levels had returned to levels slightly higher than preoperative values (Fig. 3a).

L-selectin shedding and Mac-1 induction on PMN upon C5aR peptide agonist stimulation is diminished after LR

To further confirm the reduced capability of neutrophils to respond to complement regulated proinflammatory stimuli after LR, we analyzed L-selectin shedding and the induction of Mac-1 expression on PMN before and 4 h after surgery in response to C5a-dependent stimulation in three additional patients. This functional assay revealed that both parameters L selectin shedding and induction of Mac-1 expression were significantly diminished in a dose-dependent manner after surgery compared with preoperative responses (Fig. 3b–d).

Levels of C5L2 expression on neutrophils and monocytes are correlated with the postoperative liver function

Given the reduction in C5L2 expression on monocytes and neutrophils that we had observed after LR and the parallel decrease in the chemotactic response of neutrophils to C5a stimulation, it seemed reasonable to look for possible correlations with the postoperative clinical data from LR patients. We therefore looked for correlations between C5L2 expression data and intra- and postoperative patient characteristics, type, length of surgery, presence or absence of the Pringle maneuver, postoperative liver function, and incidence of complications. Among these variables, only lower postoperative thromboplastin time and higher bilirubin plasma levels were found significantly associated with low levels of C5L2 expression on monocytes at 4 h

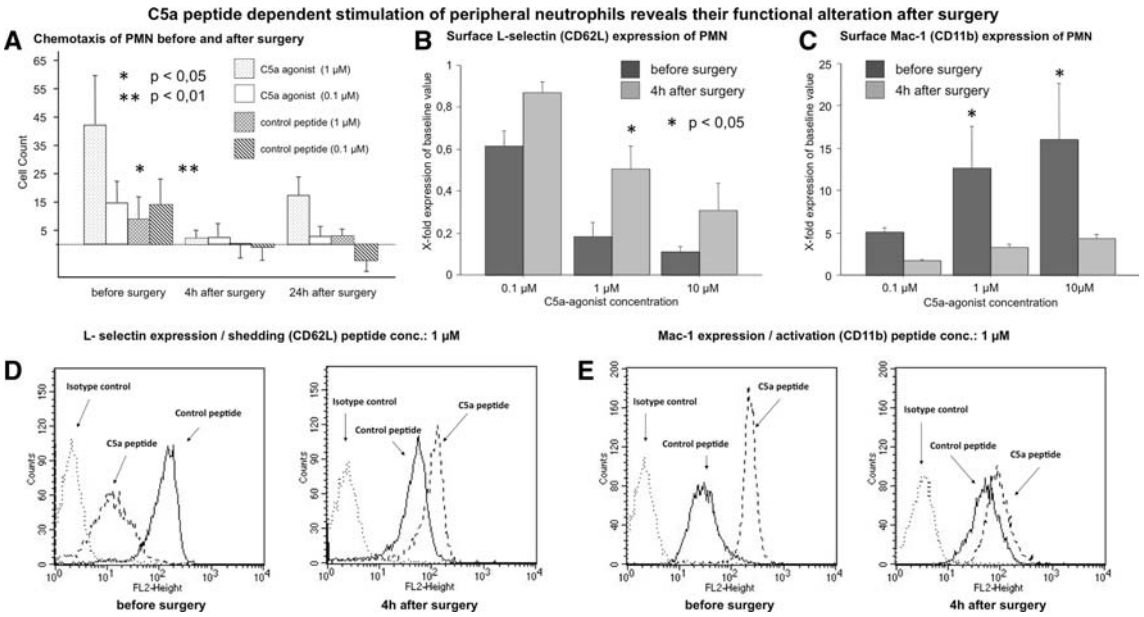

Fig. 3 C5a-dependent PMN chemotaxis, Mac-1 and L-selectin expression after LR. **a** Chemotactic responses to a C5a peptide agonist (*dotted and open columns*) and a control peptide (*cross-hatched and hatched columns*) of freshly isolated PMN from patients undergoing LR, measured before and at 4 h and 24 h after LR. C5a-dependent chemotaxis was significantly impaired, with a nadir at 4 h after LR (*second column group*); a partially restoration of function was seen at 24 h after LR (*third column group*). **b** L-selectin shedding before and 4 h after surgery in response to C5a peptide agonist stimulation (expressed in fold reduction) was significantly diminished, whereas (**c**) Mac-1 expression on PMN under the same stimulation displayed a reduced increase 4 h after LR ($n = 3$ patients). The lower panels (**d** and **e**) display examples of the corresponding fluorescence histograms with L-selectin and Mac-1 staining. Data are means ± SEM. *LR* liver resection, *PMN* polymorphonuclear cells

after surgery (cutoff value: 5 MFI [mean fluorescent intensity]) (Fig. 4), indicating a more pronounced postoperative impairment of liver function. Similar differences in these postoperative liver function values were observed in connection with the level of C5L2 expression on neutrophils at 4 h after surgery (cutoff value: 3 MFI), but the results were not statistically significant (Fig. 4). The other clinical parameters, including the Pringle maneuver, did not show significant correlations with C5L2 expression.

Discussion

In the present study, we have characterized the systemic release of anaphylatoxins in a group of patients before and after LR and have found that C3a, C4a, and C5a and their des-Arg forms showed significant distinct changes in plasma levels after LR. In most cases, the levels of these complement products returned to baseline level at 7 days after LR (Fig. 1). These findings underscore the participation of complement activation in the human systemic inflammatory response after LR. In light of the observed moderate changes of the anaphylatoxin plasma levels in the patients, it is noteworthy that healthy volunteers did not have different baseline values (Fig. 1). The underlying pathological conditions of the patients did not result in a systematic change of baseline anaphylatoxin levels. Preoperative levels of complement activation as measured by anaphylatoxin and C5b9 plasma levels did not also correlate with the patient's tumor volume. This was tested by comparing baseline activation levels in a patient subgroup with low (<50 ml, $n = 12$) tumor volume with patients with high (>50 ml, $n = 13$) tumor volume.

The changes in anaphylatoxin plasma levels were not uniform. Whereas C3a increased within 30 min after LR, C5a plasma concentrations decreased sharply and then increased thereafter. These discordant changes in anaphylatoxin concentrations could be explained by a number of mechanisms, which require further investigation. A wide range of dynamic processes, including local and systemic generation, degradation, and receptor binding, contribute to the measured pool of circulating anaphylatoxins and could affect their levels in vivo. An asymmetric activation of complement cascade pathways via crosstalk with the

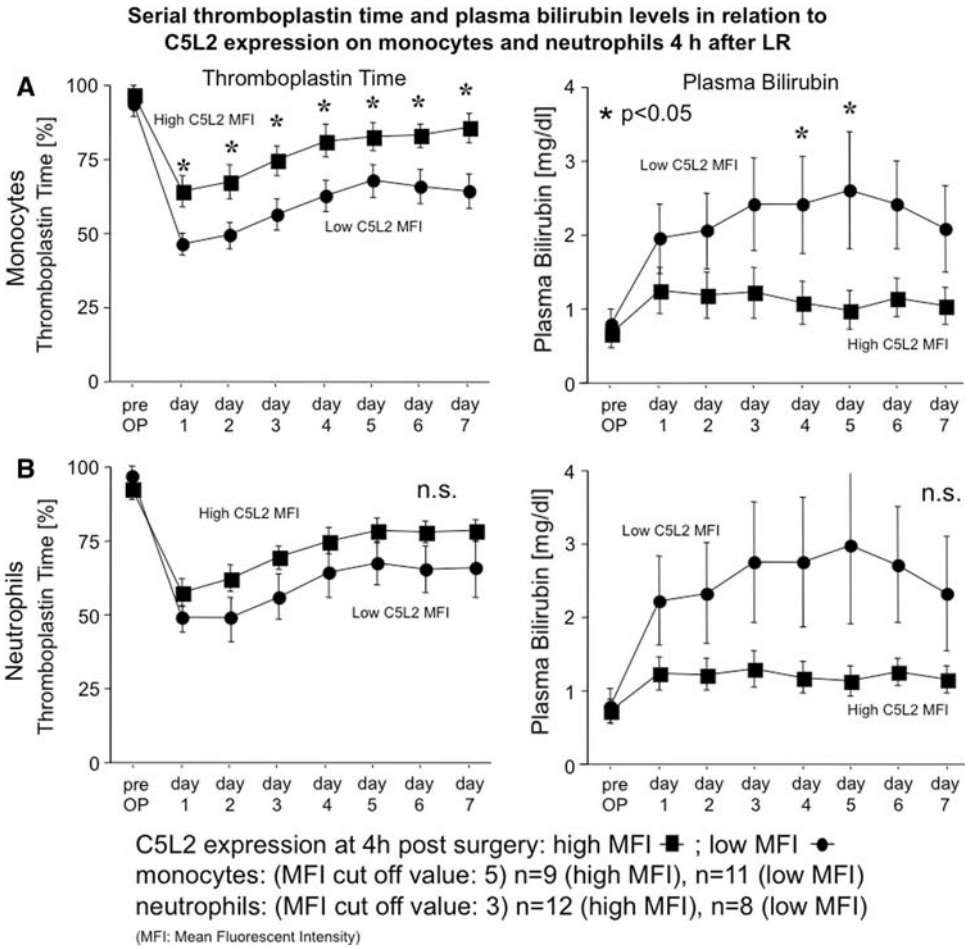

Fig. 4 C5L2 expression and liver function after liver resection. Serial thromboplastin time (*left panel*) and plasma bilirubin (*right panel*) measurements in relation to the level of C5L2 expression on (**a**) monocytes and (**b**) neutrophils (PMN) at 4 h after LR. Low C5L2 expression on monocytes (*upper panels*) at 4 h after LR was associated with a significantly more pronounced impairment of liver function after surgery as measured by thromboplastin and plasma bilirubin time course over 1 week after surgery ($n = 19$) (cutoff value: 5 MFI). **b** A similar finding was observed with C5L2 expression data on PMN, which did not reach the level of significance. Data are means ± SEM. *LR* liver resection, *PMN* polymorphonuclear cells

coagulation system also is possible [12, 13]. Recently it was reported that in the context of liver resection complement activation can occur independently of the classical or alternative pathway. Especially plasmin seemed to be partially responsible for this observation [4]. The impact of coagulation factor-dependent complement activation on the total release of complement activation products, such as C5a, has yet to be determined. However, the decrease in coagulation factors that occurred after LR (compare Fig. 4) could potentially contribute to the reduction in C5a plasma levels (Fig. 1c). Furthermore interactions of the complement system with the cellular immune system might contribute to varying degrees of complement activation. Mononuclear cells for example have been shown to represent at least two distinct cell populations, which can modulate innate immunity [14, 15]. Therefore, the predominance of mononuclear cell subtypes also might contribute to the variations and asymmetry of complement activation observed in our study population.

To unambiguously establish that complement activation had occurred after LR, we analyzed the systemic levels of the terminal sC5b9 complex. This complex showed a significant increase at 1 h after LR (Fig. 1d). We also observed an increase in the circulating C5b9 and at the same time detected a reduction in circulating C5a. These phenomena could be due to the plasma clearance of C5a, which is enhanced to such a degree that C5a generation is overridden in addition to its shorter half life. However, enhanced C5a clearance cannot be explained by the receptor expression data that we obtained for neutrophils and monocytes and, therefore, would have to be based on other mechanisms as endothelial clearance for example.

It was of interest to test an association of C5b9 or anaphylatoxin release with the occurrence of postoperative complications. Furthermore, it was expected that the extent of the surgical trauma in terms of the amount of liver resection and duration of the operation would be reflected in the extent of complement activation. Also the extent of LR is known to modify the regenerative response of the liver. This prompted us to analyze two patient groups separately (minor and segmental resections versus major resections) concerning the magnitude of complement activation after LR. This analysis of intra- and postoperative clinical parameters revealed an influence of the hepatic inflow obstruction and its duration (Pringle maneuver) on the release of sC5b9 (Fig. 1d). Duration of the Pringle maneuver beyond 25 min caused a more pronounced increase of sC5b9 plasma levels than in patients with shorter duration or without hepatic inflow obstruction. This observation stands in line with the effect of ischemia and reperfusion on complement activation in various pathophysiological situations [16]. It remains unclear why a short duration of hepatic inflow obstruction does not seem to significantly impact the release of complement activation products. It is possible that the hypoxic tolerance of the liver requires a certain threshold of hypoxia extent, which then translates in further inflammatory responses.

Apart from this finding, other correlations of intra- (e.g., extent of liver resection and Pringle maneuver) or postoperative clinical parameters and complications (e.g., morbidity, liver function, length of hospital stay) with the release of complement activation products remained below the level of significance. Probably the size of the study group was too small to establish significant correlations. Therefore, the current data do not provide evidence to support the monitoring of complement activation as an additional clinical parameter in the context of liver resection.

To determine the effect of LR on the expression patterns of C5aR and C5L2, we further analyzed the expression of these receptors on PMN and monocytes after LR. Whereas C5aR expression on monocytes and PMN did not change significantly over time, C5L2 expression was significantly diminished on both cell types at 4 h after LR (Fig. 2b). To test whether changes of C5a receptor expression are functionally relevant for these cells we measured C5a-dependent chemotactic activity of human PMN after LR and determined the consequences for L-selectin and Mac-1 expression on PMN before and after LR. A C5a peptide agonist, a full agonist of C5aR [17], was used as the stimulant. We observed a significant decrease in chemotactic activity at 4 h after surgery, with recovery to higher than baseline values by 7 days after surgery (Fig. 3), although relevant changes in C5aR1 expression were not detected (Fig. 2). Because the C5aR agonist does not bind to C5L2 (Lambris, unpublished data), and because the receptor has not been shown to modulate chemotaxis, we hypothesize that the alterations we observed in the chemotactic response may reflect different degrees of C5aR sensitization after LR. The presence of C5L2 has recently been shown to modulate C5aR-dependent signaling. A reduction in C5L2 expression on PMN was associated with impaired inflammatory cell function, and therefore, it has been suggested that C5L2 is required for C5a-mediated proinflammatory stimuli [18]. The observed divergent changes in the expression of the two C5a receptor subtypes after LR on PMN and monocytes were accompanied by a diminished chemotactic response by PMN. Furthermore L-selectin shedding and Mac-1 induction was reduced after surgery when PMN were confronted with C5a-dependent stimulation. Both observations, although based on experiments from a limited number of patients, imply down-regulation of neutrophil inflammatory activity after surgery. Thus, these data might be supportive of a proinflammatory role for C5L2 in this situation.

In respect to the clinical course we found an association of a pronounced reduction in C5L2 expression on monocytes with a more severe functional impairment of the liver in terms of plasma bilirubin levels and thromboplastin time (Fig. 4). It is noteworthy that this connection between C5L2 expression and postoperative liver function was observed at 4 h after LR. Thus, C5L2 expression levels might be of prognostic value early after LR, similar to the situation previously described in patients with septic shock [6].

Taken together, our findings support the involvement of complement activation and anaphylatoxin release in the cadre of inflammatory mediators that contribute to the systemic compensatory responses to LR. An increase of plasma C3a and C5b9 early after LR was accompanied by decreasing plasma C4a and C5a levels. Additionally C5L2 expression on PMN and monocytes changes after LR and is associated with functional alteration of circulating PMN. Furthermore, C5L2 receptor expression on monocytes at 4 h after surgery is associated with a more pronounced postoperative impairment of liver function.

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