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Neutrophil Adhesion Molecule Expression and Serum Concentration of Soluble Adhesion Molecules during and after Pediatric Cardiovascular Surgery with or without Cardiopulmonary Bypass

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Background: **Increased neutrophil activation by cardiopulmonary bypass (CPB) during cardiovascular surgery is thought to be responsible for postoperative complications. In children, the contribution of cardiovascular surgery alone to this response is not well-characterized.**

Methods: **Children undergoing surgery with CPB (CPB group, n = 35) and without CPB (control, n = 22) were studied (age, 3–17 yr). Blood was drawn 24 h preoperatively before medication, after anesthesia, after connection to CPB, at reperfusion, 4 h to 2 days after surgery, at discharge, and months after surgery. Neutrophil antigen expression and serum concentration of adhesion molecules, interleukin 8, and C5a (fragment of C5 complement) were analyzed by flow cytometry and enzyme-linked immunosorbent assay, respectively.**

Results: **With and without CPB, anesthesia and surgery induced decreased LFA-1 (CD11a–CD18), Mac-1 (CD11b–CD18), CD45, and CD54 (intercellular adhesion molecule 1) surface expression and sICAM-1 serum concentrations (all $P < 0.001$). sL-selectin serum concentration decreased with CPB ($P < 0.001$) but was not significantly altered in the control. In contrast, CD62L expression increased during CPB ($P < 0.001$). The time course of all analyzed markers was not significantly different between CPB and control, with the exception of sL-selectin ($P = 0.017$). One-day preoperative baseline values were reached days to months after surgery. Interleukin 8 and C5a serum concentrations increased after surgery in both the CPB group and the control group.**

Conclusions: **Pediatric cardiovascular surgery leads to reduced adhesiveness and activity of circulating neutrophils. This reduction is more pronounced and sustained with CPB. These data may be useful in the assessment of novel therapeutic strategies.**

IN children, during cardiovascular surgery, a number of variant factors (anesthesia, cardiopulmonary bypass [CPB], surgical trauma, ischemia–reperfusion injury, hypothermia) induce a complex humoral and cellular inflammatory response, including complement activation, release of endotoxins, and leukocyte stimulation.[1–4] This proinflammatory cascade may contribute to the development of postoperative complications, including respiratory failure, renal dysfunction, bleeding disorders, and altered liver function, and can ultimately lead to multiple organ failure. Most of the studies in children have focused on the proinflammatory response to CPB surgery.[1,4] However, during and after CPB also, an antiinflammatory response is initiated in children[3] and in adults,[5] leading to a shift to the humoral (Th2) immune response.[6,7]

Activation of neutrophilic granulocytes seems to have an important role in the pathophysiologic pathway leading to postsurgical complications.[8] These cells are able to respond rapidly to contact with foreign substances or immune complexes and to stimulation with the chemokines interleukin 8 (IL-8, CXC chemokine) and C5a (complement fragment of C5), among others.[8,9] When stimulated, surface adhesion molecules are differentially regulated. Membrane-bound L-selectin (CD62L) is shed within minutes after stimulation.[10] LFA-1 and Mac-1, both members of the integrin family, consist of heterodimeric complexes of membrane glycoproteins possessing a β_2 subunit (CD18) connected with an α_L subunit (CD11a) in LFA-1 or an α_M subunit (CD11b) in Mac-1.[11] Both LFA-1 and Mac-1 expression are up-regulated when stimulated,[11,12] enhancing attachment to intercellular adhesion molecule 1 (ICAM-1) on the endothelial cells.[13,14] ICAM-1 (CD54) is expressed on resting neutrophils at a low level and is up-regulated after stimulation.[15] The expression of the pan leukocyte antigen CD45, essential for neutrophil activation, increases with activation.[16]

Loss of CD62L and increased CD54 and integrin expression and increased soluble (s)L-selectin and sICAM-1 serum concentrations are indicators of neutrophil activation. The impact of both IL-8 and C5a on neutrophil function is pleiotropic: it exhibits proinflammatory (CD62L shedding, integrin up-regulation) but also antiinflammatory properties (reduction of neutrophil–endothelial interaction).[14]

Neutrophil activation by the CPB circuits is thought to be mainly responsible for reperfusion injury and postoperative complications.[8,17] Little is known about the influence of CPB on neutrophils. The results of clinical and experimental studies are controversial. Some suggest neutrophil activation during CPB *in vitro*[18,19] and *in vivo* in children[20,21]; others report decreased neutrophil

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activity in infants,[22] children,[23] and adults.[24] In addition, *in vitro* and *in vivo* observations conflict.[18,19]

It is not possible to draw clear conclusions about the specific effect of CPB on neutrophil activation in children because of the lack of a control group of patients who underwent similar trauma of cardiovascular surgery without CPB. The immune response to vascular surgery without CPB may cause alterations similar to those observed during CPB surgery in children[4] and adults.[25] In addition, the majority of studies were performed during a short time frame, beginning immediately before or after anesthesia and ending to 2 or 3 days after surgery, presumably before the immune response to the surgical trauma had settled. The values obtained after induction of anesthesia were regarded as the baseline, although anesthetics and analgesics clearly affect the immune system.[4,26–28] Therefore, it remains questionable whether the measured effects are due to the CPB and whether stimulation was above the normal unperturbed level of the patient.

In the current study, we characterize the time course of neutrophil activity in pediatric patients undergoing cardiovascular surgery with or without CPB within a longer time frame, starting before onset of the in-hospital medication and continuing until months after surgery.

Materials and Methods

Patients and Surgery

All patients enrolled in this study underwent elective cardiovascular surgery at the Cardiac Center Leipzig, University of Leipzig (Leipzig, Germany). The study was approved by the ethical committee of the University of Leipzig. Written informed consent was obtained from the parents of all patients. The study included blood samples from 35 children undergoing operations with CPB (CPB group) and 24 children without CPB (control). All children (age, 3–17 yr) had congenital heart diseases (CPB group: atrial [ASDII, n = 20] and ventricular [VSD, n = 10] septal defects, Ross operation [Ross, n = 5] because of aortic valve stenosis; control group: coarctation of the aorta [n = 20], persistent ductus arteriosus [n = 2]). Exclusion criteria were age less than 3 yr or more than 17 yr, genetic anomalies (*e.g.*, Down syndrome), absence of parents' consent, reoperation, and medication before the 1-day preoperative sample. The patients received similar anesthesia for induction and maintenance (midazolam, sufentanil or fentanyl, propofol, etomidate), similar medication, and similar intraoperative and postoperative care. The extracorporeal circuit was performed in a standardized manner using a roller pump (Stoeckert-Shiley, Munich, Germany) and a hollow-fiber oxygenator (DIDECO, Mirandola, Italy). Mild hypothermia was induced by cooling the priming solution (crystalloid solution, mannitol, Iono-lactat) in the extracorporeal circuit and the circulating blood with the heat exchanger. Hemodilution during surgery was approximately 32% as judged by the decrease of blood hematocrit concentrations. For further details see Tárnok *et al.*[4]

Obtaining Blood Samples

Blood samples were drawn 1 day before surgery before any medication (1 day−), after onset of anesthesia (anesthesia), immediately (mean ± SD, 14 ± 6 min; range, 10–30 min) after CPB onset (CPB1), before CPB ending during reperfusion and rewarming (CPB2), 4–6 h after the end of surgery (4 h+), 1 (1 day+) and 2 days (2 days+) after surgery and at discharge (discharge; mean ± SD, 7.6 ± 4.1 days postoperatively), and postoperatively (postOP; time after surgery: mean, 9 ± 6 months [range, 2–30 months]; obtained from 82% of all patients). In the control group, samples were drawn at similar time intervals except at CPB1 (no sample); a CPB2 value sample was drawn immediately at the end of surgery. EDTA anticoagulated blood and blood in coagulation tubes were collected for flow cytometry and serology, respectively. Serum samples were sedimented at 2,800*g*, and supernatants were frozen at −80°C in aliquots within 1 h after sampling. From the same blood samples, hematocrit values were determined.

Antigen Expression

EDTA-blood samples were stained by the whole blood technique.[29] Forty microliters blood was mixed with the appropriate volume of directly fluorescence dye–conjugated monoclonal antibodies at saturating concentrations (fluorescein-isothiocyanate or phycoerythrin). Cells were stained for 15 min at room temperature in the dark; 1 ml lysing solution (BD Biosciences, San Jose, CA) was added, mixed, and incubated for 10 min at room temperature in the dark. Cells were spun down at 300*g*, the supernatant was discarded, cells were washed twice in 1 ml phosphate-buffered saline (PBS), and cells were finally resuspended in 500 μl PBS containing 0.1% (weight/volume) paraformaldehyde (Sigma-Aldrich Chemicals, Deisenhofen, Germany). We used antibodies against CD54 (Beckman-Coulter, Hialeah, FL), CD18 (DAKO, Glostrup, Denmark), CD11b, CD11a, CD45 (all BD Biosciences), and appropriate isotype control antibodies.

Cells were measured on a dual-laser flow cytometer (FACSCalibur; BD Biosciences) calibrated with quantitative calibration beads (Spherotech Inc., Libertyville, IL), if necessary. Data were analyzed by the CellQuest software package (BD Biosciences). Neutrophils were gated based on forward and sideways scatter display. Eosinophils were removed from analysis based on their specific autofluorescence.[29] Antigen expression was determined as the mean fluorescence intensity after subtraction of the mean fluorescence intensity of the appropriate isotype control value.

Table 1. Patient and Surgical Characteristics of CPB and Control Groups

	n	Age (yr)	Weight (kg)	Sex (M/F)	Surgery (min)	CPB (min)	Cross-clamp (min)
CPB	35	7.6 ± 2.7	23.8 ± 8.3	13/22	149.0 ± 49.3	59.5 ± 38.1	35.4 ± 29.4
Control	24	7.7 ± 3.2	25.7 ± 11.9	14/10	148.9 ± 54.4		

Values are mean ± SD.

CPB = cardiopulmonary bypass.

Serum Compounds

The serum concentrations of sL-selectin, sICAM-1, IL-8, and C5a were quantified by enzyme-linked immunoassay (sL-selectin, sICAM-1, IL-8: R&D Systems, Oxon, United Kingdom; C5a: Beckman-Coulter). sL-selectin and sICAM-1 are indicators of cell activation. To compare their concentration during and after surgery with that obtained at 1 day− and between CPB and control, all values except those at postOP were corrected for hemodilution by the following equation:

$$\text{concentration}_{\text{corrected}} = \text{concentration}_{\text{sample}} \times (\text{hematocrit}_{1\ \text{day}-}/\text{hematocrit}_{\text{sample}}) .$$

Only corrected data are displayed. IL-8 and C5a concentrations were not corrected for hemodilution because their biologic activity depends on their actual serum concentration and was of interest in the study.

Statistics

Data are presented as mean ± SD. Changes with time within individual groups (CPB or control) were analyzed by Friedman test or analysis of variances for repeated measurements (ANOVA), as appropriate. The time courses of CPB and control were compared by multivariate ANOVA. Data distribution was tested for normality (Kolmogorov-Smirnov statistics). Single data sets were compared by Student *t* test or the nonparametric Mann–Whitney U test, as appropriate. Correlation was performed by Spearman rank test. All statistical analyses were performed using the SPSS program package (SPSS V.8; Knowledge Dynamics, Canyon Lake, TX).

Results

Clinical Results

Both CPB and control patients were similar with respect to patient and surgical data (table 1) and basal laboratory characteristics (table 2). In the CPB group, surgical data differed between types of surgeries with longest duration for the Ross patients (surgery, 262.6 ± 93.3 min; CPB, 141.4 ± 37.5 min; cross clamp, 96.8 ± 18.6 min) and shortest duration for the ASDII patients (surgery, 134.4 ± 43.7 min; CPB, 36.8 ± 7.5 min; cross clamp, 17.5 ± 7.9 min; all $P < 0.01$ ASDII *vs.* Ross). Mild postoperative complications were detected in 10 children from the CPB group (pericardial effusion [n = 7], fever [n = 1], pneumothorax [n = 2]) and in 5 children of the control group (pericardial effusion [n = 2], hypertension [n = 3]). No patient had preoperative, perioperative, or postoperative infections. The outcome was good for all children.

Integrins

In a typical example, the time course of integrin expression is shown for CD18 (fig. 1). During CPB surgery, CD18 expression decreased compared with the 1 day− value beginning at anesthesia and remained low until 2 days+. Baseline values were reached at discharge. Without CPB, CD18 expression had a similar time course but did not decrease to the same extent. Surgery clearly affected the expression of CD18, CD11a, and CD11b irrespective of the use of CPB (figs. 2A–C; all $P < 0.001$, except CD11b of control, $P < 0.05$; Friedman). However, the time course was not significantly different with and without CPB (multivariate ANOVA). Values determined at the same time points did not significantly differ. Integrin expression decreased immediately after anesthesia (all $P < 0.05$) and returned to baseline at 2 days+ (CD18) or at discharge (CD11a, CD11b).

CD45

CD45 expression changed significantly with time in each group ($P < 0.001$, fig. 2D), but the time course of CPB and control and values at the same time points did not significantly differ. CD45 expression decreased after

Table 2. One-day Preoperative Hematologic Parameters of CPB and Control Groups

	n	Hemoglobin (mM)	Hematocrit (%)	Erythrocytes ($\times 10^{12}$/l)	Leukocytes ($\times 10^{9}$/l)	Platelets ($\times 10^{9}$/l)
CPB	35	7.75 ± 1.04	36.4 ± 3.9	4.34 ± 0.80	6.86 ± 2.52	258.6 ± 79.6
Control	24	8.10 ± 0.76	38.8 ± 5.0	4.69 ± 0.53	6.59 ± 1.72	256.6 ± 48.2

Values are mean ± SD.

CPB = cardiopulmonary bypass.

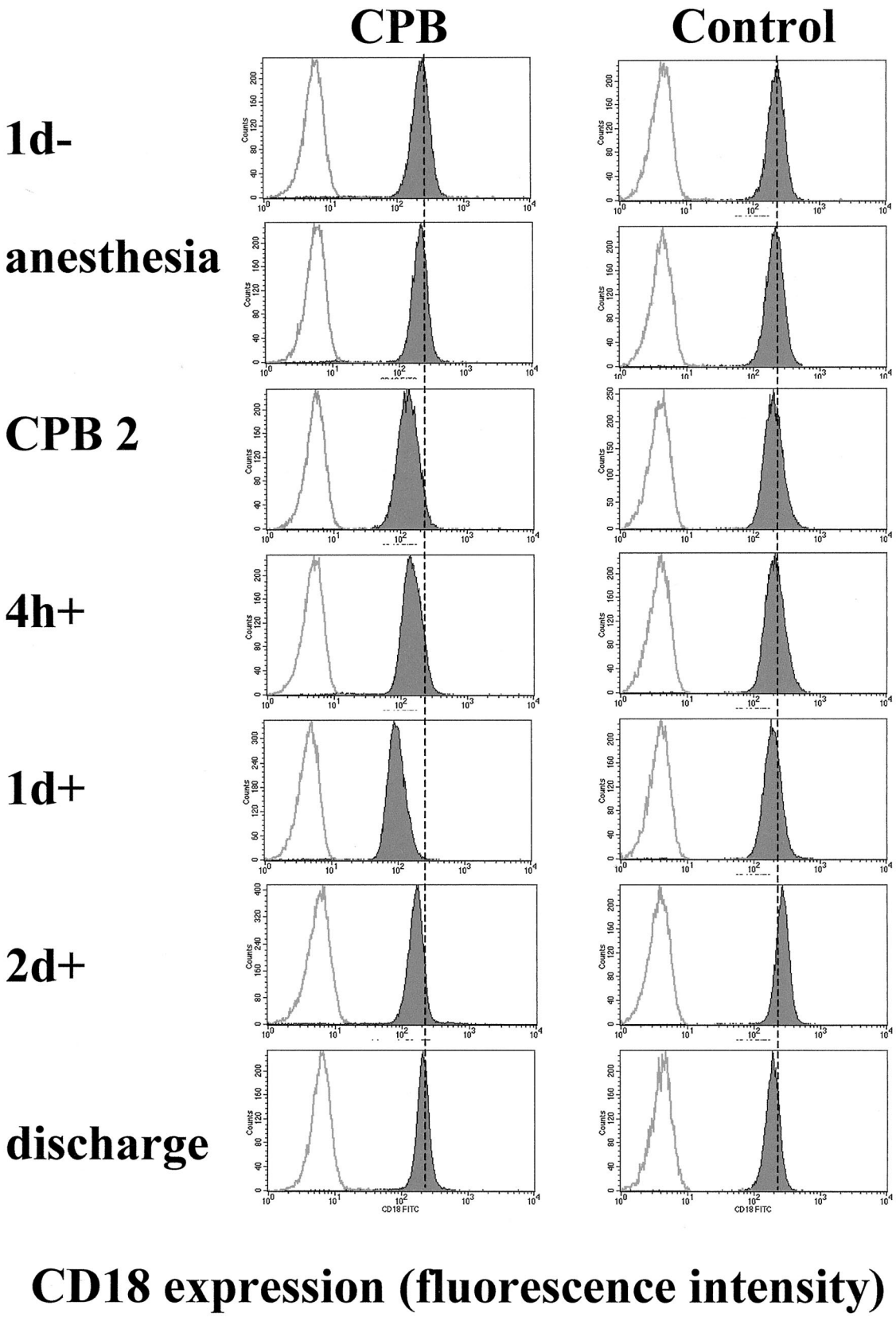

Fig. 1. Typical example of CD18 expression on neutrophils in one patient who underwent surgery with (*left*) and one who underwent surgery without cardiopulmonary bypass (CPB) (*right*). Histograms represent CD18 expression (mean fluorescence intensity, horizontal axes, filled histograms) or fluorescence intensity of neutrophils stained with an isotype control antibody (open histograms) *versus* cell count (vertical axes). The dotted lines indicate mean fluorescence intensity of baseline.

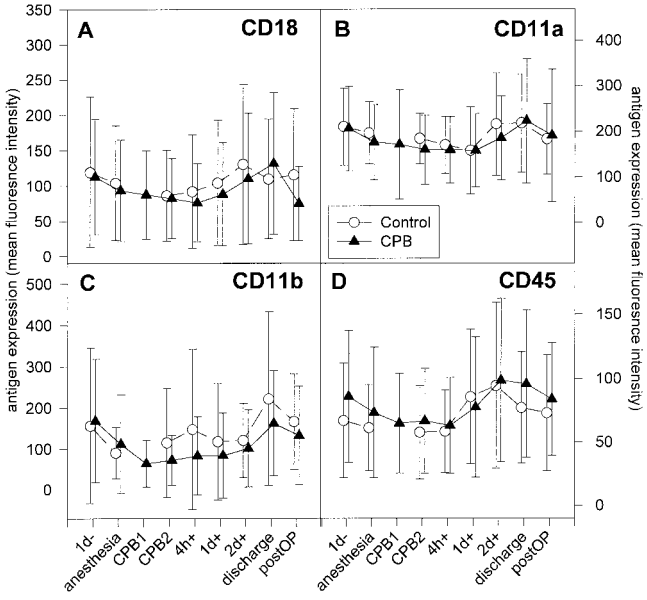

Fig. 2. Expression level of CD18 (*A*), C11a (*B*), CD11b (*C*), and CD45 (*D*) on neutrophils before, during, and after surgery. Antigen expression was determined as mean fluorescence intensity and is displayed as original value. Data show mean ± 1 SD with cardiopulmonary bypass (CPB) (closed triangles, n = 35, *C*: n = 21) or without CPB (open circles, n = 27, *C*: n = 15).

anesthesia (CPB: $P < 0.05$; control: not significant [NS]). It exceeded baseline at 1 day+ (control: $P < 0.05$) and at 2 days+ (CPB: NS; control: $P < 0.05$) and returned to baseline at discharge (control) or at postOP (CPB).

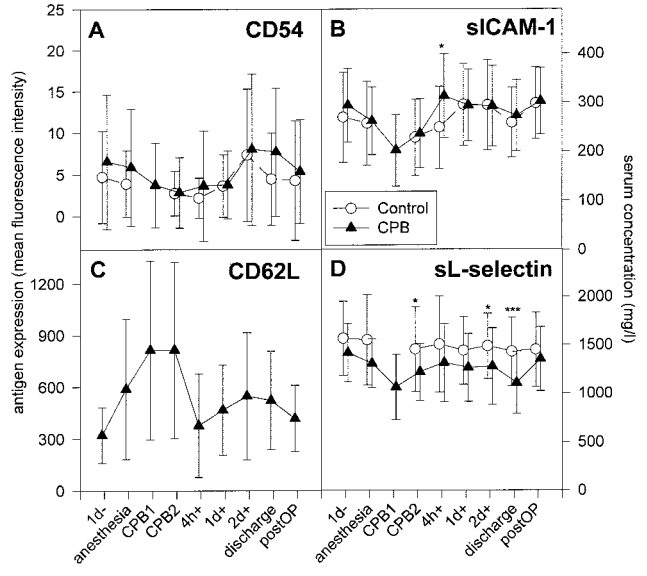

Fig. 3. Expression level of CD54 (*A*) and CD62L (*C*) on neutrophils, and sICAM-1 (*B*) and sL-selectin (*D*) serum concentrations before, during, and after surgery. For determination of antigen expression and data display, see figure 2. Data show surgeries with cardiopulmonary bypass (CPB) (closed triangles, CD54: n = 32; CD62L: n = 8; sICAM, sL-selectin: n = 35) or without CPB (open circles, CD54: n = 21; sICAM, sL-selectin: n = 24) (mean ± SD). Significant differences between the CPB and control groups are shown (*$P < 0.05$, ***$P < 0.001$).

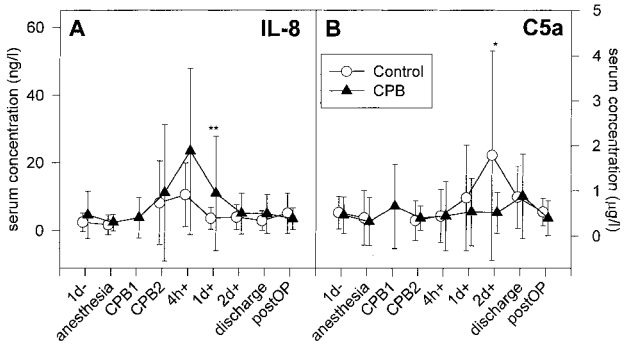

Fig. 4. Serum concentration of interleukin (IL) 8 (*A*) and C5a (*B*) before, during, and after surgery. Data show surgeries with cardiopulmonary bypass (CPB) (closed triangles: n = 35) or without CPB (open circles: n = 24) (mean ± SD). Significant differences between the CPB and control groups are shown (*$P < 0.05$, **$P < 0.01$).

Intercellular Adhesion Molecule 1

The time course of surface CD54 expression (fig. 3A) was similar with and within CPB and decreased significantly (both $P < 0.001$) with no statistical difference between the groups. CD54 expression decreased after anesthesia (CPB: $P < 0.01$; control: NS), then increased with values over baseline at 2 days+ (CPB: NS; control: $P < 0.01$), and returned to baseline at discharge (control) or at postOP (CPB).

The concentration of sICAM-1 (fig. 3B) was reduced after anesthesia and during surgery ($P < 0.001$) with no significant difference between both groups. sICAM-1 concentration decreased after anesthesia (CPB: $P < 0.01$; control: NS), then increased over baseline at 4 h+ (CPB: NS), and returned to baseline at 1 day+ (CPB) or at discharge (control). Importantly, sICAM-1 concentrations were significantly higher in the CPB group at 4 h+ compared with control.

L-selectin

Surface expression of CD62L in CPB patients significantly changed during and after surgery ($P < 0.001$; fig. 3C). It increased after anesthesia ($P < 0.05$), decreased sharply reaching baseline at 4 h+, and slightly increased (NS) and reached baseline at postOP. The time course of CD62L could only be analyzed in three control patients (data not shown) in whom it did not significantly change with time. Because of the low number of control patients, comparison with CPB patients was not performed.

CD62L expression and serum concentration of sL-selectin had complementary time courses, as expected. The time course of sL-selectin was significant only with CPB ($P < 0.001$; fig. 3D) and significantly differed between CPB and control ($P = 0.017$). With CPB, sL-selectin concentration decreased after anesthesia ($P < 0.05$), was below baseline until discharge, and reached baseline only at postOP. Without CPB, sL-selectin concentrations did not significantly differ from baseline at

any time point. At CPB2, 2 days+, and discharge, sL-selectin concentrations with CPB were significantly below control.

Interleukin 8 and C5a

Surgery with and without CPB induced increase of both IL-8 and C5a. The time courses of IL-8 (fig. 4A) and C5a (fig. 4B) were significant for each group ($P < 0.001$) but were not different between both groups. IL-8 concentrations were increased from CPB2 to 1 day+ (both groups: $P < 0.05$), decreased thereafter, and reached baseline at 2 days+. IL-8 concentration with CPB was above control at 1 day+. C5a concentrations increased at 4 h+ (both groups: $P < 0.05$) and remained increased until discharge. Baseline values were only reached at postOP. C5a concentration was higher with than without CPB at CPB2 and was lower with than without CPB at 2 days+.

Effect of Cardiopulmonary Bypass Duration

Duration of surgery, CPB, and aortic cross clamping depended on the type of surgery. Therefore, we tested whether antigen expression or concentration of the analyzed serum compounds depended on these surgical parameters. IL-8 concentration at 4 h+ correlated with the duration of aortic cross clamping ($r = 0.37$, $P < 0.05$, Spearman rank test) and with CPB duration ($r = 0.38$, $P < 0.05$). However, CD11a expression both at 4 h+ and 1 day+ correlated inversely with duration of aortic cross clamping (both $r = -0.48$, $P < 0.05$). All others values did not correlate with these surgical parameters.

Discussion

The major finding of our study is that pediatric cardiovascular surgery with and without CPB transiently inactivates circulating neutrophils. This decreased neutrophil activity is even more prominent during CPB surgery. Baseline values (*i.e.*, in our study, values in samples obtained preoperatively before any in-hospital medication) were reached only days or even months after surgery. To our knowledge, this is the first study investigating expression level and serum concentration of adhesion molecules over such a long period of time.

Our findings about adhesion molecule expression seem to differ from some earlier reports in adults.[17,30] These differences may suggest that effects of CPB and cardiac surgery are quite different between children and adults. There are a limited number of accessible publications about neutrophil activation in children during CPB surgery. Most of them concentrate on the perioperative and immediate postoperative course starting at anesthesia and drawing the last samples at 2 h,[21] 24 h,[19,31,32] 42 h,[33] or a maximum of 96 h after bypass.[23] Some of these studies indicate increased neutrophil activation during or after CPB. If we compare our data in the time frame observed by others, our observations are partially in accordance with theirs. Finn *et al.*[19] found slightly increased Mac-1 expression at reperfusion but did not detect CD62L down-regulation. Gilliland *et al.*[21] showed increased CD11b expression on neutrophils 10 min after the end of cross clamping but, in contrast to us, also increased CD18 expression. The time course of sL-selectin[31,33] and sICAM-1[31–33] from other studies is similar to our observations.

Most authors interpreted their results as indications for neutrophil activation by CPB. However, at the beginning and at end of their analysis, the immune system was presumably perturbed by anesthesia and surgical trauma. Therefore, expression values may still be altered not only as a consequence of CPB. For this reason, in the current study, baseline determination was performed at a time point when the immune system was not highly perturbed, that is, before any in-hospital medication or anesthetic premedication. Anesthetics and analgesics alone and in combination can affect neutrophil functions. The anesthetics/analgesics applied in our study depress *in vitro* neutrophil phagocytosis, oxidative burst, chemotaxis/migration, and IL-8 secretion.[26,27] Little information is available about the effect of combinations of anesthetics and analgesics used in our study on neutrophils *in vivo*. In adults, similar combinations of anesthetics depress integrin expression on neutrophils[24] and monocytes.[28] To our knowledge, in children, the effect of these anesthetics has not been reported.

To confirm preoperative baseline values, the study was extended to the outpatient follow-up months after surgery. We found that expression and serum concentrations returned to the 1 day− baseline; however, this was only after several days or, as in the case of sL-selectin, after weeks or months. This has not been reported before. Only in light of these unperturbed baseline values can the immune response during and early after CPB surgery also be interpreted as recovery from a transient immune depression.

The interpretation that circulating neutrophil activity is suppressed during and after CPB surgery in children is in accordance with reduced bactericidal activity in infants immediately after bypass until 2 days+.[22] Chemotactic activity remains unchanged,[22] and oxidative burst capacity is only increased in the immediate postoperative period but not during CPB.[12,22] In adults, circulating neutrophils are less susceptible to *ex vivo* stimulation after coronary artery bypass grafting,[17] and CD11b and CD18 expression do not change during CPB but decreased with a minimum around 4–24 h after CPB surgery.[24]

It is difficult to separate the impact of CPB on the patients' immune systems from that of the surgical trauma. As one approach, we included a group of pedi-

atric patients who underwent thoracovascular surgery without CPB. In children, such comparison has only been performed in two studies, one of them from our group.[4,23] Bührer *et al.*[23] reported that down-regulation of sL-selectin with CPB was more profound than in their non-CPB group. In our earlier study, with a different study group, we found that surgery both with and without CPB induces a proinflammatory response and down-regulation of sICAM-1 and other adhesion molecules with comparable kinetics.[4] In adults, coronary artery bypass grafting with and without CPB shows a similar extent of proinflammatory response[25] but an increased antiinflammatory reaction with CPB.[6,25] For this reason, in adults, the benefit of off-pump coronary artery bypass grafting is discussed.[34]

Several factors might contribute to this temporary decrease of circulating neutrophil activity. In the *in vivo* system, a balance of proinflammatory and antiinflammatory reactions is important to determine the extent of the inflammatory response and clinical outcome. Recent observations in children and adults show that CPB surgery induces the release of antiinflammatory cytokines[3,25] and thereby a transient antiinflammatory reaction.[6,7] The significant release of the antiinflammatory cytokine IL-10 during CPB is observed at the end of rewarming up to 4 h after surgery,[3,5] before the release of proinflammatory cytokines.[2,4] In children, IL-10 release is negligible during cardiovascular surgery without CPB.[7] IL-10 may play a protective role by suppressing the production of proinflammatory cytokines[35] and may affect in our study the low activation of circulating neutrophils. IL-10 probably contributes to the conflicting observations between simulated extracorporeal circulation and *in vivo* observations.[18] The antiinflammatory response may be induced by central nervous stress (ischemia, reperfusion) leading to the release of catecholamines and steroids, which in turn leads to IL-10 release, immunodepression,[36] and inactivation of the inflammatory cells.[35,36] In addition, CPB filters contribute to the reduced activity of neutrophils in the circulation by selective removal of activated and highly adhesive cells without promoting neutrophil activation.[12,37] Finally, activated neutrophils may adhere to and transmigrate through the activated endothelium[9] and escape from the circulation and from our analysis.

Our data could also provide indirect indications that CPB surgery has increased proinflammatory potential. After IL-8 secretion at 4 h+, CD62L is readily shed—a response that might find its reflection in the (minute) sL-selectin increase in the serum. An important source of secreted IL-8 could be activated endothelial cells because in spite of significantly increased sICAM-1 concentrations at 4 h+, CD54 expression on neutrophils is only slightly increased, and the major proportion of sICAM-1 is sequestered by activated endothelial cells.[13] In children, coinciding with this activation are the observations that the oxidative burst capacity of neutrophils[12,22] and the serum concentration of indicators of neutrophil activation (*e.g.*, lactoferrin, myeloperoxidase) in the serum[38] increase shortly after the end of CPB. However, at 1 day+, a population change has occurred, and the circulating neutrophils express increased CD62L but unchanged or even reduced[12] integrin concentrations. At this time, oxidative burst capacity returns to baseline.[12] One interpretation of this decrease is that activated leukocytes disappear from the circulation and extravasate into the tissue. This hypothesis was also expressed by others.[24] Support for this hypothesis is that in our study, IL-8 concentrations at 4 h+ inversely correlate with CD11a expression (data not shown). The net effect of all these reactions may in the periphery result in the same phenotype as in our control group.

In contrast to our observations in adults, we did not observe down-regulation of integrin expression immediately after surgery.[24] However, we recently reported in another study group a decrease in CD11b expression on neutrophils at 1 day+.[12] We do not have an explanation why this decrease was not observable in the current study.

A limitation of our study is that the immune statuses of children with different congenital heart or vascular diseases might differ. In addition, the patients in the CPB group underwent surgical procedures other than those that the control patients underwent. These different clinical situations may in turn lead to different immune responses. However, for obvious reasons, patients with congenital heart disease cannot be randomly distributed into a CPB or a control group. Aware of these problems, we included these patients into the study as controls to have an impression of how children at the same age respond to similar anesthesia and injury of the blood vessels. We are convinced that a comparison with a non-CPB group, even if it is not identical to the CPB group, serves the understanding of ongoing inflammatory processes better than the lack of comparison. This study was intended to analyze the kinetics in a long time range, which has previously not been performed. We cannot exclude that we might have missed up- or down-regulation of certain molecules, in particular during the earliest perioperative time period. It would be helpful to have a tighter time-frame of sampling during surgery; however, this is not feasible in pediatric patients. The analyses of the immune response by looking at circulating cells from the peripheral blood is a limited approach because of the lack of access to other body parts to which activated leukocytes might migrate.

The finding that cardiovascular surgery with and cardiovascular surgery without CPB display similar time courses of expression and release of adhesion molecules might result from different reasons. (1) Pediatric cardiovascular surgery with and without CPB might lead to comparable trauma on circulating leukocytes in the pe-

ripheral blood. (2) The immune response to cardiac surgery with CPB displays a complex pattern of proinflammatory and antiinflammatory responses[6,7]: a more pronounced antiinflammatory response occurs,[25] and activated leukocytes are removed by the filters.[12] (3) Finally, the proinflammatory response induced by surgery without CPB and the (probably increased) proinflammatory response induced by CPB surgery counterregulated by the antiinflammatory reaction might end up in a similar phenotype of circulating leukocytes.

Our data indicate an inability to detect CPB-specific activation and adhesion molecule expression of circulating neutrophils unequivocally. These results do not necessarily indicate that cardiovascular surgery with CPB leads to complete neutrophil deactivation, but that cardiovascular surgery in particular with CPB leads to decreased activity neutrophils that remain circulating in the child's peripheral blood. These data may be useful in the assessment of novel therapeutic strategies.

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References

1. Seghaye MC, Duchateau J, Grabitz RG, Nitsch G, Marcus C, Messmer BJ, von Bernuth G: Complement, leukocytes, and leukocyte elastase in full-term neonates undergoing cardiac operation. J Thorac Cardiovasc Surg 1994; 108:29–36

2. Wan S, LeClerc JL, Vincent JL: Cytokine responses to cardiopulmonary bypass: Lessons learned from cardiac transplantation. Ann Thorac Surg 1997; 63:269–76

3. Tárnok A, Hambsch J, Schneider P: Cardiopulmonary bypass induced increase of serum interleukin-10 levels in children. J Thorac Cardiovasc Surg 1998; 115:475–7

4. Tárnok A, Hambsch J, Emmrich F, Sack U, van Son J, Bellinghausen W, Borte M, Schneider P: Complement activation, cytokines, and adhesion molecules in children undergoing cardiac surgery with or without cardiopulmonary bypass. Ped Cardiol 1999; 20:113–25

5. Sablotzki A, Welters I, Lehmann N, Menges T, Gorlach G, Dehne M, Hempelmann G: Plasma levels of immunoinhibitory cytokines interleukin-10 and transforming growth factor-β in patients undergoing coronary artery bypass grafting. Eur J Cardiothorac Surg 1997; 11:763–8

6. Markewitz A, Faist E, Lang S, Hultner L, Weinhold C, Reichart B: An imbalance in T-helper cell subsets alters immune response after cardiac surgery. Eur J Cardiothorac Surg 1996; 10:61–7

7. Tárnok A, Schneider P: Induction of transient immune suppression and Th1/Th2 disbalance by pediatric cardiac surgery with cardiopulmonary bypass. Clin Appl Immunol Rev 2001; 1/5:291–313

8. Dreyer WJ, Michael LH, Millman EE, Berens KL: Neutrophil activation and adhesion molecule expression in a canine model of open heart surgery with cardiopulmonary bypass. Cardiovasc Res 1995; 29:775–81

9. Jagels MA, Daffern PJ, Hugli TE: C3a and C5a enhance granulocyte adhesion to endothelial and epithelial cell monolayers: Epithelial and endothelial priming is required for C3a-induced eosinophil adhesion. Immunopharmacology 2000; 46:209–22

10. Wroblewski M, Hamann A: CD45-mediated signals can trigger shedding of lymphocyte L-selectin. Int Immunol 1997; 9:555–62

11. Ding ZM, Babensee JE, Simon SI, Lu H, Perrard JL, Bullard DC, Dai XY, Bromley SK, Dustin ML, Entman ML, Smith CW, Ballantyne CM: Relative contribution of LFA-1 and Mac-1 to neutrophil adhesion and migration. J Immunol 1999; 163:5029–38

12. Tárnok A, Bocsi J, Rössler H, Schlykow V, Schneider P, Hambsch J: Low degree of activation of circulating neutrophils determination by flow cytometry during cardiac surgery with cardiopulmonary bypass. Cytometry 2001; 46:41–9

13. Smith CW, Marlin SD, Rothlein R, Toman C, Anderson DC: Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. J Clin Invest 1989; 83:2008–17

14. Luscinskas FW, Kiely JM, Ding H, Obin MS, Hebert CA, Baker JB, Gimbrone MA Jr: In vitro inhibitory effect of IL-8 and other chemoattractants on neutrophil-endothelial adhesive interactions. J Immunol 1992; 149:2163–71

15. Albelda SM, Smith CW, Ward PA: Adhesion molecules and inflammatory injury. FASEB J 1994; 8:504–12

16. Harvath L, Balke JA, Christiansen NP, Russell AA, Skubitz KM: Selected antibodies to leukocyte common antigen (CD45) inhibit human neutrophil chemotaxis. J Immunol 1991; 146:949–57

17. Ilton MK, Langton PE, Taylor ML, Misso NL, Newman M, Thompson PJ, Hung J: Differential expression of neutrophil adhesion molecules during coronary artery surgery with cardiopulmonary bypass. J Thorac Cardiovasc Surg 1999; 118:930–7

18. McBride WT, Armstrong MA, Crockard AD, McMurray TJ, Rea JM: Cytokine balance and immunosuppressive changes at cardiac surgery: Contrasting response between patients and isolated CPB circuits. Br J Anaesth 1995; 75: 724–33

19. Finn A, Moat N, Rebuck N, Klein N, Strobel S, Elliott M: Changes in neutrophil CD11b/CD18 and L-selectin expression and release of interleukin 8 and elastase in paediatric cardiopulmonary bypass. Agents Actions 1993; 38: C44–6

20. Gillinov AM, Bator JM, Zehr KJ, Redmond JM, Burch RM, Ko C, Winkelstein JA, Stuart RS, Baumgartner WA, Cameron DE: Neutrophil adhesion molecule expression during cardiopulmonary bypass with bubble and membrane oxygenators. Ann Thorac Surg 1993; 56:847–53

21. Gilliland HE, Amstrong MA, McMurray TJ: The inflammatory response to pediatric cardiac surgery: Correlation of granulocyte adhesion molecule expression with postoperative oxygenation. Anesth Analg 1999; 89:1188–91

22. Burrows FA, Steele RW, Marmer DJ, Van Devanter, SH, Westerman GR: Influence of operations with cardiopulmonary bypass on polymorphonuclear leukocyte function in infants. J Thorac Cardiovasc Surg 1987; 93:253–60

23. Buhrer C, Stiller B, Alexi-Meskishvili V, Hetzer R, Lange PE: Soluble L-selectin plasma concentrations in infants undergoing heart surgery: No association with capillary leak syndrome or need for respiratory support. Intensive Care Med 1998; 24:1093–8

24. Galinanes M, Watson C, Trivedi U, Chambers DJ, Young CP, Venn GE: Differential patterns of neutrophil adhesion molecules during cardiopulmonary bypass in humans. Circulation 1996; 94(suppl I):364–9

25. Diegeler A, Doll N, Rauch T, Haberer D, Walther T, Falk V, Gummert J, Autschbach R, Mohr FW: Humoral immune response during coronary artery bypass grafting: A comparison of limited approach, "off-pump" technique, and conventional cardiopulmonary bypass. Circulation 2000; 102(suppl 3):III95–100

26. Nishina K, Akamatsu H, Mikawa K, Shiga M, Maekawa N, Obara H, Niwa Y: The inhibitory effects of thiopental, midazolam, and ketamine on human neutrophil functions. Anesth Analg 1998; 86:159–65

27. Weiss M, Buhl R, Medve M, Schneider EM: Tumor necrosis factor-alpha modulates the selective interference of hypnotics and sedatives to suppress N-formyl-methionyl-leucyl-phenylalanine-induced oxidative burst formation in neutrophils. Crit Care Med 1997; 25:128–34

28. Hiesmayr MJ, Spittler A, Lassnigg A, Berger R, Laufer G, Kocher A, Artemiou O, Boltz-Nitulescu G, Roth E: Alterations in the number of circulating leucocytes, phenotype of monocytes and cytokine production in patients undergoing cardiothoracic surgery. Clin Exp Immunol 1999; 115:315–23

29. Lewis DE: Cytochemistry I: Cell surface Immunofluorescence: Clinical Flow Cytometry: Principles and Application. Edited by Bauer KD, Duque RE, Shankey TV. Williams & Wilkins, Baltimore, 1993, pp 143–56

30. Hill GE, Alonso A, Thiele GM, Robbins RA: Glucocorticoids blunt neutrophil CD11b surface glycoprotein upregulation during cardiopulmonary bypass in humans. Anesth Analg 1994; 79:23–7

31. Williams HJ, Rebuck N, Elliott MJ, Finn A: Changes in leukocyte counts and soluble intercellular adhesion molecule-1 and E-selectin during cardiopulmonary bypass in children. Perfusion 1998; 13:322–7

32. Boldt J, Osmer C, Linke LC, Dapper F, Hempelmann G: Circulating adhesion molecules in pediatric cardiac surgery. Anesth Analg 1995; 81:1129–35

33. Blume ED, Nelson DP, Gauvreau K, Walsh AZ, Plumb C, Neufeld EJ, Hickey PR, Mayer JE, Newburger JW: Soluble adhesion molecules in infants and children undergoing cardiopulmonary bypass. Circulation 1997; 96(suppl): II352–7

34. Jegaden O, Mikaeloff P: Off-pump coronary artery bypass surgery: The beginning of the end? Eur J Cardiothorac Surg 2001; 19:237–8

35. Eppinger MJ, Ward PA, Bolling SF, Deeb GM: Regulatory effects of interleukin-10 on lung ischemia-reperfusion injury. J Thorac Cardiovasc Surg 1996; 112:1301–5

36. Woiciechowsky C, Schoning B, Lanksch WR, Volk HD, Docke WD: Mechanisms of brain-mediated systemic anti-inflammatory syndrome causing immunodepression. J Mol Med 1999; 77:769–80

37. Smit JJ, de Vries AJ, Gu YJ, van Oeveren W: Filtration of activated granulocytes during cardiopulmonary bypass surgery: A morphologic and immunologic study to characterize the trapped leukocytes. J Lab Clin Med 2000; 135: 238–46

38. Pesonen EJ, Korpela R, Leijala M, Sairanen H, Pitkanen OM, Raivio KO, Venge P, Andersson S: Prolonged granulocyte activation, as well as hypoxanthine and free radical production after open heart surgery in children. Intensive Care Med 1996; 22:500–6