

Transient Th1/Th2 Disbalance Indicates Postoperative Effusions and Edema after Cardiopulmonary Bypass in Children

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Background: Cardiac surgery with cardiopulmonary bypass (CPB) induces substantial release of IL-10, indicating increased Th2 cell response. Therefore, in this study, we wanted to verify if this response is due to CPB or surgical trauma, and to study its relation to postoperative effusions and edema (POEE) in children.

Methods: Th1/Th2 reaction was monitored in children undergoing cardiovascular surgery with ($n = 75$) and without CPB ($n = 29$).

Results: Surgery with CPB compared to surgery without CPB induced a transient shift towards Th2. Elevated Th2

response was related to increased vascular permeability and POEE.

Conclusion: The immune suppression/Th2 response is typical for CPB, and at intermediate level is tolerable but at high level could be adverse for the patients.

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Key terms: Th2 shift; IL-10; IgE; IgG2; anti-inflammatory; postoperative complication; flow cytometry

Cardiac surgery is a substantial trauma to the immune system as a consequence of the combined effect of mechanical tissue damage, anesthesia, medication, and individual predisposition of the patient (1). Additionally, in open-heart surgery, cardiopulmonary bypass (CPB) is necessary for oxygenation, blood circulation, body cooling, and rewarming that is associated with extensive blood contact with foreign materials. Patients who undergo CPB surgery frequently develop a systemic inflammatory response syndrome (SIRS) ranging from mild complications as pericardial, pleural and or abdominal effusion, liver enlargement, and edema formation to massive generalized edema, acute respiratory distress syndrome, or even multiple organ failure. Recently, we reported on predictive serological and cellular phenotypes for postoperative effusions and edema (POEE) after CPB surgery, indicating a preoperative (allergic or inflammatory) predisposition of POEE risk patients (2,3). These individual phenotypes would allow for predictive medicine and individualized therapy (4).

Among T-helper (Th) cells, two distinct subsets, termed Th1 and Th2, can be found based on the profile of their cytokine production (5). Th1 cells typically secrete IFN- γ and predominantly promote cell-mediated immune responses and immunoglobulin (Ig) G2 production. Th2 cells are characterized by their production of IL-4, IL-5, IL-13, and IL-10, and promote humoral immunity, including elevated IgE production.

Data in adults indicate that cardiac surgery with CPB results in a disbalance of Th cell subsets towards the Th2 pathway (6,7). Children may respond in a different way than adults who possess a fully developed immune system and suffer from acquired rather than congenital heart diseases. In addition, for children, no studies are published demonstrating if Th2 response and IL-10 release are due to CPB or surgical trauma and if Th2 response is correlated with POEE. The aim of the present study was to determine whether the Th2-shift occurs in children with congenital heart disease and is specific to CPB surgery. Additionally, we tested if POEE is related to a characteristic Th1/Th2 disbalance, and thereby may show a potential target for the preventive treatment strategies.

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MATERIALS AND METHODS

This study was carried on children (age range 3–16 years) undergoing cardiac surgery with CPB (CPB) or without CPB (control). It was approved by the ethical committee of the medical faculty of the University of Leipzig and started on February 1996. Written informed consent was obtained from the parents of all pediatric patients before obtaining samples.

Sampling

Heparinized (intracellular cytokines) and native (serum cytokines and Ig) blood samples were drawn one day before surgery (1d–), after onset of anesthesia (anaesth), at thoracotomy (thoraco), immediately (mean $\pm$ SD, 14 $\pm$ 6 min; range, 10–30 min) after CPB onset (CPB1), before CPB ending during reperfusion and rewarming (CPB2), at protamine (Protamin) administration, 4–6 h after the end of surgery (+4 h), one (1 d) and two days (2 d) after surgery and at discharge (discharge; 7.6 $\pm$ 4.1 d post-operatively), and postoperatively during out-patient follow-up (3 min; time after surgery, 9 $\pm$ 6 min (range 2–30 min); obtained from 82% of all patients). In the control group, samples were drawn at similar time intervals, except at CPB1 (no sample); CPB2 value was drawn immediately at the end of surgery. Children in the CPB group underwent closure of atrial ($n = 31$) or ventricular septal defect ($n = 11$), total cavopulmonary connection ($n = 11$), valve replacement ($n = 10$), or others ($n = 12$); in the control group, children underwent correction of coarctation of aorta ($n = 24$) or persisting ductus arteriosus ($n = 5$). (Age (years): CPB group - 9.06 $\pm$ 3.68; control - 7.07 $\pm$ 3.57; $P = 0.006$; duration of surgery (min): CPB - 231 $\pm$ 86; control - 199 $\pm$ 74; $P = \text{ns}$). The majority of the children in the CPB group received perioperatively glucocorticoids.

Intracellular Cytokine Production

Th1/Th2 reaction was monitored by intracellular IL-4/IFN- γ production of CD4+ T-cells determined by flow cytometry (FCM) in 12 children with and 9 without CPB surgery. Standard FCM protocols were used for cell stimulation and the measurement of intracellular cytokines, as previously described (5). All reagents were from Hölzel Diagnostika (Köln, Germany).

Serological Th1/Th2 and POEE Analysis

Seventy five children with and 29 without CPB were included. CPB patients were subdivided to a non POEE ($n = 40$) and a POEE group ($n = 35$) based on published definitions (2) (Age (years): POEE - 9.59 $\pm$ 3.79, non POEE - 8.56 $\pm$ 4.60, $P = \text{ns}$; duration of surgery (min): POEE - 265 $\pm$ 96, non POEE - 196 $\pm$ 60, $P = 0.002$; duration of CPB (min): POEE - 96 $\pm$ 53, non POEE - 64 $\pm$ 35, $P = 0.009$). POEE patients suffered from postoperative effusions, massive edema, and liver enlargement; non POEE had no symptoms or mild edema ($n = 5$). The main medication of the children was not significantly different. Serum components of Th2 response (IL-4, IL-10 (both ELISA, Bender MedSystems, Vienna, Austria), IgE (ELISA,

Pharmacia Behring, Marburg, Germany)) and Th1 response (IFN- γ (Bender MedSystems), IgG2 (Radial Immune Diffusion, The Binding Site, Heidelberg, Germany) were measured.

Statistical Analysis

Statistical analysis was performed with Statistical Program for Social Sciences (SPSS, Knowledge Dynamics, Version 10, Chicago). All parameters were assessed for normality by the Kolmogorov–Smirnov test. Differences were tested by the unpaired Student's *t*-test or Wilcoxon test, as appropriate. Time courses were compared by analysis of variances for repeated measures (ANOVA). All reported *P* values are two-tailed.

RESULTS

Th Cells During CPB

The flow cytometric measurements show (typical example Fig. 1A) that in patients undergoing surgery with CPB, the mean fluorescence intensity (MFI) of peripheral Th-lymphocytes producing IL-4 (Th2) increased but IFN- γ (Th1) decreased. Without CPB these changes were not observed and Th1/Th2 ratio deviated in a small range, whereas with CPB IL-4/IFN- γ ratio significantly increased (at CPB2 and +1 d), demonstrating a Th2 shift (Fig. 1B.). In spite of this clear shift to the Th2 phenotype, systemic release of the Th2 cytokine IL-4 and also IFN- γ remained below the limits of detection throughout the surgery up to discharge (data not shown).

IL-10 Release and Increased IgE/IgG2 Ratio are CPB Specific

The release of the Th2 cytokine IL-10 significantly increased (peaked between CPB2 and +4 h) with CPB surgery but was not detectable without CPB (Fig. 2A.). In CPB group, the total serum IgE (Th2) increased significantly postoperatively (between protamine and +1 d) (Fig. 2C.) when compared with control. In contrast, IgG2 (Th1) did not differ in patients with or without CPB (Fig. 2B).

Th2 Shift is Stronger in POEE Patients

As shown in Figure 2D, perioperative IL-10 (between CPB2 and +4 h) and the postoperative IgE serum levels (between +4 h and +2 d) were significantly higher in POEE patients when compared with non POEE patients (Fig. 2E), while IgG2 levels were not significantly different (Fig. 2E).

DISCUSSION

In general, our results demonstrate in contrast to earlier interpretations that surgery with CPB is not dominated by an immune-stimulatory reaction (8), but specifically induces an anti-inflammatory pathway mediating humoral immune response. Thus, we found that in children, intracellular cytokine expression indicates transient Th2-shift during CPB surgery; this leads to the conclusion that anesthesia, analgesia, and surgical trauma alone do not lead to the Th1/Th2 disbalance. These results are the first showing a Th2 shift in children during open-heart surgery,

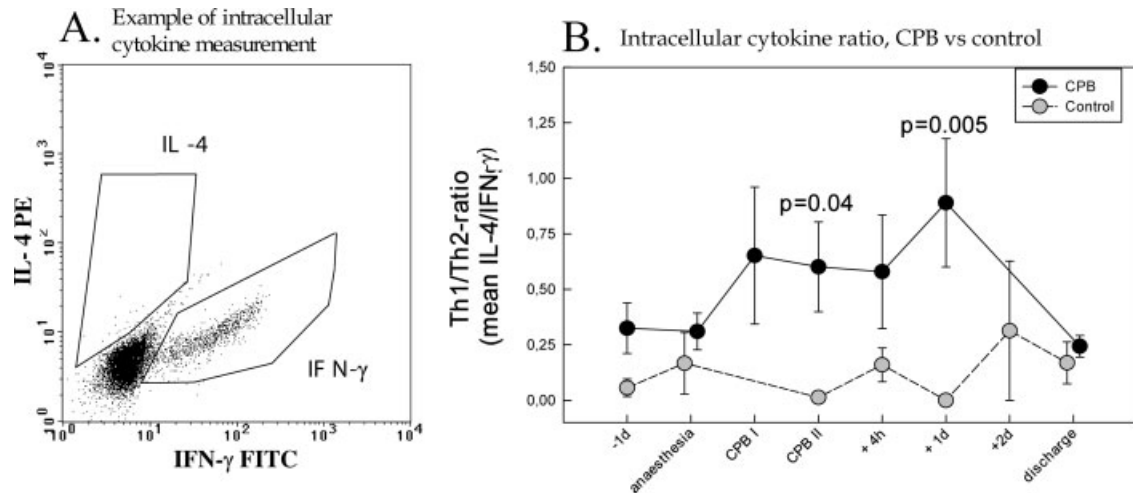


FIG. 1. Th1/Th2 disbalance during cardiovascular surgery. A: Typical example for an FCM analysis of IL-4 and IFN- γ producing Th cells ($CD3^{+}4^{+}$). Lymphocytes were gated based on forward and sideward scatter display and CD4 expression (not shown). B: Mean fluorescence intensity ratio of IL-4 and IFN- γ in Th cells ($CD3^{+}4^{+}$) during and after surgery. Mean of surgery without ($n = 9$, control) or with CPB ($n = 12$) $\pm$ SEM. Blood samples were stimulated with PMA and ionomycin for 4 h in the presence of Monensin, and then stained for the surface antigens CD3 and CD4 and the intracellular cytokines IFN- γ and IL-4.

which is similar to that in adults (7), and elevated Th2 shift is related to POEE after CPB surgery.

The interpretation of the CPB response (with a highly specific IL-10 release) as a Th2 shift is further underlined by the increased postoperative total serum IgE serum levels with unchanged IgG2 levels. Postoperative increase of IgE indicates that CPB did not cause a general immune suppres-

sion, because humoral immune response is not suppressed but possibly even enhanced. Macrophage function could be reduced by IL-10-induced downregulation of HLA-DR expression and immunostimulatory cytokine production, with an increased susceptibility to infections (9).

Because of the predominance of an anti-inflammatory or Th2 response during CPB surgery, the question was

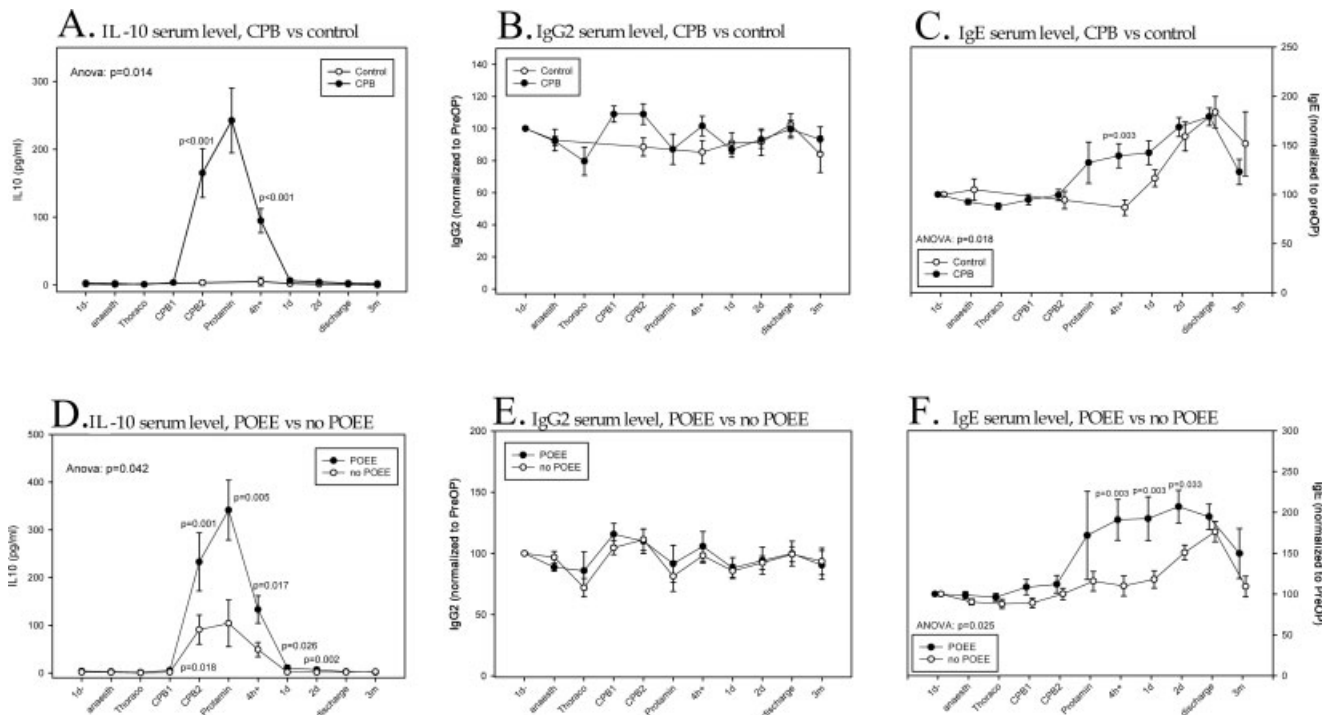


FIG. 2. Time course of the serum level of IL-10 and IgG2 and IgE ratio in patients with vs. without CPB (A-C) or with vs. without POEE (D-F). A,D: Serum IL-10 level is perioperatively significantly higher with CPB and with POEE. B,E: Serum IgG2 level is not different in CPB and control as well as POEE and control patients. C,F: Serum IgE level is early after surgery, significantly higher with CPB and with POEE. Data points show mean $\pm$ SEM, P -values indicate statistical comparison of the groups at the same time-point (Mann-Whitney U-Test).

raised if Th2 shift is associated with POEE. Our data indicate that in children, elevated IL-10 and IgE secretion are associated with POEE. The immediate (within hours) strong increase of IL-10 during CPB contact (possibly due to the rapid activation of IL-10 producing cells) in the POEE group shows an increased preoperative pool or pre-activation of these cells (2,3). The possibility of highly accurate and specific preoperative prediction of POEE risk may be related with the serological or cellular phenotypes, as we have shown earlier (2,3).

The higher increase of IgE after the surgery and increased amount of serum histamine levels preoperatively in POEE patients suggest an allergic atopic status (2,3). One of the key molecules that induce IL-10 release could be histamine, as it affects many of its cellular sources (10). Consequently, the elevated vascular permeability due to the increased histamine level and the release of IL-10 immediately after CPB may be responsible for developing POEE.

The specific immune response to CPB in children in our study is comparable with the earlier results in adults, with the immunosuppressive phase in trauma and sepsis (9,11). The cytokine IL-10 plays a central role in the immune sequel to CPB and it correlates with postoperative outcome, as reported earlier for children (11). It seems, therefore, feasible to assume that influencing IL-10 affects postoperative outcome. In light of these data, it seems questionable whether treatment strategies aiming to decrease proinflammatory response such as perioperative glucocorticoid administration are beneficial or not. Treatment with glucocorticoids or corticosteroids, both leading to increased IL-10 release during CPB surgery in adults, did in children not reduce POEE symptoms but prolonged postoperative bleeding (12).

In conclusion, our results show that the immune suppression/Th2 response is typical for CPB, and at intermediate level is tolerable but at high level could be unfavorable for the patients. Therefore, it seems useful to attenuate overshooting anti-inflammatory response to CPB with pro-inflammatory cytokines (9), and reduction or

avoidance of glucocorticoids or treatment for allergic symptoms may prove beneficial. Combining the presented results with preoperative prediction of POEE risk may open a new way for an early individualized therapy and preventive medicine (2–4). Furthermore, future has to show whether IL-10 release and Th1/Th2 disbalance are indicators or whether they are, in fact, the major inducers of the adverse response of the patient's immune system.

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