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Immunodepression after CPB: Cytokine dynamics and clinics after pediatric cardiac surgery – A prospective trial

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ABSTRACT

Background: Corrective surgery for congenital heart defects is known to trigger a severe immune reaction. There has been extensive research on the effects of inflammation after cardiopulmonary bypass (CPB). Interestingly, monocytes are observed to be non-responsive to stimulation with lipopolysaccharide (LPS) under these conditions, indicating a state of immunodepression, which lays the ground for second hit infections after cardiosurgery with CPB.
Objectives: The aim of this prospective study was to analyze immunodepression after pediatric cardiopulmonary bypass and to differentiate the effects of monocytic anergy on postoperative outcome.
Methods: In a prospective trial, we quantified the immune responses in 20 pediatric patients (median age 4.9 months, range 2.3–38.2 months; median weight 7.2 kg, range 5.2–11.7 kg) with congenital ventricular septal defect undergoing heart surgery with CPB. Ex vivo LPS-induced protein expression of IFN-γ, IL-1β, IL-1Ra, IL-6, IL-8, IL-10, IL-12, IL-17, TNF-α, and MCP-1 was measured before (T1), immediately after (T2) and 4 h after (T3) cardiopulmonary bypass surgery using Luminex technology.
Results: The innate immune system responds to CPB with an almost complete depression of monocytic function. Inflammatory IL-12, TNF-α, IL-1β, IL-6, IL-8 and IFN-y are completely suppressed. IL-10, IL-1Ra and MCP-1 are still produced during suppression with IL-1Ra being overly secreted during reversion. Suppression of TNF-α expression after LPS-stimulation correlates closely with longer mechanical ventilation time ($r = -0.619$, $p = 0.004$).
Conclusion: Cardiosurgery with CPB causes a state of immunodepression making pediatric patients more vulnerable to second hit infections. MCP-1, IL-10, and IL-1Ra play an important role in monocyte recovery, eventually permitting new therapeutic options for controlling immunodepression and inflammation. Standardized glucocorticoid therapy should be evaluated carefully for each individual patient.

1. Introduction

1.1. Background

The inflammatory reaction following the contact of blood with artificial surfaces, e.g. during cardiopulmonary bypass (CPB), has been studied extensively. Inflammatory cytokines have been shown to be predictive markers of postoperative complications such as infection [21,48], capillary leak syndrome [9,29], and organ failure [7,46]. Much less is known about the occurrence of immunodepression during and after cardiopulmonary bypass. Persistently low monocyte HLA-DR expression (an indicator of immunosuppression) is independently associated with mortality [31,7]. Inflammation and immunodepression are closely related; a primarily locally limited inflammatory response may escalate to a systemic inflammation or immunoparalysis [19,48]. However, only a few studies differentiate monocyte capacity during immunodepression after CPB.

1.2. Definition and pathophysiology of immunodepression

The concept of immunodepression reflects primarily the incapacity of monocytes to secrete cytokines and present antigens to T cells. Immunodepression has been observed after trauma [26,33], surgery [48,55], stroke [35], hemorrhage [57], and cardiac surgery with CPB [2]. While transient immunodepression can be understood as counterbalancing an inflammatory overreaction, persistent immunoparalysis

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has been shown to be an independent cause of infection, sepsis, and death [21,48].

Cytokines such as IL-10, prostaglandin E2 (PGE_2), transforming growth factor-β (TGF-β), haemoxygenase, catecholamines, and cortisol have been suggested as mediators of immunodepression. Anti-inflammatory IL-10 is presumed to initiate the auto regulation of an excessive inflammatory response [1]. IL-10 inhibits the DNA transcription of IL-1β, IL-6, and TNF-α [51] by arresting the signaling pathway from tyrosine kinase p56lyn to Ras [18]. PGE_2 produced by cyclooxygenase 1 and 2 is also associated with a reduced innate immune response. Elevated PGE_2 suppresses the expression of Ia antigens on the monocyte surface thereby increasing cAMP and reducing IL-1 expression on macrophage membrane [30]. The heat shock protein 32, also referred to as heme oxygenase 1, is also supposed to trigger immunodepression [44]. Some studies found that TGF-β may control inflammation [41], probably by inhibiting the lipopolysaccharide (LPS)-induced differentiation to dendritic cells, thus causing T-cell anergy [15].

Norepinephrine, epinephrine, and cortisol also bind to surficial monocyte receptors and inhibit pathways of pro-inflammatory cytokine genes (Bone (5)) [5]. While cortisol represses the pro-inflammatory cytokines IL-1, IL-6, and IL-8, it also activates anti-inflammatory IL-1Ra and IL-10. Cortisol in particular is thought to sustain a state of immunoparalysis, i.e. a complete and long-lasting immunodepression. Volk et al. indicate that methylprednisolone administered during CPB may aggravate immunodepression [49].

1.3. Methods of measuring immunodepression

There are two primary laboratory methods for measuring immunodepression. Generally accepted and widely used is the quantification of HLA-DR receptors on the surface of monocytes correlating closely with their antigen presenting capability to T-cells. A reduced expression of HLA-DR molecules indicates a decrease in monocyte functionality [13]. Although the method still lacks standardization, single and multicenter trial evidence show that flow cytometric measurement of HLA-DR expression is a good approximation for monitoring immunodepression [13]. The method used in this study measures the capacity of monocytes to perform protein biosynthesis after stimulation with LPS, thus permitting us to further differentiate the function of suppressed monocytes. The surface of monocytes bears a variety of receptors other than HLA-DR and a variety of cellular pathways regulate the monocytic immune response. Even in the state of immunodepression, the monocytic production capacity differs depending on the type of activation factor [16]. Accordingly, not all pathways are equally disrupted during suppression. Anergy of monocytes is certainly associated with the density of HLA-DR expression on the monocyte surface, but it is modified by additional surface antigens.

1.4. Function of selected cytokines

Monocytes are activated via a large variety of surface pattern recognition receptors (PRR), e.g. scavenger receptors for the binding of low-density-lipoprotein (LDL), mannose and glucan receptors for carbohydrates, and toll-like-receptors (TLR-2, TLR-4) for LPS from the outer membrane of Gram-negative bacteria. Activated monocytes produce cytokines, which can be vaguely subclassified according to their accentuated pro- (e.g. IL-1, IL-6, IL-8, IL12, and TNF-α) or anti-inflammatory (e.g. IL1Ra and IL-10) effect. Depending on the type of stimulus and stimulated target cells, the effects of cytokines differ widely. We chose to analyze an array of 10 pro- and anti-inflammatory cytokines and chemokines to represent monocyte function. Table 1 gives a summary of their origins and effects.

2. Subjects and methods

The study protocol was approved by the ethics commission of

Table 1
Description of selected cytokines.

Cytokine	Produced by	Target Cells	Immunological effects	Molecular-weight (kDa)
IL-1β	Monocytes, T-cells, B-cells, Natural killer cells (NKC), endothelium, epithelium	T and B cells, monocytes, granulocytes, hepatocytes	Pro-inflammatory; up-regulation of ICAM, ELAM,IL-6,IL-8;acute phase response; induces hematopoiesis [17]	19.348 (P01584)
IL-1Ra	Monocytes	Endothelium	Anti-inflammatory; antagonist for IL-1a- and IL-1β- receptor; induces no direct cellular response; up-regulation by IL-10; acute response protein [40]	22.055 (P18510)
IL-6	T- cells, B-cells, monocytes, endothelium	B-, T-, NK-cells,thymocytes	Pro-inflammatory; autoregulatory effects on TNF-α, IL-10, T-cells; induces B-cell differentiation [54,58]	23.718 (P05231)
IL-8	T-Zellen, monocytes, granulocytes	T-, B-cells, mast cells,thymocytes	Chemotaxis, recruitment and adhesion of neutrophils and monocytes to endothelium [11];	11.098 (P10145)
IL-10	T-cells, mast cells, ceratinocytesmonocytes,	T-, NK-cells, mast cells, monocytes	Downregulates function of TH1 and TH2 cells; antagonist of IL-12; induces IL-1Ra; enhanced by cortisol [51] and catecholamines [42]	22.517 (P22310)
TNF- α	Monocytes, T-cells, NKC,	Monocytes, granulocytes, vascular endothelium, nucleated cells	Pro-inflammatory cytokine; induces apoptosis [10]	25.644 (P01375)
MCP-1 (CCL2)	Endothelium, monocytes	T-, B- NK-cells, thymocytes, monocytes	Chemotaxis of monocytes (from bone marrow) and basophils to site of inflammation; activation of leucocytes, chemotaxis and adhesion [12]; activated by PDGF; up-regulated during sepsis [59]	11.025 (P13500)

Data on molecular weights origin from the protein database uniprot/proteinknowledgebase.org.

Charité-Universitätsmedizin Berlin (EA/082/07). Written informed consent was obtained from all parents before study enrollment.

2.1. Subjects

Between May 2008 and November 2009, 20 pediatric patients (body weight range 5.2–11.7 kg) with congenital ventricular septal defect undergoing surgical closure on CPB were enrolled in the trial. All subjects were simultaneously enrolled in a randomized controlled trial to examine the influence of CPB temperature on immune response [43]. Excluded were patients with cardiac anomalies with a significant hemodynamic effect such as pulmonary valve stenosis, tetralogy of fallot and univentricular heart physiology.

2.2. Study design

The study was performed at the German Heart Institute Berlin in Berlin, Germany. Preoperative, surgical, and postoperative management have previously been described in detail [43]. Briefly, all patients older than six months were premedicated with midazolam (0.5 mg/kg PO). Anesthesia was induced with sulfentanil (1–2.5 μg/kg/h), 0.2% etomidate (0.2 mg/kg), pancuronium (0.1 mg/kg) and maintained with sulfentanil (0.8 μg/kg/h) and diprivane (5 mg/kg/h). All patients received a urinary catheter, one peripheral venous catheter, a central venous catheter, and a femoral or radial arterial cannula.

Elective closure of the VSD was realized through a median sternotomy under CPB with antegrade intermittent cold crystalloid cardioplegia. Standarized CPB using polyvinyl tubing, roller pumping (mast-mounted pump, Stöckert Instruments, München, Germany), and a hollow-fiber membrane oxygenator (Capiox RX05, Terumo cooperation, Tokyo, Japan) was performed. The extracorporeal circuit was primed with a balanced electrolyte solution (Ionosteril, Fresenius Kabi, Bad Homburg, Germany). Heparin was administered at a rate of 3 L/m^2 body surface area. In 15-patients, a Gore-Tex patch (Bard Souvage, Tempe, AZ, USA) was placed to close the VSD, and in five patients, the VSD was directly closed without a patch.

Persistent cardiac defects were observed in three subjects, which were immediately repaired during the same operation (patch enlargement of the pulmonary artery, mitral valve reconstruction, and revision of VSD suture because of aortic valve insufficiency).

After the operation, all patients were transferred to the intensive care unit in stable hemodynamic condition. Piritramide (0.1 mg/kg) and metamizole (10 mg/kg) were administered for analgesia. Three patients required longer ventilation times (24 h, 72 h and 74 h) and were therefore, sedated with midazolam (3–3.9 μg/kg/min). Extubation criteria were defined as PaO_2 > 80 mmHg, FiO_2 of 0,4 or $PaCO_2$ of 35–40 mmHg during CPAP.

2.3. Blood sampling protocol and laboratory method

Using the Instant Leukocyte Culture System (ILCS) (EDI/Myriad RBM, Austin, Texas, USA) whole blood samples were collected from the central venous line immediately after the induction of anesthesia (T1), immediately after weaning from CPB (T2), and 4 h after weaning from CPB (T3). Complete blood counts, blood chemistries, and coagulation studies were performed the day before surgery (baseline), after admission to the intensive care unit, and during the first postoperative day. The ILCS method allows for an immediate snapshot of the functionality of the monocytes. Without centrifugation, the samples were stimulated using standardized LPS (100 ng/ml type E. coli, O55:B5). The stimulated samples were incubated at 37 °C for 24 h. The LPS dose was determined based on an experimentally tested dose-effect curve with submaximal activation of the monocytes. The stimulation was halted by pressing down on a filter membrane to separate the supernatant from the cells. The concentrations of IFN-γ, IL-1β, IL-1Ra, IL-6, IL-8, IL-10, IL-12, IL-17, MCP-1, and TNF-α were measured using a particle-based immunoassay with fluorescent labeling (MPXHCYTO-60 K, Millipore, Billerica, MA, USA) and detected by flow cytometry (Luminex Corp, Austin, TX, USA) as previously described [43]. As the half-life of all selected serum cytokines are about 60 min or below, it can be presumed that cytokines secreted *in vivo* have disappeared at the time of measurement.

2.4. Study outcomes

As primary clinical outcome the changes in cytokine levels after incubation with LPS were observed. Secondary outcome was the duration of mechanical ventilation (time of intubation before surgery until extubation in the ICU).

2.5. Statistical methods

All data was tested for normal distribution using the Kolmogorov-Smirnov and Shapiro-Wilk test. Cytokine kinetics was analyzed using the Wilcoxon test for non-parametric analysis with repeated measures. Results are shown as box and whisker blots. Due to large interindividual differences in cytokine production after stimulation with LPS, the correlation analyses focuses entirely on ratios, i.e. on kinetics of cytokine concentrations T2/T1, T3/T2 and T3/T1. The non-parametric correlation analysis is based on Spearman. Results are shown as bar diagrams. Values were considered statistically significant when the p-value was less than 0.05. Data was analyzed using SPSS 21.0 and MS Excel 2007.

3. Results

3.1. Demographic profile and operative data

The demographic profile of all patients, blood test results, and the intraoperative characteristics are summarized in Tables 2, 3, and 4, respectively. None of the patients had been mechanically ventilated before the operation. None showed clinical symptoms of pneumonia or elevated CRP and there was no case of postoperative paralysis of the diaphragm (phrenoplegia). Median duration of VSD closure operation was 123 min (72–300 min), median duration of CPB was 71 min (33–154 min).

3.2. Kinetics of immunodepression: decrease of responsiveness to LPS

3.2.1. Time of measurement T1 (preoperative): complete monocyte capacity

Preoperatively (T1), we noted considerable interindividual differences in the responsiveness of monocytes to LPS stimulation. IFN-γ concentrations differ up to 350-fold (668 pg/ml, range 24–8448 pg/ml). LPS induced a strong burst of IL-6 (9906 pg/mL, range 4346–35,105 pg/mL), IL-8 (9497 pg/mL, range 4279–40,000 pg/mL) and MCP-1 (9165 pg/ml, range 5922–15,000 pg/mL) secretion, but comparatively weaker secretion of IL-1Ra and IL-10 (452 pg/ml, range 77–1768 pg/mL; 451 pg/mL, range 110–1494 pg/mL), respectively. Differences in the responsiveness to LPS did not correlate with differences in age, weight, CRP, leukocyte count, or body temperature.

Table 2
Demographic profile of children.

Demographic data (median and range)	
Age (month)	4.9 (2.27–38.2)
Weight (kg)	7.2 (5.2–11.7)
Body surface (m^2)	0.36 (0.29–0.51)
Gender (m/f)	12/8

Table 3
Operative data.

Operative data (median and range)	
OP time (min)	123 (72–300)
CPB-time (min)	71 (33–154)
Cross clamp time (min)	47(18–98)
Cardioplegic solution (ml/kg)	84 (30–200)
Fluid balance CPB (ml/kg)	18 (−8–49)

Table 4
Blood sample measurements.

1st blood drawing (preoperative) (means and SDs)	
Hemoglobin (g/dl)	11.90 ± 1.0
Leukocytes (*10^9/l)	13.90 ± 6.5
Monocytes (*10^9/l)	1.10 ± 0.53
CRP (mg/l)	0.16 ± 0.24
2ndblood drawing (h after CPB)	1.25 ± 0,57
Hemoglobin (g/dl)	10.36 ± 2.91
Leukocytes (*10^9/l)	15.95 ± 5,56
Monocytes (*10^9/l)	1.15 ± 0.55
Neutrophils (*10^9/l)	10.00 ± 4.50
CRP (mg/l)	4.08 ± 2.62
3rd blood drawing (h after CPB)	15.3 ± 4.21
Hemoglobin (g/dl)	12.14 ± 3.00
Leukocytes (*10^9/l)	14.40 ± 4.28
Monocytes (*10^9/l)	1.91 ± 0.89
Neutrophils (*10^9/l)	10.50 ± 3.70
CRP max (mg/l)	7.89 ± 3.54

3.2.2. Time of measurement T2 (end of CPB): immune depression

Immediately after weaning from CPB, monocytic responsiveness to LPS was significantly suppressed ($p < 0.05$ for T2/T1) concerning all cytokines with the exception of IL-17 ($p = 0.056$). In contrast to the observation of large differences in reaction to LPS at T1, the kinetics of T2/T1 were relatively similar amongst the pediatric patients. We noted a shift from inflammatory to immunosuppressive cytokines for all children (Fig. 1).

3.2.3. Time of measurement T3 (4 h after CPB): paralysis and regeneration

Approximately four hours after CPB, we noted a reversion of total suppression (T3 significant higher than T2) for IL-6 ($p = 0.008$), IL-8 (0.002), IL-1Ra ($p = 0.0001$), TNF-α ($p = 0.001$), and MCP-1 ($p = 0.001$). Monocytes showed full recovery for MCP-1 and IL-1Ra secretions. IL-1Ra concentrations increased 3-fold in comparison to T1 values, and was the only cytokine of our array analysis to immediately

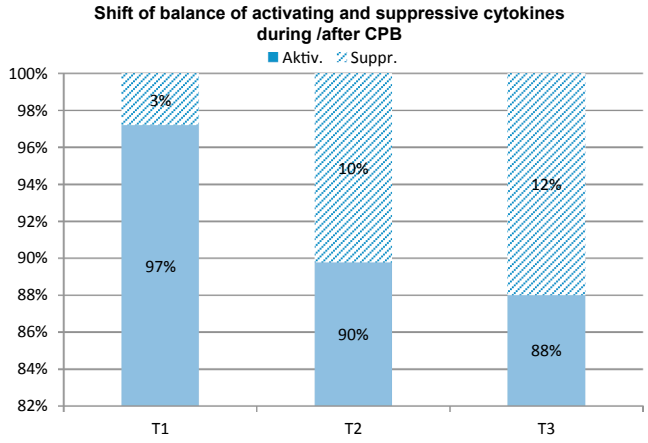

Fig. 1. Shift of balance of selected activating/suppressing cytokines after LPS stimulation during/after CPB. Suppressive: IL-10, IL-1Ra. Activating: IL-1β, IL-6, IL-8, TNF-α. Data based on cytokine concentrations in nmol/ml. Molecular weights are found at uniprot/proteinknowledgebase.org (see Table 1).

increase after CPB. Monocytes tended to recover for IL-8, IL-17, and IL-10, reaching concentrations of 50% or higher compared to initial values. During this relatively short observation period T1–T3, monocytes remained suppressed for IFN-γ, IL-1β, IL-12, and TNF-α secretions (T3/T1 below 10%, $p < 0,05$) (see Fig. 2).

3.3. Cytokine response patterns

In Fig. 3, cytokine dynamics are summarized during paralysis and recovery. The reversion of immunodepression 4 h after CPB is represented by the ratio of T3/T1 on the x-axis. The reduction of monocyte responsiveness is represented by the T2/T1 as shown on the y-axis.

Our analyses suggested an essential role of anti-inflammatory cytokines IL-1Ra, IL-10, and MCP-1 during recovery from SIRS and immunodepression. IL-10 was not totally suppressed and IL-1Ra was overly produced during recovery. Monocytes were suppressed for IL-12, IL-6, TNF-α, IL-1β, and IFN-γ during T2 through T3. MCP-1 and IL-17 were partially inhibited during surgery (T2) and MCP-1 approached already it's initial value at T3.

3.4. Clinical outcome

Our data reveal a close correlation between immunodepression of TNF-α ($r = -0619$, $p = 0004$) and length of mechanical ventilation (median 16 h, 4–75 h). Whereas serum cytokines (not focus of this paper) show high significance with the duration of CPB (IL6_serum_2 $r = 0764$, IL1-Ra_serum_4 $r = 0730$, IL10_serum_2 $r = 0717$, IFNy_serum_2 $r = 0584$, all $p < 0,01$; IL8_serum_2 $r = 0528$, $p < 0,05$), the statistics are less conclusive for cytokine levels after LPS. In essence, neither the duration of CPB nor aortic cross clamp time show a significant association with the deactivation of monocytes. However, there are few exceptions for individual cytokines: IFN-γ correlates positively with operation time ($r = 0470$, p 0036), CPB time ($r = 0466$, p 0038) and aortic clamp time ($r = 0559$, p 0010), not however with CK-mb ($r = 0104$, p 0663). The situation is different for CK-mb as an indicator for surgical trauma. IL-6 and TNF-α are significantly negatively associated with CK-mb (I IL6_T2/T1 $r = -0486$, p 0030; $r = -0568$, p 0009, respectively), not however with operation time, CPB time or aortic clamp time (IL6_T2/T1: $r = -0298$, p 0201; $r = -0098$, p 0682; $r = -0153$, p 0518; TNF-α_T2/T1: $r = -0298$, p 0201; $r = -0436$, p 0055; $r = -0429$, p 0059, respectively).

4. Discussion

In this study, we showed that cardiosurgery with CPB induces immunodepression with a tendency towards recovery after termination of CPB (T2), when the minimum monocyte capacity was measured. The design of our study (all patients operated on CPB) does not allow further conclusions, e.g. whether the kinetics of IL-1Ra, IL-10 and MCP-1 are similar for operations without CPB or for the reversion of immunodepression in general. We also observed a high variability of cytokine burst after LPS stimulation, which has been described earlier [37]. Duval et al. showed that monocytic response for IL-6, IL-8, and IL-1Ra to LPS stimulation was dependent on the complexity of the congenital heart defect [14]. Moreover, genetic polymorphism is also accountable for the variability in cytokine release as previously described by Boehm et al. for TNF-α release in response to LPS after CPB that was independent of a promoter variant [3]. Correspondingly, variability of up to 10-fold in TNF-α secretion due to genetic polymorphisms has been measured [47]. Another possible reason for the variability of cytokine secretion is the age of the patient. In a cross-sectional study on healthy newborns, infants, and young adults, Härtel et al. demonstrated that cytokine response correlates differentially with age [22]. Our evaluation did not show a relationship between weight or age with cytokine capacity after LPS stimulation. In our study cohort,

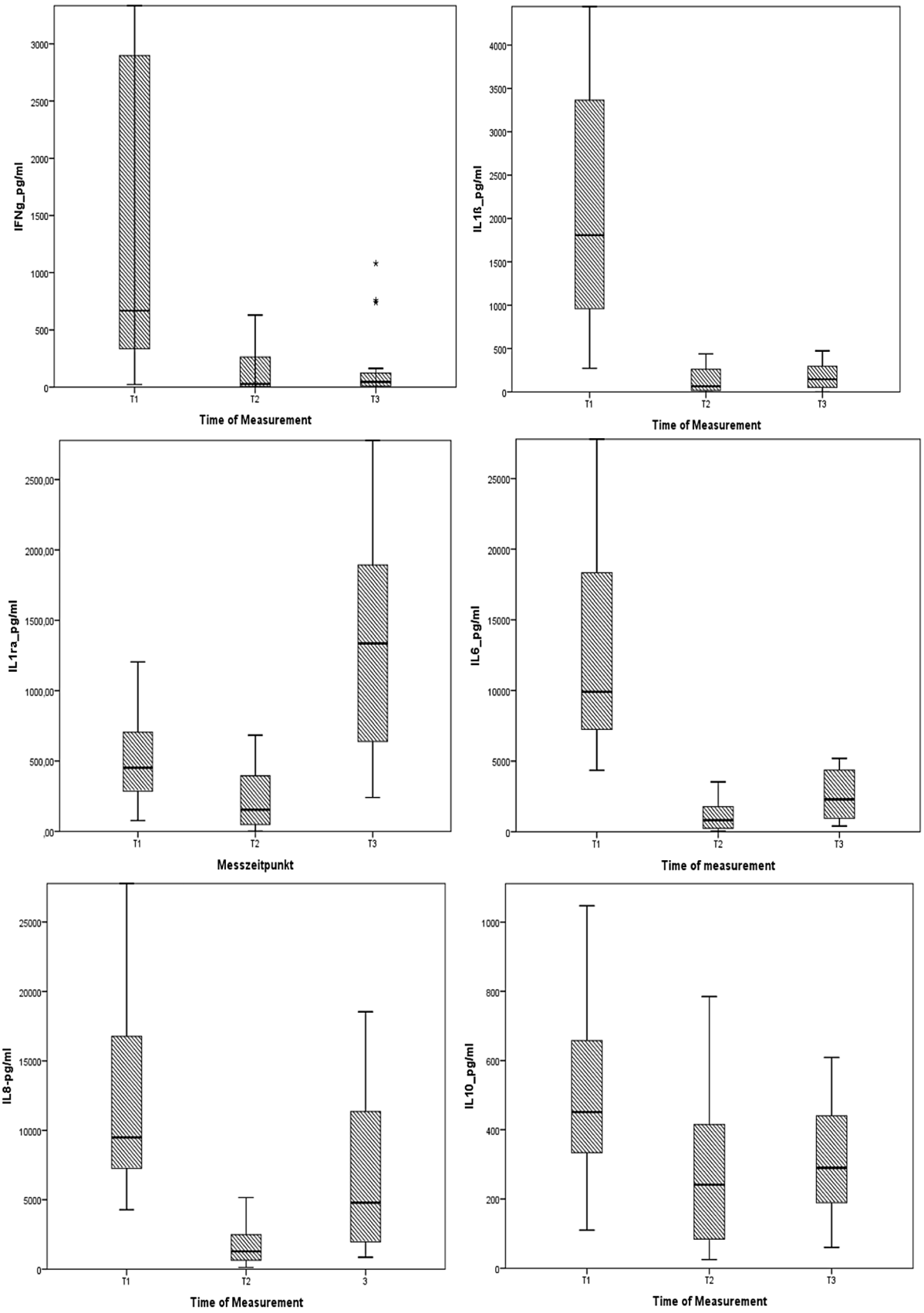

Fig. 2. Ex vivo cytokine secretion after LPS stimulation.

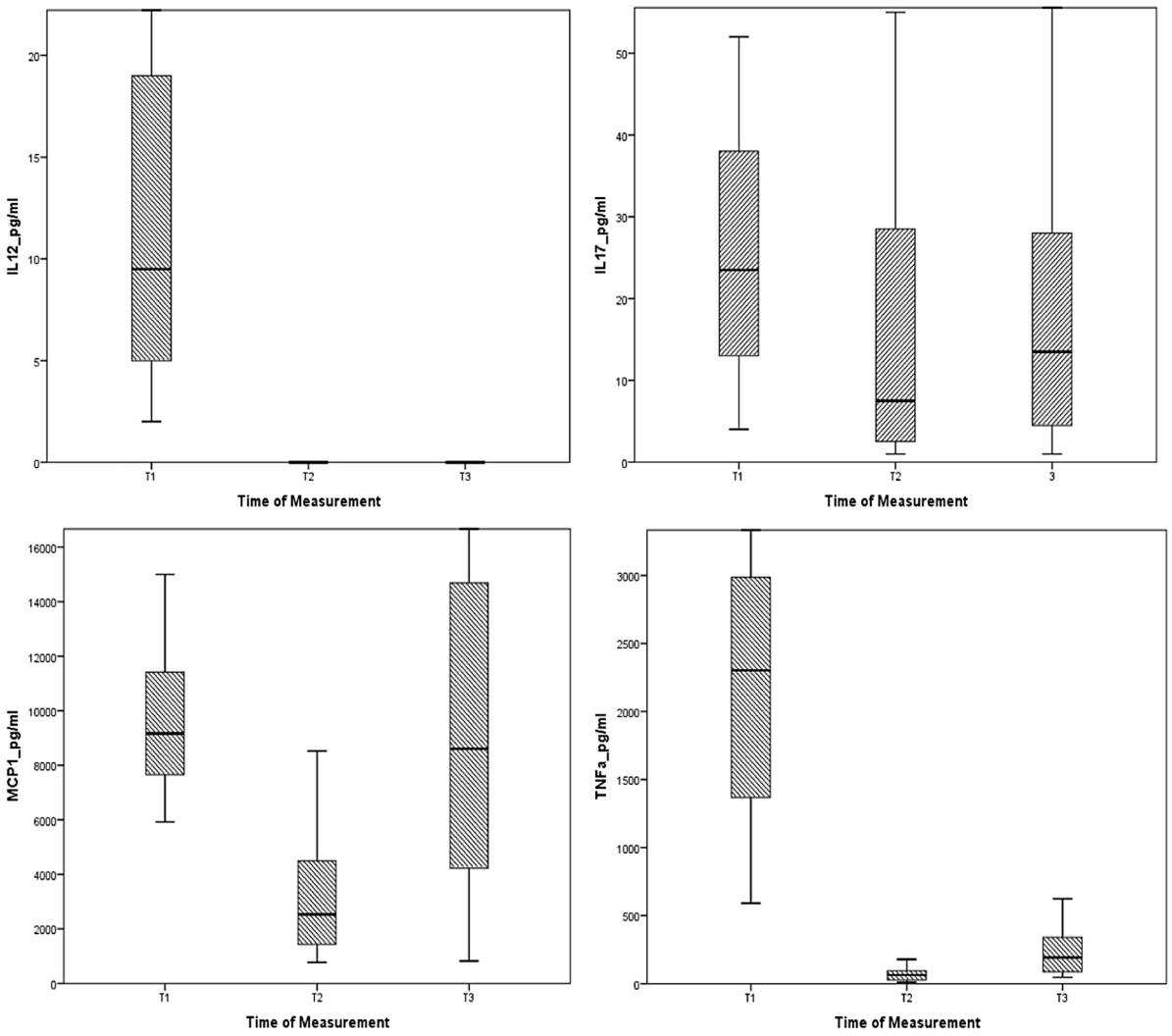

Fig. 2. (*continued*)

the immune responsiveness to LPS in T1 (before anesthesia), the degree of monocytic anergy (immune suppression) in T2 and T3, as well as the level of plasma cytokine levels (immune activation) have shown to be independent from the age of our patients. This result parallels the scientific evidence that cytokines are part of the innate immune system [23]. However, we cannot conclude age independence of immune responsiveness other than for our cytokine panel. Associated with younger ages were longer CPB durations ($r = -0.504$, $p = 0.024$) and longer periods of mechanical ventilation after surgery ($r = -0.541$, $p = 0.014$), due to the additional operative complexity associated with lower body weight. Age itself, however, did not have any correlation with a higher cytokine release after LPS stimulation or higher *in vivo* baseline values before surgery.

Activating cytokines: The suppression of activating cytokines after pediatric CPB has been observed relatively uniformly. In contrast to immunoparalysis during sepsis, immunosuppression after CPB is described as transient but bears the risk of aggravating a second hit [8,32,24,27], which is in line with our results. Especially, TNF-α has been observed to remain suppressed, indicating ongoing immunodepression. Deactivation of IL-1β, IL-6, and IL-8, and a rise in IL-10 after CPB has been observed by Grundmann et al. [20]. Similar results were obtained by Hensler et al., who reported a rise in IL-10 and concurrent down regulation of IL-1, IL-6, and IL-8 following major surgery [56]. Li et al. reported in a study on pediatric patients undergoing cardiac surgery a suppression of inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8 and a simultaneous increase in IL-10 after incubation with LPS [32].

Suppressive cytokines: Suppressive IL-10 has been suggested to down-regulate excessive immune activation. In the current study, monocytic capacity for IL-10 was relatively stable (40% decrease), i.e. the degree of suppression did not depend on the duration of CPB. This aspect is assessed differently in the literature. In accordance with our results (no correlation between IL-10 *in vivo* and IL-10 concentrations after LPS stimulation) Döcke et al. found a limited effect of IL-10 on immunodepression, similar to the findings of Volk et al. [50]. Meisel and his team did not detect IL-10 during immunodepression after stroke, and suggest a burst of acetylcholine as the trigger of suppression [35]. In contrast, Kawasaki et al. and Tarnok et al. suspect an immediate increase of IL-10 and a simultaneous suppression of TNF-α as the cause of immunoparalysis during surgery. Investigating patients undergoing CPB, Cornell et al. stated a twofold increase in IL-10 in patients suffering from immunoparalysis (median IL-10 = 7.12 pg/ml versus 3.93 pg/ml in the immunocompetent group, $p = 0.03$) [8]. In our study, there are supposedly other overlying factors causing the strong depression or concealing the effect of IL-10 (e.g. cortisone).

We measured a transient decline of IL-1Ra during CPB (T2), followed by a threefold increase four hours later (median 452 pg/ml at T1, 154 pg/ml at T2, 1336 pg/ml at T3). Only a few studies have addressed the mechanism of IL-1Ra during immunodepression after cardiosurgery with CPB. Cornell et al. observed no differences in the secretion of IL-1Ra after LPS stimulation in both immunocompetent and immunodepressed groups [8]. Others measured significantly elevated

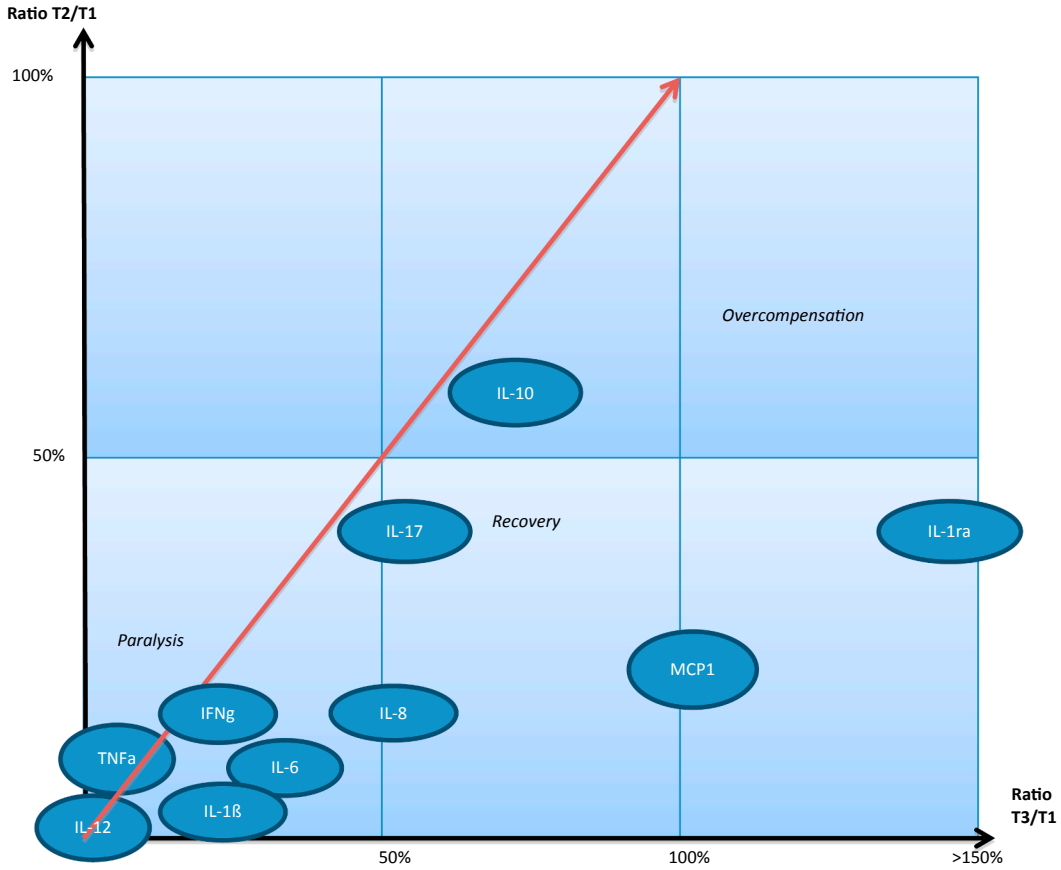

Fig. 3. Secretion patterns of cytokines after LPS stimulation in relation to base values (T1) T2/T1 ratio of mean cytokine concentrations representing immunodepression and T3/T1 its reversion.

IL-1Ra concentrations after CPB *in vivo*, however, they did not examine monocytic function after stimulation with LPS [34]. Tanzi et al. examined *in vivo* IL-1Ra concentrations of patients after stroke and suggested that IL-1Ra elevation correlated closely with the risk of infection after stroke [45].

Clinical outcome: in this study, even transient immunodepression matters: TNF-α and mechanical ventilation time are closely negatively correlated ($r = -0619$, $p = 0004$). When searching for perioperative triggers of immunosuppression, we find that surgical trauma as measured by CK-mb is closely associated with the suppression of IL-6 and TNF-α, not or to a fewer extent with operation time, CPB time or aortic clamp time. We found very few publications addressing immunodepression after pediatric cardiosurgery with CPB. Some studies measured the relevance of persistent immunodepression after surgical trauma [52,19, adults]. Other authors contributed immunodepression mainly to CPB and did not differentiate between other perioperative triggers [20].

Concurrence of inflammation and immunodepression: Equivalent measurements of *in vivo* plasma concentrations at T1-3 and 24 h after CPB (T4) were used for comparison of *in vivo* and *ex vivo* (LPS stimulated monocyte production) cytokine concentrations. As expected, we observed that the induction of immunodepression was accompanied by early onset inflammation, as seen in an increase of pro-inflammatory cytokines *in vivo* (data published in [43]). While CPB duration did not correlate with the deactivation of monocytes with the exception of IFNy, it was closely related to higher *in vivo* cytokine concentrations (IL-6, $r = 0.764$; IL-1Ra, $r = 0.730$; IL-10, $r = 0.717$; IFN-γ, $r = 0.584$; $p < 0.01$).

We detected the induction of inflammation as well as suppressed monocyte responsiveness during the first two hours of cardiac surgery (Fig. 4).

The presented findings do not comply with the concept of compensatory anti-inflammatory response syndrome (CARS) introduced by Bone in the late 1990s [4], referring to the systemic deactivation of the immune response following a severe infection as a mechanism to restore immune homeostasis by HLA-DR down regulation [25]. A subsequent immune response following cardiothoracic surgeries has also been described, presumably initiated by IL-10 induction [5].

Our findings are more consistent with analysis of gene expression of anti- and pro-inflammatory cytokines after stimulation with endotoxins. Here, any inflammatory immune response seems to be accompanied by parallel anti-inflammation [53]. In a similar way, Osuchowski et al. refuted a sequential SIRS-CARS theory as a model of early sepsis. Their results are comparable to our findings in the sense that anti-inflammatory (IL-10 and IL-1Ra) and pro-inflammatory (MCP-1, TNF-α, IL-6, IL-1β) mediators are induced simultaneously [38]. Likewise, Duval et al. reported a parallel activation of *in vivo* cytokines and decline in monocytic capacity after pediatric cardiac surgery [14].

Clinical Implications: The presented data stating immunodepression in children undergoing cardiopulmonary bypass implies that immunosuppressive therapies with glucocorticoids before or during pediatric cardiac surgery should be evaluated carefully. Glucocorticoids are widely used in pediatric CPB surgery with the intention to attenuate SIRS, likewise in our center. However, it's application in preventive means is controversial as the influence on clinical outcome is inconclusive and an increase of side effects such as second hit infections and hyperglycemia assumed [39]. Our data support the notion that steroids in pediatric cardiac surgery must be used critically as it might aggravate or even cause the described immunodepression in the current study. A prospective placebo controlled trial investigating the effects of glucocorticoids on clinical outcome and immunodepression is urgently needed.

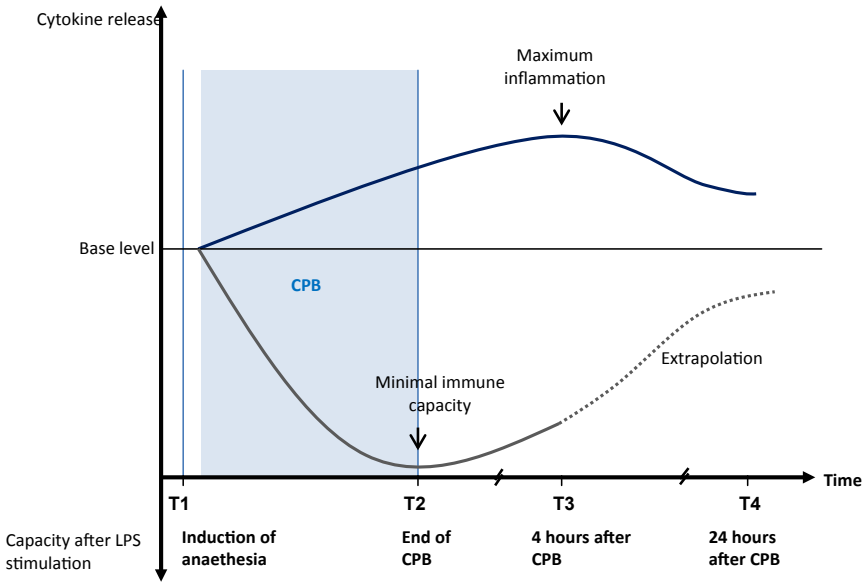

Fig. 4. Concurrence of inflammation und immune suppression during and after (values are extrapolated) upper line: over all measured cytokine concentrations in whole blood samples [43]; lower line: whole cytokine concentrations measured in instant leukocyte culture systems 24 h after LPS stimulation simulating monocyte activation.

Our data confirm the complexity of immunomodulatory therapy. During recreation IL-1Ra is produced disproportionately. Subcutaneously applied IL-1Ra has been shown to reduce markers of inflammation, e.g. in the setting of acute coronary syndrome [36]. Dependent on the confirmation of IL-1Ra as a dominant player for reversion of immunodepression by future studies, recombinant IL-1Ra might open new possibilities for treating excessive inflammation.

4.1. Limitations

The LPS model for innate immune capacity: LPS is a valid stimulant taking into account that a burst of inflammatory cytokines and complement factors increase the permeability of the intestinal wall for gram-negative endotoxin. Endogenous LPS has been shown to represent a serious risk factor for cardiac surgery patients [28,6]. The validity of monocyte responsiveness as a model for immune suppression has been confirmed in various publications [24,55]. However, this method has several limitations: The immune response induced is LPS-specific, i.e. it focusses to a large extent only on monocytes. LPS is not standardized in terms of the volume applied, target activation values, the type of LPS, the usage of various other endotoxins, as well as the duration and temperature of incubation. Interindividual differences in monocyte responsiveness to LPS are enormous and up until now, no reference values are available.

Time-Temperature protocol of measurements: Concerning this study, additional subsequent measurements of monocyte function would have been useful: a trend to the reversion of suppression for many interleukins is recognizable, still not statistically significant. “Second hits” could have been missed in the following days after surgery. However, both clinical course and T4 measurements *in vivo* after 24 h suggest that immune activation and suppression have both been reversed.

As the children were simultaneously enrolled in a study to investigate the effects of hypothermic- (32 °C) in comparison to normothermic- (36 °C) CPB, intraoperative temperature varied within our study population [43]. To the best of our knowledge, no data on the effects of hypothermia on monocyte capacity in a clinical setting exists. Although we did not observe any effect of CPB temperature on cytokine secretion by monocytes in the limited analysis with a small population of 10 subjects per group (data not shown), under-estimating the effects of temperature cannot be ruled out. However, we demonstrated previously that CPB temperature had no significant consequence on *in vivo* cytokine concentrations [43].

5. Conclusions

Cardiosurgery with CPB significantly reduces the capacity of monocytes to respond to antigen stimulation. Whereas the clinical symptoms of inflammation are more easily detected, the emergence of concurrent immunodepression can be easily missed. Our study suggests a critical role of cytokines IL-1Ra, IL-10, and MCP-1 during recovery from SIRS and immunodepression. Because suppression of monocytic production of TNF-α *in vitro* relates to the need for prolonged mechanical ventilation, standardized glucocorticoid therapy should be evaluated carefully for each individual patient as it might aggravate immunodepression.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cyto.2017.03.017.

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