Follow-up papers – Experimental

Changes in the cytokine network and complement parameters during open heart surgery

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Abstract

Objective: During cardiac surgery with cardiopulmonary bypass (CBP) there is a systemic inflammatory reaction, involving enhanced release of inflammatory cytokines and complement. However, few studies have analysed the levels of anti-inflammatory mediators and chemokines after CPB. In this study we investigated the complexity of the cytokine network particularly focusing on the balance between interleukin (IL)-10 and inflammatory cytokines and chemokines. Methods: Blood samples from 20 patients (seven females; 13 males, age 30–81 (median 65) years) who underwent CPB, were collected before, and at several time points after surgery, and analyzed for plasma levels of inflammatory and anti-inflammatory cytokines and parameters of complement activation. Results: A marked increase in the anti-inflammatory cytokine IL-10, rather than in inflammatory cytokines, characterized the initial phase after CBP. As for the early inflammatory response the most prominent feature was a rise in the inflammatory chemokines IL-8 and monocyte chemoattractant protein-1, while the increase in tumor necrosis factor-α was rather modest. In contrast to the rapid ‘rise and fall’ in most of the markers, significantly raised IL-6 levels persisted throughout the study. Immediately after CPB there was also a marked increase in complement activation, with return to baseline levels on the first postoperative day. Conclusion: The present study shows a complex pattern of changes in the cytokine network and complement parameters during CPB with a marked rise in both inflammatory and anti-inflammatory mediators. However, in contrast to cytokine pattern during various infections, the initial phase after CPB was dominated by a marked rise in anti-inflammatory cytokines (i.e. IL-10).

Keywords: Inflammatory response; Cardiopulmonary bypass; Cytokine; Complement

1. Introduction

The occurrence of a systemic inflammatory response during cardiac surgery and cardiopulmonary bypass (CPB) has been well established, and the heart itself has been shown to release inflammatory mediators following ischemia [1]. The inflammatory reaction during CPB seems to involve several innate immunity responses such as the cytokines (e.g. tumor necrosis factor (TNF)-α and interleukin (IL)-8) and complements [2,3]. Notably, these responses have also been implicated in the development of postoperative morbidity after CPB including infectious complications and organ dysfunction [4]. In fact, inflammatory cytokines such as TNF-α, IL-1 and IL-6 have been found to promote cardiac failure in various animal models [5]. However, while several studies have analysed the concentrations of inflammatory cytokines such as TNF-α and IL-6, fewer studies have examined levels of anti-inflammatory mediators, e.g. IL-10. Hence, in several inflammatory disorders the potential pathogenic effect of inflammatory cytokines will depend on the balance in the cytokine network, particularly on the levels of counteracting anti-inflammatory mediators [6]. Moreover, although increasing evidences suggest an important role for chemotactic cytokines or chemokines during inflammation by directing and activating chemokines into inflamed tissue [7], the literature is virtually devoid of data on chemokines during open heart surgery.

We hypothesized that CPB is not only characterized by an increase in inflammatory mediators, but also by an anti-
inflammatory response and in the present study this hypothesis was investigated by differential experimental approaches, particularly focusing on the balance between IL-10 and inflammatory cytokines and chemokines.

2. Materials and methods

2.1. Patients and procedures

Twenty patients, seven females and 13 males, aged from 30 to 81 (median 65) years (SD ± 12.5) were included in the study. Seven patients underwent coronary artery bypass grafting (CABG), two patients valve replacement or plasty, seven patients combined CABG grafting and valve replacement or plasty, and four patients underwent ‘other procedures’ (i.e. resection of the ascending aorta and/or the aortic arc, eventually combined with aortic valve replacement or CABG). Two of the last four patients were operated in deep hypothermia and circulatory arrest (CHCA). All the operations were elective and all patients were in a stable clinical condition without any evidence of unstable angina or other acute conditions. None of the patients used immunosuppressive drugs prior to operation and none had any signs of clinical infection during the study period. For comparison, blood samples were also collected from 20 sex- and age-matched healthy blood donors. The regional ethical committee approved the study, and signed informed consent was obtained from each patient.

2.2. Surgical technique

In all patients, the operative approach was a median sternotomy with CPB and systemic hypothermia. Two patients were operated in deep hypothermia and circulatory arrest (‘other procedure’, see above). Crystallloid cardioplegia and topical cooling with ice slush were used routinely. All CPB circuits were heparin coated including the Spiral Gold Oxgenator (Baxter Health-Care Inc., Irvine, CA), immediately immersed in melting ice and centrifuged within 5 min at 1600 g for 10 min. Plasma was stored in multiple aliquots at −80 °C until analysis and was thawed only once.

2.3. Anesthesia

Anesthesia was induced with thiopentone, fentanyl and pancuronium, and continued with a mixture of isoflurane and fentanyl (Alpharma, Oslo, Norway). The patients were artificially ventilated with a mixture of nitrous oxide and oxygen and extubated in the intensive care unit. For postoperative pain relief, the patients were given morphine (Nycomed Pharma, Oslo, Norway) or ketobemidon (Pharmacia & Upjohn, Stockholm, Sweden), supplemented with paracetamol (Alpharma). Subcutaneous injection of low molecular heparin (Dalteparin, ‘Fragmin’, Pharmacia & Upjohn), was given daily after the operation. The patients received antibiotic prophylactics with three doses of intravenous cephalothin (Eli Lilly & Co., Indianapolis, USA) 2 g × 3 for at least 24 h and until all drains or monitor lines were removed.

2.4. Blood sampling protocol

Blood samples were collected from the radial artery after induction of anesthesia before surgery, at the end of the operation, 2 h after surgery and on the first and second postoperative day. Fourteen and 21 days after the operation, peripheral venous blood was collected. Blood (both arterial and venous) was collected into sterile EDTA tubes (Becton Dickinson, San Jose, CA), immediately immersed in melting ice and centrifuged within 5 min at 1600 g for 10 min. For all parameters, all samples from a given patient were analysed in the same microtiter plate to minimize run-to-run variability. The intra- and interassay coefficients of variation were < 10% for all EIAs.

2.5. Cytokine and complement analysis

IL-6, IL-8 and monocyte chemoattractant protein (MCP)-1 were measured by enzyme immunoassays (EIAs) as recommended by the manufacturer (R&D Systems, Minneapolis, MN). TNF-α and IL-10 were quantified by an EIA (BioSource Europe, Nivilles, Belgium) [8]. Stromal cell-derived factor-1α (SDF-1α) was analyzed by EIA as described by Damás et al. [9].

The complement activation products C3bc (i.e. the sum of C3b, iC3b and C3c) and TCC (the terminal C5b-9 complement complex) were measured by EIA, based on neoepitope-specific monoclonal antibodies to the activation products as previously described [10,11]. The results are given in arbitrary units (AU) based on a standard of normal human serum activated with zymosan.

For all parameters, all samples from a given patient were analysed in the same microtiter plate to minimize run-to-run variability. The intra- and interassay coefficients of variation were < 10% for all EIAs.

2.6. Statistical analyses

Differences between groups were analysed using the Mann–Whitney U-test. Differences between more than two groups were analysed by the Kruskal–Wallis H-test followed by the Mann–Whitney U-test. The Friedman test was used for repeated measurements. Wilcoxon’s signed-rank test was used to compare individual time points with baseline. In some comparisons the total cytokine or complement response during the entire study period was calculated as area under the curve (AUC) for each patient. The P-values are two-sided and considered significant when less than 0.05.
3. Results

3.1. Cytokine and complement parameters at baseline

As shown in Fig. 1, prior to CPB the patients had significantly increased levels of IL-6, IL-8 and TNF-α compared to healthy controls, with particularly high IL-6 levels. In contrast, IL-1β was significantly decreased in these patients at baseline (Fig. 1). No changes were seen in complement parameters, MCP-1, SDF-1α or IL-10 levels comparing healthy controls and preoperative values in these patients (Fig. 1).

3.2. Cytokine levels after CPB

Several significant patterns were revealed during operation (Fig. 1). First, there was a marked (~50-fold) and rapid increase in the anti-inflammatory cytokine IL-10, with maximum levels immediately after the operation, reaching preoperative levels after two days. Also SDF-1α showed a rapid increase, followed by a return to baseline levels after 1–2 days, but in contrast to IL-10 the levels were comparable to concentrations in healthy controls throughout the observation period. Secondly, also the chemokines IL-8 and MCP-1 showed a rapid increase after operation, but the rise was more modest (~6-fold increase), and for most of the patients the maximum levels was reached 2 h after operation. Thirdly, also IL-6 showed a marked increase (~4-fold), reaching maximum levels 2 h after the operation. However, in contrast to IL-10, IL-8 and MCP-1, these high levels persisted until 2 days after operation, and even after two weeks, IL-6 was (~2-fold) increased compared with baseline levels. Also IL-1β showed a ‘IL-6-like’ pattern reaching maximum level 2 h after operation (~3.5-fold increase), but in contrast to IL-6, the IL-1β levels were only slightly elevated compared to concentrations in healthy controls throughout the study period, reflecting decreased levels prior to operation. TNF-α levels showed only minor changes during operation with a slight, but significant increase (~1.3 fold) 2 h after the operation.

3.3. Complement parameters after operation

During CPB there was also a marked and almost linear increase in TCC (~4.5-fold) and C3bc (~7-fold), reaching maximum levels immediately after the operation, returning to baseline level on the first postoperative day (Fig. 2). However, for both C3bc and TCC there was also a moderate but significant increase during the second and third postoperative week (Fig. 2).

3.4. Cytokine and complement levels in relation to clinical variables

The present study population represented a somewhat heterogeneous group of patients with regard to variables such as ages, type of operation and CPB duration time. However, while we found no relationship between cytokine levels and ages or CPB duration time, patients undergoing ‘other procedures’ (n = 4, see Section 2) had higher levels of TCC and IL-10 expressed as AUC (see Section 2) compared with the other patients (P < 0.05 for both parameters), possibly reflecting deep hypothermia and circulatory arrest in this group of patients. Moreover, although we found no association between the degree of autotransfusion (i.e. volume of retransfused mediastinal shed blood) and levels of cytokines or complements, this procedure could potentially have influenced the actual levels of these parameters [12,13].

4. Discussion

In the present study we demonstrate a complex set of changes in the cytokine network and complement parameters after CPB. We found that CPB is characterized by a marked and early increase in the level of the anti-inflammatory cytokine IL-10 rather than an initial rise in inflammatory cytokines. Moreover, we report that the inflammatory response after CPB is characterized by a marked rise in chemokines such as IL-8 and MCP-1 rather than an increase in ‘traditional’ inflammatory cytokines such as TNF-α and IL-1. Finally, in contrast to the rapid ‘rise and fall’ in most of the mediators, markedly increased levels of IL-6 persisted throughout the study period.

IL-10 appears to be a general suppressor of immune responses. In infectious diseases and septic shock, this cytokine is synthesized later than inflammatory cytokines in both T cells and monocytes suggesting a regulatory role in the later phases of the immune response [14,15]. In contrast, a major finding in the present study was that the early cytokine response during CPB was dominated by a marked increase in IL-10. Moreover, while markedly raised IL-10 levels in several infections may persist for several days representing poor prognosis [16], IL-10 returned to preoperative levels 2 days after CBP. Whereas TNF-α is known to be a potent inducer of IL-10 as a counteracting mechanism [17], this seems not to be the case during CPB, with a marked increase in IL-10 before a moderate rise in TNF-α levels, suggesting other IL-10 ‘inducing events’ such as enkathen of prostaglandin E2 and catecholamines [18,19]. Whatever the mechanisms, the pronounced and early anti-inflammatory response during CPB may have several consequences. On one side, this response may by inhibiting inflammatory cytokines and leukocyte chemotaxis, limit tissue damage and inappropriate inflammation during operation. On the other hand, such an early anti-inflammatory response may also predispose to infectious complications in these patients and the exact consequences of these responses will have to be further clarified.

As for the inflammatory cytokines, the most pronounced responses were seen for the inflammatory chemokines IL-8 and MCP-1. In contrast, levels of TNF-α showed only
moderate changes during operation, and although there was a significant rise in IL-1β, the concentration of this cytokine was near to normal throughout the study period. Hypoxia and oxidative stress is known to be potent inducers of IL-8 and MCP-1 at least partly through activation of the transcriptional factor NF-κB [20,21]. However, NF-κB activa-

Fig. 1. Serum levels of (A) IL-10, (B) TNF-α, (C) IL-β, (D) IL-6, (E) IL-8, (F) MCP-1 and (G) SDF-1α in 20 patients undergoing operative approach with median sternotomy, cardio-pulmonary bypass (CBP) and systemic hypothermia. Blood samples were taken before (Pre) and immediately (OP) and 2 h (h) after operation, on the first and second postoperative day (d), and 2 and 3 weeks (w) after operation. Shaded area indicates ranges in 20 sex- and age-matched healthy controls. *P < 0.05, **P < 0.01 and ***P < 0.001 versus baseline. *P < 0.05 and **P < 0.01 versus healthy controls. Data are given as medians and 25th–75th percentiles.
tion may also induce TNF-α synthesis [20,21], suggesting that other mechanisms may also be operating in the marked induction of IL-8 and MCP-1 during CPB. For example, vascular shear stress has been reported to enhance MCP-1, but to impair TNF-α synthesis [22,23]. Nonetheless, this rise in both CC- (i.e. MCP-1) and CXC-chemokines (i.e. IL-8) during CPB may contribute to the inflammatory response after this operation by promoting recruitment and activation of both granulocytes, T cells and monocytes into various organ systems after CPB.

We confirm a previous report of a marked and rapid increase in C3bc and TCC during operation [24]. C3bc serves as indicator for both the classical, lectin, and alternative pathway activation, whereas TCC indicates activation of the terminal pathway to its end [25]. Interestingly, we also show an increase in these parameters 2 and 3 weeks after CPB, and the biological consequences of this late increase in complement activation will have to be further clarified.

The present study shows a complex pattern of changes in the cytokine network and complement parameters after CPB with a marked rise in both inflammatory and anti-inflammatory mediators. However, in contrast to cytokine pattern during various infections, the initial phase after CPB was dominated by a marked rise in anti-inflammatory cytokines (i.e. IL-10). If this early anti-inflammatory response represents a physiological mechanism to limit tissue damage and inflammation during operation, or if it represents a pathogenic response, predisposing to postoperative infections will have to be clarified in forthcoming studies.

References


