Complement Activation and Cytokine and Chemokines Release During Mediastinitis

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Background. **Mediastinitis after open heart operation is an infrequent, but life-threatening complication with a reported incidence rate between 1% and 4%. Hospital mortality is estimated at 10% to 35%. The aim of the present work was to study the systemic inflammatory reaction as judged by complement activation and cytokine and chemokines release in patients with mediastinitis after open heart operation.**

Methods. **Seven patients with clinical signs of mediastinitis were included. Three patients had undergone coronary artery bypass grafting, whereas 4 patients had combined coronary artery bypass grafting, valve replacement, or valvuloplasty. Blood samples were drawn before induction of anesthesia and at the time of reoperation, and thereafter daily during the hospital stay. Controls comprised similar patients with an uneventful postoperative course.**

Results. **The terminal SC5b-9 complement complex concentration in the mediastinitis patients was substantially higher compared with the controls ($p < 0.001$), and the terminal SC5b-9 complement complex values showed no overlap between the two groups. Interleukin-8, stromal cell-derived factor-1α and IL-6 concentrations were also significantly higher in the mediastinitis group than in the control group ($p < 0.001$), but with considerable overlap between the groups. Interleukin-1β, interleukin-10, and monocyte chemoattractant protein-1 concentrations were slightly higher in the mediastinitis group, and no differences were seen for the tumor necrosis factor-α.**

Conclusions. **During mediastinitis, the complement is activated and the cytokines and chemokines, interleukin-6, interleukin-8, and stromal cell-derived factor -1α are released. These proteins may be involved in the pathogenesis of this complication. Terminal SC5b-9 complement complex may be an indicator to discriminate mediastinitis patients from those with uneventful course.**

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Mediastinitis after open heart operation is an infrequent complication with a reported incidence of 1% to 4%, associated with increased morbidity, mortality, and cost. Management generally consists of reoperation with debridement and drainage, followed by prolonged hospitalization for antibiotic therapy. The risk of mortality is reported to be 10% to 35% [1–4]. The cause and pathophysiology of mediastinitis is complex and many risk factors have been identified, such as invasion of the body by microorganisms that activate the immune system and bring about widespread metabolic changes and tissue injury [5–8]. The cytokines and chemokines are key mediators of the acute phase response, and complement activation is a uniform reaction of the host to a variety of stress including operation, cardiopulmonary bypass (CPB), and infection [9–14].

Although several studies have reported the cytokine response to infection after CPB, such as bacterial septicemia, the literature is virtually devoid of studies examining cytokine and chemokines responses during mediastinitis. Therefore the aim of the present study was to investigate the systemic inflammatory reaction as judged by cytokine and chemokines release, as well as complement activation in patients with mediastinitis after open heart operation.

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Patients and Methods

The study was approved by the regional ethical committee, and a signed informed consent was obtained from each patient.

Primary Operation

In all patients the primary operative approach was a median sternotomy, with CPB and systemic hypothermia, using crystalloid cardioplegia and topical cooling with ice slush. All CPB circuits, including the Spiral Gold Oxygenator (Jostra, Germany), were heparin coated with Baxter Duraflo II (Baxter Healthcare Inc, Irwine, CA). The initial heparin dose was 4 mg/kg to achieve activated clotting time more than 480 seconds. After CPB, protamine sulfate (Leo, Løvens kemiske Fabrik, Copnhagen, Denmark) was administered to reestablish the preoperative activated clotting time level. Mediastinal shed blood was retransfused.

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Mediastinitis Patients

A total number of 941 adult patients underwent open heart operations in the period from October 1, 1999 to August 1, 2001 (21 months). Of these, 9 patients (0.96%) had mediastinitis develop that required reoperation. Seven of the 9 patients (1 female and 6 males, aged 47 to 75 years; median, 59 years) were included in the study. Two patients did not enroll on admittance. Three patients had undergone coronary artery bypass grafting, 1 patient had valve replacement, and 3 patients had both coronary artery bypass grafting and valve replacement. Diagnosis was clinically based on sternal instability, dehiscence of the sternotomy, or purulent drainage from the wound.

Exploration was performed, and pus encountered and cultured. Debridement was followed by continuous irrigation and drainage of the re-closed wound with antibiotic solution, until the effluent drainage had been free from bacterial growth for 2 days. Irrigation time ranged from 9 to 19 days (median, 12 days).

Revision

Surgical revision for mediastinitis was performed using the Robiscek approach [15], a method of closing an osteoporotic sternum after open heart operation. Heavy wire is woven vertically over and under the sternal costal junctions. Horizontal wires pass outside the vertical wires to permit snug closure of sternal edges. The Robiscek operation with debridement, irrigation, and resuturing of the sternum is the standard procedure for surgical treatment of mediastinitis at our department. Only if this technique provides healing, then plastic surgical reconstruction with a muscle flap is used. For the revision of mediastinitis, 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 during the operation and extubated on the operating table. For postoperative pain relief, the patients were given morphine (Nycomed Pharma, Oslo, Norway) or ketobemidon (Pharmacia & Upjoin), supplemented with paracetamol (Alpharma, Oslo, Norway). Subcutaneous injections of low-molecular weight heparin (Dalteparin, Fragmin, Pharmacia & Upjohn, Stockholm, Sweden) were given daily after the operation.

Postoperative Bacterial Findings and Treatment With Antibiotics

Staphylococcus aureus was found in 5 patients and *Staphylococcus epidermidis* in 1. Although 1 patient had no bacterial growth, the pus was dominated by granulocytes indicative of bacterial mediastinitis. All patients were treated postoperatively with intravenous antibiotics starting at the time of revision and continuing for 4 to 6 weeks. All 7 patients were treated with dicloxacillin in combination with netilmicin. In 3 patients meronem, vancomycin, or tobramycin were also given.

Control Patients

The control group consisted of 20 consecutive patients undergoing CPB, all who had uneventful postoperative courses. Seven of these patients underwent coronary artery bypass grafting, 2 patients had valve replacement or plasty, 7 patients had combined coronary artery bypass grafting and valve replacement or valvuloplasty, whereas 4 patients underwent various other procedures. Blood samples were obtained 3 weeks after CPB, which corresponded to the median (20.3 days), (range, 10 to 28 days), interval from operation to diagnosis of sternal infection. Thus, the controls reflect background levels of complement activation and cytokine release due to the primary operation per se at the time point where the diagnosis of mediastinitis was made. The protocol did not intend to compare mediastinitis with other postoperative infectious complications, and therefore only noninfected controls were included.

Blood Sampling Protocol

Blood samples were collected from a radial artery or brachial vein after induction of anesthesia and before reoperation, and then daily until day 11 (the observation period); ie, all 7 patients for the first 7 days, 6 patients for 10 days, and 5 patients for 11 days. The blood samples were collected directly into sterile ethylenediaminetetraacetic acid-pretreated tubes (Becton Dichenson, San Jose, CA), immediately immersed in melting ice and centrifuged within 5 minutes at 1600 *g* for 10 minutes. Plasma was stored in multiple aliquots at −70°C, and was thawed only once. Before analyzing stromal cell-derived factor (SDF)-1α, plasma was centrifuged at 11,000 g for 10 minutes to remove platelets.

Cytokine and Complement Analysis

Interleukin (IL)-1β, IL-6, IL-8 and monocyte chemoattractant protein-1 were measured by enzyme immunoassays (R & D Systems, Minnepolis, MN). Tumor necrosis factor-α and IL-10 were quantified by an enzyme immunoassays (BioSource Europe, Nivilles, Belgium) as previously described [16]. For SDF-1α analysis, wells were coated overnight with monoclonal mouse antihuman SDF-1α (clone 79018.111; R & D Systems; 2 μg/mL in sterile phosphate-buffered saline). Subsequent steps included biotinylated polyclonal human antihuman SDF-1α (200 ng/mL), horse-radish peroxidase streptavidin and mixture of H_2O_2 and tetramethylbenzidine as substrate. Standard was recombinant SDF-1α. All reagents were purchased from R&D Systems.

The complement activation products C3bc (ie, the sum of C3b, iC3b and C3c) and terminal SC5b-9 complement complex (TCC) were measured by enzyme immunoassays based on neoepitope-specific monoclonal antibodies to the activation products as previously described [17]. The results are given in arbitrary units based on a standard of normal human serum activated with zymosan and defined to contain 1,000 arbitrary units/mL.

For all measurements, all samples from a given patient were analyzed in the same microtiter plate to minimize

Table 1. Plasma Levels of Cytokines, Chemokines, and Markers of Complement Activation in Mediastinitis Patients During the Course of the Disease Compared With Age-Matched and Sex-Matched Cardiopulmonary Bypass Operation Control Patients Without Mediastinitis

	Cardiopulmonary Bypass Control Group	Day						
		0	1	3	5	7	9	11
n = 20		7	7	7	7	7	6	5
Tumor necrosis factor-α (pg/mL)	11 ± 5	13 ± 8	18 ± 9	17 ± 11	15 ± 15	13 ± 8	16 ± 8	13 ± 10
IL-Iβ (pg/mL)	0.6 ± 0.2	1.6 ± 1.1[b]	1.1 ± 0.6[a]	0.9 ± 0.4[a]	0.9 ± 0.5	1.0 ± 0.4	0.9 ± 0.3	1.0 ± 0.8
IL-6 (pg/mL)	9 ± 6	36 ± 7[b]	41 ± 14[c]	37 ± 12[c]	29 ± 9[b]	28 ± 9[a]	26 ± 10[a]	24 ± 11
IL-8 (pg/mL)	8 ± 7	22 ± 17[c]	28 ± 11[c]	33 ± 25[c]	40 ± 38[c]	99 ± 209[c]	54 ± 52[c]	34 ± 23[c]
IL-10 (pg/mL)	2 ± 2	10 ± 13	14 ± 15[a]	6 ± 8[a]	6 ± 6	6 ± 8	8 ± 9	3 ± 3
Monocyte chemoattractant protein-1 (pg/mL)	172 ± 68	214 ± 88	310 ± 104[b]	279 ± 178[a]	219 ± 112	233 ± 148	264 ± 98[a]	239 ± 112
Stromal cell-derived factor-α (ng/mL)	2.5 ± 1.4	4.6 ± 2.2[b]	5.7 ± 1.7[c]	6.7 ± 3.1[c]	7.8 ± 4.8[c]	6.1 ± 3.1[c]	6.4 ± 5.7[c]	3.8 ± 1.5
C3bc (AU/mL)	12 ± 4	14 ± 6	15.3 ± 3.6	12.7 ± 5.9	13.8 ± 5.9	15.2 ± 6.3	12.9 ± 4.3	13.4 ± 3.6
Terminal SC5b-9 complement complex (AU/mL)	0.9 ± 0.2	1.8 ± 0.5[c]	1.8 ± 0.4[c]	1.6 ± 0.4[c]	1.6 ± 0.4[c]	2.1 ± 1.0[c]	1.9 ± 0.5[c]	1.7 ± 0.7[c]

[a] $p < 0.05$ versus CPB operation control patients without mediastinitis; [b] $p < 0.01$; [c] $p < 0.001$.

Data are given as mean ± standard deviation.

AU = arbitrary units; control group = normal rates control; IL = interleukin.

run-to-run variability. The intra-assay and inter-assay coefficients of variation were less than 10% for all enzyme immunoassays.

Statistical Analysis

Differences between groups were analyzed using the Mann-Whitney *U* test. The Friedman's test was used for repeated measurements, and if significant, the Wilcoxon's signed rank test was used to compare individual time points with base line. The *p* values were two-sided. Because of the large number of statistical tests performed, the *p* values were graded as less than 0.05, less than 0.01, and less than 0.001, and the values below 0.001 were considered significant. Higher values ($p < 0.05$ and < 0.01) were interpreted with caution as trends.

Results

Follow-Up Data

All patients were followed up by readmission after 1 year, or by questionnaires or telephone interview. Survival was checked against the Norwegian death registry. All patients are alive and fully re-situated 1 to 2 years after operation. Our results with the combined re-suture and irrigation technique have been good.

Cytokines and Chemokines

The concentrations of the chemokines IL-8 and SDF-1α and the cytokine IL-6 were substantially higher (200% to 400%) before reoperation in the mediastinitis patients than in the controls ($p < 0.001$) and were persistently elevated throughout the observation period (Table 1). In contrast, the concentrations of the cytokines IL-1β and IL-10 and the chemokines monocyte chemoattractant protein-1 were increased in the mediastinitis patients for only the first days of the observation period and did not show the same degree of significance ($0.05 > p > 0.001$) (Table 1). For tumor necrosis factor-α there were no differences between the groups (Table 1). There were no statistically significant changes in any of these measurements in the mediastinitis patients during the observation period.

Complement Activation

There was no significant difference in the C3bc concentration between mediastinitis patients and controls. In contrast, TCC was significantly increased (~100%) at the time of reoperation in the mediastinitis patients ($p < 0.001$), and the elevated levels persisted throughout the observation period (Table 1). Of all measured factors, TCC was the only one showing no overlap between the values in the mediastinitis patients and in the controls. Neither C3bc nor TCC changed significantly in the mediastinitis patients during the observation period.

Comment

Postoperative mediastinitis is a serious complication in cardiac operation with substantially increased morbidity and mortality. Diagnosis is based on clinical features, echocardiography, computed tomographic scanning, blood tests, and blood culture findings. However, negative blood cultures have been reported in the literature. The mechanism for the development of the mediastinitis is not fully understood. In an attempt to study the systemic inflammatory reaction of this condition, we measured activation of complement and release of cytokines and chemokines in patients that had mediastinitis develop after open heart operation.

Mediastinitis initiates a general inflammatory re-

sponse, manifested by fever and reflected by elevated biochemical markers such as leukocyte counts and acute phase reactants [18, 19]. Inflammatory mediators like cytokines, chemokines, and complement activation products may well contribute in the pathogenesis of this condition. However, except from a suggested predisposition to mediastinitis in individuals with a polymorphic variant of the tumor necrosis factor gene [20], the literature is virtually devoid of studies in this field.

The present study is the first to emphasize that activation of complement and release of cytokines and chemokines take place during mediastinitis. Although this observation per se is rather expected, the patterns observed and the degree of systemic involvement is of particular interest. Thus, although markedly raised levels of IL-1 and tumor necrosis factor-α are found during acute infectious complications to CPB, such as septicemia, this was not found during mediastinitis. Actually, the inflammatory response during mediastinitis seems to be dominated by the CXC-chemokines IL-8 and also to a lesser degree IL-6. The latter is one of the main cytokines released after various types of operation [11, 21]; this also plays an important role during the acute-phase response and is suggested to be the major inducer of C-reactive protein [22]. Moreover, IL-8, classified as CXC-chemokines, induces chemotaxis and activation of T cells and granulocytes [23], and the persistent elevation of these chemokines during mediastinitis may potentially reflect the involvement of granulocytes in this disorder. In contrast to the rise in several inflammatory cytokines, changes in the anti-inflammatory cytokine IL-10 were modest further supporting a net inflammatory response during mediastinitis.

In addition to IL-6 and IL-8, there was a substantial and persistent elevation in SDF-1α during mediastinitis. SDF-1α is CXC-chemokines with both inflammatory and anti-inflammatory properties [24], and except for a report in HIV-infected patients [25], to our knowledge this is the first report of SDF-1α levels in plasma during infection in humans. SDF-1α and its receptor, CXC receptor 4, are important for stem cell migration and hematopoiesis [26, 27], and although the biological significance of the increase of these chemokines in mediastinitis is uncertain, a hematoproliferative role contributing to the leukocyte response during this infection may be implied.

The concentration of TCC was significantly higher in the mediastinitis group compared with the control patients, whereas no difference was seen for C3bc. The present data supports previous findings that TCC is an indicator of complement activation in vivo [28]. Notably, there was no overlap between the TCC values in the mediastinitis group and the control group, in contrast to the cytokines and chemokines, whereas the interindividual variation was more pronounced both in the controls and in the mediastinitis patients. Our data are in accordance with the previous finding that TCC is closely related to the development and prognosis of severe septicemia [29]. The complement system is normally kept under strict control by regulatory proteins that restrict activation to the local site of infection. Systemic activation implies that the system is out of control, and damage may occur in remote organs. The activation seen in mediastinitis underscores the systemic response of this initially local process and suggests that complement may contribute in the pathogenesis of this condition.

There were no significant changes in any of the measurements studied within the patient group during the observation period. This reflects the prolonged inflammatory response in mediastinitis, which does not decline despite microbial control, underscoring the potent systemic inflammation in these patients.

In conclusion, our data suggests that a substantial cytokine (particularly IL-6) and chemokines (particularly IL-8 and SDF-1α) release takes place during mediastinitis. Furthermore, systemic complement activation as measured by TCC formation was significantly enhanced in patients with mediastinitis.

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