Relationship of the proinflammatory cytokines to myocardial ischemia and dysfunction after uncomplicated coronary revascularization

The proinflammatory cytokines have been implicated in mediating myocardial dysfunction associated with myocardial infarction, severe congestive heart failure, and sepsis. We tested the hypothesis that cytokine levels are elevated after uncomplicated coronary artery bypass grafting and associated with episodes of postoperative myocardial ischemia and dysfunction. Coronary artery bypass grafting was performed under general anesthesia with moderate systemic hypothermia and cold-blood potassium cardioplegic solution. Tumor necrosis factor- α and interleukin-6 levels were determined by bioassays, and interleukin-8 levels were measured by a sandwich enzyme-linked immunosorbent assay. Myocardial function and ischemic episodes were assessed by intraoperative transesophageal echocardiography and perioperative 12-channel Holter monitoring. A total of 22 patients were studied, with no deaths or complications. Arterial tumor necrosis factor- α rose in a bimodal distribution, peaking at 2 and 18 to 24 hours after the operation (at 20.2 ± 6.4 pg/ml, [mean $\pm$ standard error of the mean]) and 5.8 ± 1.6 pg/ml, respectively; before cardiopulmonary bypass: 0.90 ± 0.20 pg/ml, $p < 0.001$ for both peaks) then progressively declined to levels before bypass. Arterial interleukin-6 was maximally elevated immediately on termination of cardiopulmonary bypass and peaked again 12 to 18 hours after cardiopulmonary bypass (at 7520 ± 2439 pg/ml and 6216 ± 1928 pg/ml, respectively; before bypass: 746 ± 187 pg/ml, $p < 0.0001$ for both peaks). Arterial interleukin-8 levels were more variable but followed a similar pattern, peaking in the early period after cardiopulmonary bypass and again at 16 to 18 hours after the operation (at 4110 ± 1403 pg/ml and 1760 ± 1145 pg/ml, respectively; before bypass: 461 ± 158 , $p < 0.05$ for both peaks). By multivariate analysis, the aortic crossclamp time was independently predictive of postoperative cytokine levels. Left ventricular wall motion abnormalities were associated with both interleukin-6 and interleukin-8 levels, worsening scores being associated with increasing levels (for interleukin-6, $p = 0.003$; for interleukin-8, $p = 0.05$). Postoperative myocardial ischemic episodes were associated with interleukin-6 levels, six of seven (85 %) patients with episodes of myocardial ischemia after a peak in interleukin-6 concentrations ($p < 0.01$). We conclude that proinflammatory cytokines are elevated after uncomplicated coronary revascularization and may contribute to postoperative myocardial ischemia and segmental wall motion abnormalities. (J THORAC CARDIOVASC SURG 1994;108:626-35)

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Both cardiopulmonary bypass (CPB) and extracorporeal membrane oxygenation induce a systemic inflammatory response characterized by the activation of chemotactic factors, oxygen free radicals, and proinflammatory cytokines.^{1,2} This so-called post-pump inflammatory response has been linked to stunned myocardium, respiratory distress syndrome, renal failure, pancreatitis, and neurologic injury.^{3,4} Recent evidence suggests that the proinflammatory cytokines have significant cardiovascular activity, both by regulating nitric oxide homeostasis⁵ and by mediating interactions between leukocytes and endothelium.⁶ We investigated the time course of proinflammatory cytokine levels after CPB, and correlated their levels with postoperative myocardial ischemia and function.

Materials and methods

Patient selection. Institutional Review Board approval was obtained from the Committee on Human Research. Patients considered eligible for study were those undergoing nonreoperative coronary revascularization who had given written, informed consent at the San Francisco Veterans Affairs Medical Center from October 1991 to February 1992. Patients were excluded if they previously had undergone CPB for any reason, if evidence of an acute myocardial infarction was present within 1 week of operation, if concomitant cardiac procedures were performed at the time of operation (such as aortic valve replacement), if other chronic illness was present (such as renal insufficiency or immunodeficiency syndromes), or if they were steroid dependent.

Coronary artery bypass grafting. Patients were prepped and draped in standard fashion, and an appropriate length of great saphenous vein was harvested. Simultaneously, a median sternotomy was performed, and either the left or both internal mammary arteries were mobilized. Patients were systemically heparinized and the activated clotting time was maintained above 400 seconds for the duration of the CPB period. CPB was instituted with the use of a single two-stage venous cannula placed through the right atrial appendage and an arterial catheter placed in the ascending aorta. The coronary sinus was cannulated with a catheter placed through a stab incision in the right atrium. Patients' temperatures were cooled to 26° to 28° C, the aorta was crossclamped, and the heart was arrested with an infusion of cold-blood potassium cardioplegic solution perfused through the aortic root. Arrest was maintained with intermittent antegrade cold-blood potassium cardioplegic solution given at approximately 20-minute intervals. Rewarming commenced during completion of the final distal anastomosis, and the crossclamp was subsequently removed. The proximal anastomoses were performed with partial occlusion of the ascending aorta. Patients were weaned from CPB, the heparin was reversed with intravenous protamine sulfate, the cannulas were removed, hemostasis was obtained, and the chest was closed. Postoperative care was routine, and no control of inotropes, ventilator management, or any other aspects of the convalescence was controlled for the purpose of the study.

Cytokine levels. Intraoperative blood samples for cytokine determinations were drawn from the pulmonary artery, right

superior pulmonary vein, systemic artery, and the coronary sinus just before institution of CPB with all cannulas in place and immediately after termination of CPB. Postoperative samples were drawn from the systemic arterial line and the pulmonary artery catheter initially on an hourly basis for the first 6 hours and then at 12, 18, 24, 36, and 48 hours post-CPB. Blood samples (5 ml) were immediately placed into a sterile polypropylene test tube containing 25 μ l of heparin (Elkins-Simm, Inc., Cherry Hill, N.J.) and centrifuged at 5000 rpm for 15 minutes at 4° C. The plasma was transferred to a sterile 5 ml polypropylene test tube and stored at -70° C until it was bioassayed.

Tumor necrosis factor- α (TNF- α) concentrations were assessed with the WEHI 164 subclone 13 cell line. Plasma samples were serially diluted in 96-well plates with a multi-channel pipettor. Dilutions were made in Roswell Park Memorial Institute medium 1640 (Gibco Laboratories, San Francisco, Calif.) containing 1% fetal bovine serum and 1 mmol/L l-glutamine in a final volume of 100 μ l. A standard curve of serially-diluted human recombinant TNF- α (rTNF- α) was simultaneously included in each assay. The WEHI cells were suspended at a concentration of 5×10^5 cells/ml in Roswell Park Memorial Institute medium 1640 containing 10% fetal bovine serum, 30 μ g/ml gentamicin, 1 mmol/L l-glutamine, and 0.5 μ g/ml Actinomycin D (Calbiochem, LaJolla, Calif.). A total of 100 μ l of this suspension was added to each sample-containing well. The plates were incubated at 37° C in a humidified atmosphere containing 5% CO₂ for 18 to 20 hours. MTT-Tetrazolium (20 μ l of a 5 mg/ml solution; Sigma Chemical Co., St. Louis, Mo.) was added to each well, and the plates were allowed to incubate for an additional 4 hours. Finally, 150 μ l was aspirated from each well with a Bio-Tek plate washer (Bio-Tek Instruments, Inc., Winooski, Vt.), to which was added 100 μ l of 0.04 N HCl/isopropanol to dissolve the dark blue tetrazolium crystals. The plates were protected from light and allowed to stand overnight at room temperature. Absorbance was read at 550 nm on a Bio-Tek enzyme-linked immunosorbent assay (ELISA) reader and TNF- α concentrations were calculated on the basis of the rTNF- α standard.

Interleukin (IL-6) concentrations were assessed with the B-9 cell line. Similar to the TNF- α bioassay, samples were serially diluted in 96-well plates with Iscove's Modified Dulbecco's medium (Gibco Laboratories) containing 5% fetal bovine serum, L-glutamine 1 mmol/L, penicillin 100 U/ml, and streptomycin 100 μ g/mL. A standard of serially diluted human recombinant IL-6 (rIL-6) was run in each assay. The cultured B-9 cells were washed twice and resuspended at a concentration of 5×10^4 cells/ml in the previously described media supplemented with 2-mercaptoethanol 100 mmol/L. A total of 100 μ l of the cell suspension was added to each sample well, and the plates were incubated at 37° C in a humidified atmosphere containing 5% CO₂ for approximately 72 hours. The plates were processed as previously described for the TNF- α assay except that incubation with MTT-tetrazolium lasted for 6 hours. IL-6 concentrations were calculated on the basis of the rIL-6 standard.

IL-8 concentrations were determined as described by DeForge and Remick.⁷ Rabbit polyclonal antibody to recombinant mononuclear cell-derived (72 amino acid form) human IL-8 (rIL-8) was raised, and the immunoglobulin G was isolated from the antisera with the use of a protein A-agarose column (Pierce, Rockford, Ill.). ELISA plates (Nunc-Immuno Plate

Table I. Perioperative variables

Age at operation (yr)	64 ± 2
Male gender	22 (100)
Diabetes mellitus	4 (18)
Hypertension	12 (55)
Prior myocardial infarction*	10 (45)
Unstable angina	2 (9)
Congestive heart failure	0
Preoperative ejection fraction (%)	52 ± 5
Days of preoperative hospitalization (days)	2.1 ± 0.4
Distal anastomosis (n)	3.2 ± 0.2
Proximal anastomosis (n)	2.1 ± 0.2
Internal mammary arteries taken down (n)	1.1 ± 0.1
Vein graft segments used (n)	2.1 ± 0.2
Bypass time (min)	95 ± 6
Crossclamp time (min)	60 ± 4
Postoperative ICU days (n)	1.9 ± 0.3
Postoperative inotropic agents used (n)	1.3 ± 0.3

Perioperative variables used for statistical analysis. Numbers represent either mean ± SEM, or n (%) as indicated. ICU, Intensive care unit.

*All myocardial infarctions were old (greater than 1 month).

†Two patients were transferred from the coronary care unit for coronary revascularization, otherwise all patients underwent elective operation.

Maxisorb, Neptune, N.J.) were coated with 50 ml/well of anti-IL-8 diluted to 1 µg/ml and incubated overnight at 4° C. The plates were washed three times with phosphate-buffered saline solution containing 0.05% vol/vol polysorbinate 20 (Tween-20), blocking solution (phosphate-buffered saline solution containing 2% BSA) was added, and the plates incubated for 1 to 2 hours at 37° C. An IL-8 standard curve was prepared with rIL-8, and the samples and standards added to the plates and incubated for 1 hour at 37° C. The plates were then washed, biotinylated rabbit anti-human IL-8 antibody was added, and the plates were incubated at 37° C for 30 minutes. Avidin-horse-radish peroxidase (Dako, Carpinteria, Calif.) was diluted 1:5000 and added. The plates were again incubated for 30 minutes at 37° C. The plates were washed, and substrate solution was added orthophenylenediamine dichloride 0.67 mg/ml (Dako), 0.0125% H₂O₂ in citrate phosphate buffer 0.25 mmol/L, pH 5.0). Color development proceeded for 4 to 5 minutes at room temperature before being stopped by the addition of 50 µl of H₂SO₄ 3 mol/L. The absorbance was then measured at 490 nm on an ELISA reader (Bio-Tek), and the concentration was calculated on the basis of the standard curve of rIL-8. The lower limit of sensitivity for the assay was 45 pg/ml.

Transesophageal echocardiography. Immediately after tracheal intubation, a gastroscope tipped with either a 3.5 MHz (Diasonics, Inc., Milpitas, Calif.) or a 5 MHz phase-array transducer (General Electric, Milwaukee, Wis.) was introduced into the esophagus. The transducer was positioned and maintained at the level of the mid-papillary muscles to obtain a short-axis view of the left ventricle. Echocardiographic data were recorded on video tape continuously from completion of endotracheal intubation to the onset of CPB and from completion of the last proximal anastomosis to skin closure. The short axis, cross-sectional images were divided into anterior, posterior, lateral, and septal segments with the papillary muscles used as guides. The wall motion of each segment was graded at 3- to 5-minute intervals according to the following scale: 0 = normal, 1 = mild hypokinesis with myocardial thickening, 2 = hypoki-

nesis, 3 = akinesis, 4 = dyskinesis. A wall motion score (WMS) (range 0 to 4) was assigned to each analyzed echocardiographic image by the following formula:

$$\text{WMS} = [\frac{1}{4} (\text{anterior}^2) + \frac{1}{4} (\text{posterior}^2) + \frac{1}{4} (\text{lateral}^2) + \frac{1}{4} (\text{septal}^2)]^{1/2}$$

Electrocardiographic measurements. Patients were monitored continuously with an ambulatory two-lead (CC5, modified CM5) solid-state electrocardiographic recorder (QMED-Monitor one TC; QMed, Clark, N.J.) for 1 to 2 days before operation, in the intraoperative period and 7 days after the operation. The incoming signal was digitized at 256 MHz and fed to a 65CO₂ microprocessor (Marquette Electronics, San Francisco, Calif.) for analysis, where signal amplitude was compared with a fixed internal reference used for calibration. Validated complexes were analyzed to determine the onset, peak, J-point, and J + 60 ms point. The ST segment level average was updated on a beat-by-beat basis, and amplitude data were quantified in 1 mm steps. An ischemic episode was defined as reversible ST segment depression from a baseline of ≥ 1 mm or greater or an ST segment elevation of 2 mm or greater at the J point, lasting for at least 1 minute. Baseline was considered the most stable ST segment within 2 hours of an ischemic episode. All episodes were validated by two independent investigators blinded to patient identity or clinical course. In addition, a 12-lead electrocardiogram was obtained before the operation and daily for the first 7 postoperative days. Preoperative abnormalities including evidence of prior infarction, left ventricular hypertrophy, conduction abnormalities, dysrhythmia, and non-specific ST wave abnormalities were recorded.

Statistical analysis. Statistical analysis was run on personal computers (IBM Corp., Boca Raton, Fla.) with commercially available software (SAS Institute, Cary, N.C.). We used χ^2 analysis to test categorical data, whereas continuous variables were analyzed with univariate regression analysis. Variables statistically significant at the $p = 0.05$ level were entered into a stepwise multiple regression analysis to test for independence with a strategy of maximizing R². Combinations of categorical and continuous data were analyzed with logistic analysis.

Results

Patient characteristics. A total of 22 patients were in the study (Table I); the patient demographics were representative of Veterans Affairs population undergoing coronary revascularization. In the postoperative period, one patient had a post-pump psychosis, manifested by delusions and combative behavior, which completely resolved with supportive therapy. Computed tomography of the brain obtained on the second postoperative day was negative. It is noteworthy that this patient had the highest IL-8 concentration of the study group, peaking at 1,237,122 pg/ml in the early postoperative period. One additional patient, who underwent elective coronary revascularization in preparation for peripheral revascularization, had nonembolic lower extremity ischemia and required urgent peripheral revascularization 1 week after coronary revascularization. This patient had a lower extremity wound infection at the site of the peripheral

Fig. 1. TNF- α levels after uncomplicated coronary artery bypass grafting.

vascular reconstruction, which resolved with appropriate management. No deaths or other complications otherwise occurred in the study group.

Cytokine levels. Arterial TNF- α rose in a bimodal distribution after discontinuation of CPB (Fig. 1), peaking at 2 and 18 to 24 hours after the operation at 20.2 ± 6.4 pg/ml and 5.8 ± 1.6 pg/ml, respectively (pre-CPB: 0.90 ± 0.20 pg/ml, $p < 0.0001$ and $p < 0.001$, respectively). Thereafter, TNF- α levels progressively declined to pre-CPB levels by 48 hours. Similarly, arterial IL-6 levels (Fig. 2) were elevated in a bimodal distribution, being maximally elevated immediately on discontinuation of CPB, declining to pre-CPB levels at 5 to 6 hours, and peaking again at 12 to 18 hours (at 7520 ± 2439 pg/ml and 6216 ± 1928 pg/ml, respectively; pre-CPB: 746 ± 187 pg/ml, $p < 0.0001$ for both peaks). Arterial IL-8 levels (Fig. 3) were more variable but followed a pattern similar to that of TNF- α and IL-6, peaking in the early post-CPB period and again at 16 to 18 hours after the operation (at 4110 ± 1403 pg/ml and 1760 ± 1145 pg/ml, respectively; pre-CPB: 461 ± 158 , $p < 0.05$ for both peaks).

TNF- α , IL-6, and IL-8 levels were significantly higher in all the sampled vascular beds at termination of CPB as compared with pre-CPB controls (TNF- α levels, Fig. 4). In the early post-CPB period, systemic venous TNF- α levels were significantly higher than simultaneously drawn pulmonary venous samples, but this relationship was reversed during the subsequent 48 hours, where systemic arterial levels were higher and often peaking earlier than simultaneously drawn samples from the pulmonary artery (Fig. 5). A delayed increase occurred in IL-8 compared with IL-6 and TNF- α , suggesting that a stor-

age pool for IL-8 might be functional or that IL-8 production may be secondarily induced. Further studies are required to define the temporal rises in the various cytokines.

By stepwise logistic regression analysis of perioperative variables, (Table I), only the aortic crossclamp time was an independent predictor of postoperative cytokine levels (Fig. 6). In this regard, an increasing crossclamp time predicted a greater proinflammatory cytokine response, with IL-6 levels being the most reliably predicted (IL-6: $F = 8.5$, $p \leq 0.01$; TNF- α : $F = 7.4$, $p \leq 0.05$; IL-8: $F = 6.1$, $p \leq 0.07$).

Cytokines and left ventricular function. Intraoperative transesophageal echocardiographic wall motion scores (WMS) were collected continuously and analyzed every 3 to 5 minutes for an average of 105 ± 3 and 27 ± 1 minutes before and after CPB, respectively. As a consequence, only those cytokine levels drawn either before the operation or during the first hour after discontinuation of CPB were used to analyze the transesophageal echocardiography data. The average intraoperative WMS for the entire group did not change after CPB (pre-CPB WMS of 0.87 ± 0.07 ; post-CPB WMS of 0.89 ± 0.10 , $p =$ not significant), although individual patient variation did occur. The absolute WMS did not correlate with circulating levels of measured cytokines either before the operation or within the first hour after CPB.

After CPB, however, eleven patients improved their WMS (change of -0.27 ± 0.14), whereas eleven patients worsened their score (change of $+0.27 \pm 0.08$). With the use of multiple regression analysis, the change in WMS correlated independently with both IL-6 (Fig. 7)

Fig. 2. IL-6 levels after uncomplicated coronary artery bypass grafting.

Fig. 3. IL-8 levels after uncomplicated coronary artery bypass grafting.

and IL-8 levels but not with $\text{TNF-}\alpha$ levels (for IL-6: $R^2 = 0.36$, $p = 0.003$; for IL-8: $R^2 = 0.21$, $p = 0.05$). It is noteworthy that patients who increased their WMS after CPB (i.e., had a decline in ventricular function) had almost a 10-fold higher level of circulating IL-6 levels as those who improved their score (IL-6 of 4838 ± 1191 pg/ml versus 548 ± 156 pg/ml, respectively; $p < 0.001$). These data suggested that elevations in IL-6 and IL-8 levels can predict a decline in myocardial contractility, although are not predictive of the absolute WMS.

Cytokines and myocardial ischemia. Patients were monitored with an ambulatory solid-state electrocardiogram for a mean of 3.4 ± 0.3 hours in the intraoperative period and continuously until postoperative day 7. Because cytokines levels were determined for the first 48 postoperative hours, however,

only those ventricular dysrhythmia and ischemic episodes that occurred within this period were included in the statistical analysis. After the operation, premature ventricular contractions occurred in 14 patients (63%) with a median frequency of two per hour (range 0 to 48 per hour). Ventricular tachycardia (more than three continuous beats) occurred in nine patients (41%) and were uniformly clinically silent. No episodes of ventricular fibrillation occurred in the study group. Seven patients (32%) had a total of 11 episodes of ST segment changes defined as myocardial ischemia by the criteria stated previously. The average onset of the ischemic episodes was 4.9 ± 4.7 hours (range 0 to 42 hours) post-CPB, with a total ischemic time of 62 ± 20 minutes per patient. The average ST segment change was 1.01 ± 0.01 mm.

Six patients had ischemic episodes associated with peak IL-6 elevation, usually after the first elevation but, in one instance, after the second elevation (Fig. 2). Six additional IL-6 elevations

Fig. 4. TNF- α levels measured from various vascular beds before and directly after termination of CPB. In all instances, post-CPB levels were significantly elevated compared to their pre-CPB levels ($p < 0.01$). PV, Pulmonary vein; SA, systemic artery; PA, pulmonary artery; CS, coronary sinus.

Fig. 5. Systemic arterial and pulmonary arterial TNF- α levels in a single patient after CPB. Systemic arterial cytokine levels peaked earlier, and often rose higher, than simultaneously drawn pulmonary arterial levels.

were not associated with ischemic episodes, for an overall Mantel-Haenszel χ^2 probability distribution of 6.27 ($p < 0.01$). It was noteworthy that the absolute level that the IL-6 level reached did not correlate with either the duration of the ischemic episode or with the degree of the ST segment change, suggesting that a rise in circulating IL-6 is clinically more significant than the absolute level. Neither TNF- α nor IL-8 levels were independently associated with ischemic episodes, although both correlated with ischemic episodes by simple correlation analysis. Similarly, neither premature ventricular contractions nor episodes of ventricular tachycardia were independently associ-

ated with cytokine levels, although they did correlate by univariate analysis.

Discussion

The proinflammatory cytokines, such as TNF- α , IL-1, IL-6, and IL-8, are a class of endogenous proteins that exert important influences on immune, hematologic, and metabolic responses to injury.⁸ They are active at low concentrations and are produced by a variety of cells,

Fig. 6. Relationship between the aortic crossclamp time and early IL-6 levels after CPB. Solid line: regression (expected values) line; dotted line: 95% confidence interval.

Fig. 7. Relationship between early post-CPB IL-6 levels and the change in transesophageal echocardiography WMS after coronary revascularization. More negative transesophageal echocardiography WMS signify improvement in score. Solid line: regression (expected values) line; dotted line: 95% confidence interval. Echo, Echocardiography.

including lymphocytes, monocytes, and neutrophils.⁹ Cytokines have important autocrine, paracrine, and endocrine functions and play an important role in the pathogenesis of septic shock and chronic illness.^{10, 11} Not all cytokines are damaging, however; some, such as transforming growth factor- β , having unique cytoprotective properties.¹² The proinflammatory cytokines have a wide spectrum of cardiovascular activity, mediated both through the regulation of nitric oxide homeostasis¹³ and through effects on vascular endothelium.⁶ Cytokines also play a role in chronic cardiovascular processes, such as in

the cachexia of severe congestive heart failure,¹⁴ the immune lesions of Kawasaki's disease,¹⁵ and the morphologic changes associated with rheumatic heart disease.¹⁶

The association between inflammation and myocardial ischemia and injury has been recognized for over 50 years¹⁷, and remains a topic of continued investigation. CPB initiates a generalized systemic inflammatory response characterized by the activation of complement, neutrophils, endotoxin, elastases, and the proinflammatory cytokines.¹ This so-called post-pump inflammatory

response, which also follows extra corporeal membrane oxygenation,² is postulated to be caused by the contact of blood with foreign materials and exposure to abnormal shear forces.³ Inflammation which follows CPB or extra corporeal membrane oxygenation may significantly contribute to myocardial stunning,^{4, 18} as well as be responsible for common post-pump syndromes such as respiratory distress syndrome, renal failure, pancreatitis, and neurologic dysfunction.¹⁹ Cytokine-mediated intimation, as a cause for myocardial ischemia and dysfunction, has been implicated in a growing number of conditions, including sepsis,^{20, 21} myocardial infarction,²² the reperfusion phenomenon,¹² and acute allograft rejection.²³ CPB may contribute to postoperative myocardial stunning, ischemia, and injury wholly on the basis of initiating a generalized systemic inflammatory response.

The present study confirms that the levels of common proinflammatory cytokines TNF- α , IL-6, and IL-8 are elevated after CPB, a finding that has been noted in varying degrees by other investigators.^{24, 28} We noted that after 60 minutes of aortic crossclamping, cytokine levels were almost linearly related to the total crossclamp time (Fig. 6), perhaps reflecting the inflammatory consequences of longer periods in which blood is exposed to foreign materials or abnormal shear forces. This relationship may have been skewed by a single patient with an aortic crossclamp time of greater than 100 minutes, and the relationship, if any, requires further investigation. A relationship between the total bypass time and IL-8 levels has been noted by Finn and associates,²⁸ which may, in part, explain the increase in early hemodynamic instability and late end-organ damage generally associated with longer CPB bypass periods. In addition, the dependence of cytokine levels on CPB or crossclamp time may explain the variation in cytokine levels reported by various investigators,¹ in addition to those variations commonly introduced by sampling differences and methods of assay. In general, however, the proinflammatory cytokines appear to be induced by CPB, and their levels appear to be associated with the relative length of the CPB period.

The proinflammatory cytokines may be characterized as possessing early and late hemodynamic effects which are mediated through their regulation of nitric oxide homeostasis¹³ and alteration of the vascular endothelium,⁶ respectively. Cytokines induce nitric oxide production in endothelium and smooth muscle cells in response to stressful stimuli^{13, 29} through an enzyme which has recently been purified and characterized.³⁰ IL-6, for example, has significant negative inotropic effects, which occur within minutes of its administration and which are inhibited by blocking nitric oxide production.⁵ Nitric

oxide mediates many of the vascular actions of the cytokines in shock,^{31, 32} possesses significant regulatory effects on coronary vascular tone,^{33, 35} and promotes the reperfusion phenomenon.³⁶ The early hemodynamic effects of the proinflammatory cytokines therefore appear to be mediated by regulation of nitric oxide homeostasis and may result in a syndrome not unlike that noted in sepsis, that of systemic vasodilation, myocardial dysfunction, vascular hyporesponsiveness to vasoconstricting agents, and a potential for end-organ damage.¹⁰

The clinical correlates of increased proinflammatory cytokine production are perhaps reflected in the transesophageal echocardiographic WMS and electrocardiographic measurements. Changes in WMS after CPB correlates with circulating levels of IL-6 (Fig. 7), higher levels being associated with a worsening WMS, whereas lower levels were associated with preserved scores. It was noteworthy that the change in the WMS, and not the absolute score, was the correlating variable, suggesting that baseline ventricular function is independent of circulating proinflammatory mediators, whereas worsening myocardial contractility may in part be related to an elevation in IL-6. Myocardial ischemic episodes, defined as ST segment depression of more than 1 mm or elevation more than 2 mm at the J point lasting for more than 1 minute, correlated primarily with the appearance of the second cytokine peaks. The definition of ischemic episodes used in this study is admittedly a liberal one but has been the standard definition used in studies of the SPI Perioperative Myocardial Ischemia group. The definition undoubtedly accounts for the apparently high prevalence of myocardial ischemia despite complete revascularization reported in this study, but there were little hemodynamic consequences to the ischemic episodes and no instances of perioperative myocardial infarction despite comprehensive postoperative evaluation. The correlation between cytokine elevations and both WMS and episodes of myocardial ischemia suggest that the proinflammatory cytokines may be among some of many variables that affect postoperative myocardial contractility, among which are the loading conditions of the heart, the completeness of revascularization, the effectiveness of the myocardial protection, the effects of administered medications, the state and reversal of anticoagulation, intrinsic platelet function, the levels of other acute phase reactants, and intrinsic sympathetic function.

Using a suspended papillary muscle preparation, Finkle and associates⁵ also noted a negative inotropic effect of the cytokines, an effect which appeared early after cytokine exposure and was blocked by inhibiting nitric oxide synthase. Cytokines have a role in mediating myocardial stunning,^{37, 39} in part, through their known nega-

tive inotropic effects through nitric oxide synthase.⁵ Proinflammatory cytokines also possess important hemodynamic effects through their up-regulation of vascular endothelium, both by increasing leukocyte adhesiveness to the endothelium⁴⁰ and by promoting platelet procoagulation.⁴¹ TNF- α and IL-1 regulate leukocyte adhesiveness through the induction of adhesion molecules, endothelial-leukocyte adhesion molecule-1 and intracellular adhesion molecule-1. Stimulation of endothelial cells with TNF- α or endotoxin, for example, up-regulates expression of the adhesion molecules,⁴⁴ whereas blocking leukocyte adhesion to endothelium by monoclonal antibodies reduces the reperfusion phenomenon.⁴⁵ Cytokines also promote the procoagulant properties of endothelium by inducing production of platelet-activating factors, while simultaneously decreasing production of thrombomodulin,⁴¹ and promote both leukocyte adhesion and platelet procoagulation on vascular endothelium, leading to endothelial cell dysfunction and resultant ischemia and injury.⁴⁶ Such effects require both protein synthesis and regulation of adhesion molecules, and peak 3 to 4 hours after initial endothelial stimulation.³⁶ The late hemodynamic effects of the proinflammatory cytokines therefore appear several hours after their exposure and appear to be mediated principally through the vascular endothelium.

An improved understanding of the cellular biology of post-pump inflammation may not only contribute to improving the safety and results of the CPB procedures but may lead to a better understanding of those clinical conditions in which inflammation is thought to play a major role. Cardiac surgeons are in a unique position of contributing to this fertile field by creating a controlled and reproducible model of acute inflammation on a daily basis. Strategies that might be investigated include the use of monoclonal antibodies directed against either the proinflammatory cytokines or adhesion molecules, promotion of cytoprotection by transforming growth factor- β or heat shock proteins, regulation of nitric oxide synthase, and others. Successful understanding of post-pump inflammation may lead to the development of applications with wide utility in clinical conditions ranging from sepsis to organ transplantation.

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