Transcriptomic and Proteomic Patterns of Systemic Inflammation in On-Pump and Off-Pump Coronary Artery Bypass Grafting

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**Background**—Coronary artery bypass grafting (CABG) using cardiopulmonary bypass (CPB) provides controlled operative conditions but induces a whole-body inflammatory response capable of initiating devastating morbidity and mortality. Although technically more demanding, deliberate avoidance of CPB in off-pump surgery attenuates the physiological insult associated with CABG.

**Methods and Results**—To systematically assess the molecular mechanisms underlying the better-preserved remote organ function, we studied gene expression patterns in leukocytes and plasma proteomic response to on-pump and off-pump CABG. Proteomic analysis confirmed (tumor necrosis factor-α, interleukin [IL]-6, IL-10) and expanded (eg, interferon [IFN]-γ, granulocyte colony–stimulating factor [G-CSF], monocyte chemotactic protein-1, macrophage inflammatory protein-1β) the mediators released on CPB, whereas blood leukocyte transcriptomics suggested that circulating leukocytes are not primarily responsible for this response. Interestingly, release of some cytokines (eg, IL-6, IFN-γ, G-CSF) was observed on off-pump surgery to a similar extent but with delayed kinetics. A total of 45 of 4868 transcripts were identified to be significantly altered as a result of initiation of CPB. Systematic analysis of transcriptional activation by CPB revealed primarily genes involved in inflammation-related cell–cell communication (such as L-selectin or intercellular adhesion molecule-2) and signaling (such as IL-1, IL-8, or IL-18 receptors and toll-like receptors 4, 5, and 6), thus confirming a “primed” phenotype of circulating peripheral blood mononuclear cells.

**Conclusions**—Gene array and multiplex protein analysis, only in concert, can illuminate the molecular mechanisms responsible for systemic sequelae of CPB and indicate that circulating leukocytes overexpress adhesion and signaling factors after contact with CPB, which potentially facilitates their trapping, eg, in the lungs and may promote a subsequent tissue-associated inflammatory response. (Circulation. 2005;112:2912-2920.)

**Key Words:** immunology ■ cardiopulmonary bypass ■ inflammation ■ leukocytes ■ molecular biology

Coronary artery bypass grafting (CABG) is one of the most frequently performed operations worldwide. Because the incidence of coronary artery disease and, thus, the number of CABGs performed continue to rise, any improvement in the safety and efficacy of the procedure, however small, would have a major impact on outcome in absolute terms.1,2 CABG involving cardiopulmonary bypass (CPB) has associated deleterious effects, such as central nervous system complications, coagulopathy, and a variable degree of end-organ dysfunction. Although off-pump cardiac surgery was a necessity before the advent of CPB and cardioplegia and persisted in centers with limited resources,3 deliberate avoidance of CPB, albeit with inherent technical limitations, has nowadays attracted interest as a means to prevent systemic sequelae of extracorporeal circulation.2,4 Recent randomized prospective studies involving several thousand patients suggest potential reductions in morbidity and mortality in off-pump compared with on-pump CABG,5–8 in particular in high-risk patients, such as those with recent myocardial infarction, poor left ventricular function, or comorbidities, eg, chronic renal failure or obstructive lung disease.5,9 There is persuasive evidence that in addition...
to less manipulation of the aorta and heart, the avoidance of CPB in off-pump coronary artery bypass attenuates the systemic inflammatory response thought to propagate myocardial ischemia/reperfusion as well as remote organ injury. With respect to the mechanisms responsible for the inflammatory response to CPB, contact of circulating immune-competent cells with surfaces of the extracorporeal circuit are considered crucial, although other sources, such as heart, lung, or gut and liver, have been suggested to contribute.\textsuperscript{10}

These paradigms are based on results from a variety of studies that have been conducted to assess the response of single genes or proteins to CABG with or without the use of CPB. However, any synoptic view of data derived from a group of studies is hampered by the fact that interactions between various genes or sources cannot be detected. Furthermore, because of the complex nature of the cytokine network, comprising a multitude of redundant but also antagonistic pathways, changes in single genes cannot describe the functional state of the innate immune system adequately. To overcome these shortcomings, simultaneous assessment of a broad spectrum of mRNA transcripts using the microarray technology or multiplex profiling of inflammatory proteins present in the systemic circulation has been proposed to allow gene expression phenotyping in similar complex clinical settings, such as autoimmune disease.\textsuperscript{11}

Thus, we compared gene expression patterns from peripheral blood leukocytes and circulating protein levels of inflammatory mediators by combined use of microarray total RNA and multiplexed cytokine analysis. Using this concerted approach of transcriptomic and proteomic analyses, we confirmed and expanded cytokines released in response to initiation of CPB and observed “priming” of circulating leukocytes, whereas the latter are seemingly not the primary source for the inflammatory response accompanying CPB.

**Methods**

**Patient Selection**

Twenty-two white patients undergoing either conventional CABG with CPB (n=12; 9 male, 3 female) or minimally invasive coronary bypass grafting (n=10; 8 male, 2 female) were enrolled. All study was approved by the Institutional Ethical Board of the University, and each patient provided signed informed consent. Exclusion criteria were age more than 80 years, and requirement of additional surgery, such as carotid endarterectomy or valve replacement.

**Multiplex Protein Analysis**

Whole blood was collected into heparinized phlebotomy tubes for each patient after induction of anesthesia but before the beginning of surgery (preoperative), after skin closure (early postoperative, ie, at 223±25 minutes in on-pump and 152±22 minutes in off-pump surgery, respectively), and at 24 hours, ie, at discharge from the intensive care unit (late postoperative time point). Each sample was immediately centrifuged at 4°C and 400g for 10 minutes. The serum samples were separated and multiple aliquots stored at −80°C until proteomic analysis. The Multiplex bead kit was purchased from BioRad Laboratories, Inc and included cytokines (interleukin [IL]-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, tumor necrosis factor [TNF]-α, and interferon [IFN]-γ), growth factors (granulocyte colony-stimulating factor [G-CSF], granulocyte-monocyte colony-stimulating factor [GM-CSF]), and chemokines (monocyte chemotactic protein [MCP]-1 and macrophage inflammatory protein [MIP]-1β). Standard curves for each mediator were generated, ranging from 2 to 8000 pg/mL. Undiluted serum samples (50 μL) were incubated with 50 μL of antibody-coupled microsphere sets (5000 beads per mediator per well) for 1 hour at room temperature. Freshly diluted secondary detection antibody (25 μL; 1 μg/mL) were added and incubated at room temperature for 1 hour. Streptavidin-phycocerythrin (50 μL; ×1) was added, followed by an incubation for 10 minutes at room temperature. After each step (incubation of samples with microsphere sets, incubation with secondary detection antibody, and streptavidin-phycocerythrin), a filtering step and 3 washing steps using a vacuum manifold were performed. Thereafter, 125 μL of assay buffer was added to each well and analyzed on a Bioplex Protein Array System (BioRad Laboratories, Inc) according to the manufacturer’s instructions. Because IFN-α was not part of our multiplex panel but the transcripts were induced in the microarray experiments, a commercial ELISA (R&D Systems) reacting with multiple subtypes of IFN-α but not cross-reacting with IFN-γ was used to measure plasma concentration. All protein concentrations are given in picograms per milliliter.

**Transcriptomical Analysis**

Microarrays were run for 8 and 5 randomly selected patients in the on-pump and off-pump groups, respectively, for the 3 time points; ie, a total of 39 microarray experiments were conducted. For transcriptomical analysis, PaxGene tubes (Qiagen) were used for leukocyte sampling under controlled venous stasis (<30 seconds, 40 mm Hg) and total RNA isolated according to the manufacturer’s instructions from the PaxGene Blood RNA Kit. RNA purity was confirmed by spectrophotometry (A\textsubscript{260}/A\textsubscript{280} 1.6, A\textsubscript{260}/A\textsubscript{320} 2.0), ethidium bromide–stained RNA agarose gel, and a multiple exon-spanning real-time polymerase chain reaction (RT-PCR) amplification of a housekeeping gene (GAPDH). Experiments were performed using the SIRS-Laboratory GmbH in-house research microarray, which comprised 5308 probes addressing 4868 transcripts corresponding to 3704 human genes relevant to inflammation, immune response, and related processes as well as 78 reliable control probes (see Supplementary Notes: “Microarray Experiment Description” online according to MIAME guidelines MGED\textsuperscript{12}).

Total RNA (10 μg) was reverse transcribed using Superscript-II reverse transcriptase from Invitrogen in the presence of aminovalyl-dUTP from Sigma and labeled by use of the AlexaFluor 647 system. AlexaFluor 647–labeled cDNA was cohybridized with AlexaFluor 555–labeled cDNA obtained from the same amount of total RNA isolated from the immature monocyctic cell line SigM5 obtained from American Type Culture Collection and subjected to culture under standard conditions. After incubation in a hybridization apparatus (HS 400, TECAN, for 10 hours at 42°C, formamide-based-hybridization buffer system), arrays were washed according to the manufacturer’s instructions and dried, and hybridization signal intensities were measured immediately with an Axon 4000B scanner (Axon Instruments).

Microarray data preprocessing of hybridization signals included (1) spot detection and background subtraction, (2) spot flagging according to defined signal-to-noise threshold values, and (3) normalization and transformation of the signals obtained from different channels. For the former 2 steps, the GenePix Analysis Software was used; for the third step, we applied the approach of Huber and colleagues,\textsuperscript{13} including variance-stabilized transformation. Expression of genes with invalid spots were labeled as missing values (see Supplementary Notes: “Microarray Experiment Description” online).

For confirmation of transcriptomical changes observed in the microarray experiment by real-time PCR, first-strand complementary DNA synthesis was performed using 1 μg of isolated RNA from...
a randomly selected set of 4 patients for each group. RNA was mixed with 250 ng of 18 oligo-dT primer, denatured at 70°C for 10 minutes, and chilled on ice for 5 minutes. Reverse transcription was performed in a final volume of 30 µL containing 10 mmol/L DTT, 0.5 mmol/L each dNTP, 2 U of RNasin (Fermentas) and 200 U of SuperScript II (Invitrogen) in ×1 reverse-transcription buffer (Invitrogen) at 42°C for 1 hour. The enzyme was then inactivated by incubation at 65°C for 10 minutes. Gene-specific primers for selected cytokines that were either induced (ie, IPN-α) or not induced on the transcript level but released into plasma (ie, TNF-α, IL-4, IL-6, IL-10, INF-γ, G-CSF, MCP-1, MIP-1β) but not observed to be induced in the microarray analysis were designed by use of Primer 3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/ primer3_www.cgi) to obtain an annealing temperature of 55°C and an amplicon length between 100 and 200 bp.

RT-PCR amplification was performed with a Rotor-gene 3000 (Biotage) in 10-µL reaction volumes consisting of 4 µL cDNA, 0.5 µmol/L of each forward and reverse primer, and 5 µL of SYBR green Mix (Quagen GmbH). An initial step of 94°C for 15 minutes was followed by 45 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 45 seconds. Fluorescence acquisitions in the SYBR green and ROX (internal reference dye) channels were performed at the end of the extension step. The melting protocol ranged from 50°C to 95°C after a stepwise increment of 1°C held for 15 seconds, and 72°C for 45 seconds. Fluorescence acquisitions in the SYBR green and ROX (internal reference dye) channels were performed at the end of the extension step. The melting protocol ranged from 50°C to 95°C after a stepwise increment of 1°C held for 30 seconds. Each sample, as well as a negative template control, was performed in triplicate for each of the primer pairs assayed.

PCR Analysis
Using the Rotor-gene 3000 software version 6, relative sample amounts were obtained. For each patient, the sample before surgery was assigned the arbitrary value of 1; values for both postoperative samples were calculated relative to the first sample.

Because we failed to document increases in the amount of mRNA transcripts for cytokines found to be released into the plasma, a positive control experiment, ie, stimulation of whole-blood cultures with endotoxin concentrations relevant for clinical conditions such as sepsis (10 ng/mL), was conducted. In these experiments, RNA induction and plasma cytokine concentration were measured for TNF-α and IL-6 by means of RT-PCR and ELISA, respectively.

Statistical Analysis

Microarray Data
In the analysis, 4081 reliable probes (77%), ie, probes with sufficient mean spot quality and with robust expression variation throughout the experiment, were included after removal of 1227 probes from the data set. Specifically, omitted probes included 475 probes (9%) with mean signal intensity not above the background level (ie, probes with spot intensities of <82 random fluorescence units on average) and 752 probes (14%) with only marginal expression variation throughout the experiment.14 Missing values (approximately 1% of the data matrix) were imputed by use of the k-nearest neighbor algorithm13 (see Supplementary Notes: “Microarray Experiment Description” online).

Three comparisons were performed applying appropriate statistical approaches: expression of 1 transcript in on-pump versus off-pump groups with previous surgery (unpaired t test, “gene by gene”); postoperative versus preoperative expression in (2) patients subjected to CABG/CPB and (3) in patients undergoing off-pump revascularization; (2D l-sample principal component [pc] test,16 “gene-by-gene”, for 2 and 3). To control the false discovery rate occurring in multiple comparisons, a vector of q values was estimated to each vector of probability values17 (see Supplementary Notes: “Microarray Experiment Description” online).

Selected genes were ordered by hierarchical cluster algorithm using correlation distances and the average linkage method.

Multiplex Protein Data
Protein expression data were analyzed by use of the exact same approach, ie, unpaired t test and pc test in a “gene-by-gene” approach as specified for microarray data. Data were log-transformed to obtain a symmetrical distribution.

For the data analysis, including pattern recognition, software routines were developed by the authors using the Matlab standard and statistic toolboxes (The MathWork Inc).

Results

Clinical Characteristics of Patients Undergoing On-Pump and Off-Pump Revascularization
Patients subjected to either on-pump or off-pump surgery were comparable with respect to age (on-pump, 65±11 y; off-pump, 60±11 y) and body weight (on-pump, 76±7 kg; off-pump, 86±18 kg). All patients enrolled belonged to NYHA class II and had comparable left ventricular function (ejection fraction: on-pump, 61±12%; off-pump, 61±8%) although, as expected, coronary artery disease affected more vessels in on-pump (3±0) than off-pump patients (1±0; P<0.01). The required time for off-pump (152±22 minutes) was significantly shorter than for on-pump revascularization (223±25 minutes; P<0.01). Consequently, mechanical ventilation tended to be more prolonged in on-pump (358±128 minutes) than off-pump surgery (254±192 minutes; P=NS). Aortic cross-clamping time averaged 1 hour in on-pump surgery (57±13 minutes).

Microarray Analysis of Gene Expression Patterns in Peripheral Blood Leukocytes
Steady-state transcript concentrations of the panel of inflammation-related transcripts were comparable between the 2 groups before surgery, because no probe achieved a q value less than 0.96 (see “Microarray Experiment Description” online).

Time-dependent changes on on-pump surgery affected approximately 24% of probes. A set of 48 probes, corresponding to 45 transcripts (ie, reflecting 3 redundant probes targeting different regions of the same gene to increase robustness of the microarray), with a probability value of <0.002 (corresponding to a q value of <0.13) were considered as being significantly changed (thus, 6 false-positive decisions were accepted). In contrast, time-dependent changes on off-pump surgery affected approximately 5% of probes. From this set, 4 probes with the lowest probability value were selected, all with a q value of <0.4, corresponding to 1 or 2 false-positives; none of these probes corresponded to the ones found to be altered by CABG/CPB.

The gene expression patterns of the 52 selected probes for both groups were ordered by hierarchical clustering for patients subjected to on-pump surgery (Figure 1, left). Expression patterns from off-pump patients were grouped in the same order (Figure 1, right).

The examination of the dendrogram and the master gene list (see “Microarray Experiment Description” online) revealed 38 probes (corresponding to 35 transcripts) upregulated and 14 transcripts downregulated on on-pump (n=48) or off-pump (n=4) surgery. Among genes found to be upregulated, the expression of 6 genes (PDE7A-LRP1) continuously increased after surgery. The largest category identified 32 transcripts with a maximum early after surgery followed by a subsequent gradual decrease.
Interestingly, some genes found to be upregulated with initiation of CPB tended to be induced on off-pump surgery as well (e.g., TLR-6, Flotilin-2, serine/threonine kinase11-interacting protein) but to a lesser extent (thus not reaching statistical significance) and with delayed kinetics.

Although these genes reflect a generalized stress response to surgery/anesthesia, induction of other genes was restricted to initiation of CPB. The latter genes, such as transcripts encoding for adhesion molecules (L-selectin, intercellular adhesion molecule [ICAM]-2), for cytokine receptors (IL-1-R, IL-8-R, IL-18-R) and toll-like receptors (TLR-4, -5, -6), or regulatory proteins (BCL2), were primarily involved in cell–cell communication, signaling, or apoptotic demise (see Microarray Experiment Descriptions online). For validation of expression patterns as assessed by microarray technology, a panel of transcripts was confirmed by independent methodology (i.e., real-time PCR): Among the various cytokine mRNAs studied, only transcripts encoding IFN-α (as assessed by microarray and RT-PCR) and IL-10 (selectively detected by real-time PCR) were observed to be upregulated (Table).

Proteomic Analysis of the Inflammatory Response to Coronary Revascularization With or Without CPB

Assessment of serum concentrations for the panel of cytokines, chemokines, and growth factors studied also revealed divergent responses regarding order of magnitude or time course of release to coronary revascularization, depending on the use of CPB. Although some cytokines were not affected by the surgical stress event (e.g., IL-5, IL-7, IL-13, and IL-17), other cytokines/chemokines required initiation of CPB for their induction (e.g., TNF-α, IL-10, MCP-1, and MIP-1β). However, some cytokines (e.g., IL-6, and IFN-γ) were induced to a similar extent in response to either on-pump or off-pump surgery, but with a strikingly different time course: These mediators appeared immediately during surgery with initiation of CPB in the circulation and already decreased on the first postoperative day, whereas their expression on off-pump surgery was delayed but comparable with respect to the order of magnitude (Figure 2). This comparable increase in IL-6 is also in line with a comparable plasma CRP on day 1 (on-pump, 80±51; off-pump, 80±18 mg/L).
Although IFN-α transcripts were induced in peripheral blood mononuclear cells (PBMCs), plasma concentrations were unchanged in patients undergoing CABG/CPB over time (median [interquartile ratio], pg/mL: previous CABG/CPB, 10.2 [18.2]; early postoperative, 10.2 [14.5]; late postoperative, 10.2 [10.9]).

Under experimental conditions in which cytokines were released from the PBMC pool, ie, in a whole-blood culture system stimulated with clinically relevant endotoxin concentrations, steady-state transcripts corresponded well to plasma cytokine concentration (positive control, Figure 3).

### Discussion

Cardiovascular surgery involving CPB is associated with an ischemia-reperfusion injury to the heart and lungs and induces a complex inflammatory response affecting not only the heart and lungs but also remote organs such as kidney, gut, and brain. In recent decades, the risk of cardiovascular surgery has been reduced substantially, although devastating inflammatory complications continue to contribute to early complications despite improved bypass technology.\(^5\)\(^-\)\(^10\)

Nevertheless, although contact of circulating cells with non-physiological surfaces setting in motion the innate immune response has traditionally received attention as a mechanism for immediate post-CPB remote injury (“CPB-SIRS”), more recent evidence suggests that a rapid, putatively adaptive antiinflammatory response is induced simultaneously.\(^10\)

This antiinflammatory response might limit untoward effects of CPB but might also result in impaired wound healing and immunodeficiency, giving rise to postoperative infectious or even septic complications, a leading cause of more delayed perioperative morbidity and mortality.\(^15\) Thus, a synoptic view of data derived from a multitude of “single-gene” experiments would suggest that on-pump surgery induces a complex systemic inflammatory response, with activation, sequestration, and elimination of circulating leukocytes reflected in the appearance of a multitude of proinflammatory mediators in the circulation. In addition, a compensatory antiinflammatory reaction to the initial inflammatory insult complicates the response to CPB.

However, “single gene” approaches are inherently limited to assess such complex pathophysiological networks, most notably in light of the current understanding of confounding genetic factors, such as polymorphisms in inflammation-related genes. Thus, the analysis of this complex, partially redundant, but also antagonistic pattern of transcriptional activation or suppression requires novel tools to assess a broad spectrum of interacting genes in a multiplexed manner in combination with sophisticated data pattern recognition.\(^11\)\(^-\)\(^19\)

These techniques may not only allow a better understanding of the pathophysiology but may also lead to individualized therapy\(^20\) for the benefit of patients prone to inflammatory complications. Regarding the latter, we have demonstrated the suitability of the microarray technique used to monitor the complex inflammatory host response in sepsis,\(^19\) whereas the use of the information obtained, eg, to guide therapeutic interventions (“theragnostics”) in the systemic inflammatory response to infectious or noninfectious causes, remains to be demonstrated.

Despite an immense potential to improve understanding of (patho)physiological pathways, microarray technology has methodological limitations, eg, because of the lack of standardization of RNA sampling from whole blood, as well as because of changes in blood cell composition. Moreover, compartmentalized inflammatory reactions are not necessarily reflected in gene expression in circulating PBMCs, because these cells may traffic among bone marrow, blood lymphoid organs, and inflamed tissues.\(^21\) Furthermore, statistical analysis of changes in a multitude of genes resulting in large amounts of raw data requires sophisticated tools for data pattern recognition. Thus, strategies are required to deal with the specific problems associated with microarray technology: the negligible variation in individual gene expression pattern irrespective of the relative proportions of RNA-containing blood cells\(^22\) as well as the statistical approach used minimizes false-positive results in the present study. The expression patterns were validated specifically by confirming amounts of altered as well as unvaried genes with conventional techniques. Furthermore, we aimed to address the issue of compartmentalized inflammation in cells other than PB-
Figure 2. Time course of plasma cytokines and corresponding mRNA steady-state concentration in RNA isolated from PBMCs. Dots connected by solid line represent the time course for median protein concentration in plasma samples (in pg/mL), whereas vertical lines indicate the corresponding interquartile range. Gray areas mark the interquartile range for steady-state mRNA transcripts in total RNA isolated from PBMCs. For all cytokines, chemokines, and growth factors presented, significantly enhanced protein expression was confirmed after surgery, whereas none of the corresponding mRNA transcripts were found to be significantly altered, indicating that circulating PBMCs are not the primary source of the inflammatory response reflected in occurrence of cytokines in plasma. Although in “on-pump” surgery, the increase was found already early after surgery, the increase was either missing or occurred in a delayed manner on the first postoperative day for “off-pump” patients. No significant changes were observed for the other cytokines present on the biochip. Gene transcripts and encoded proteins are arranged according to the patterns obtained in cluster analysis of the microarray data.
patients subjected to off-pump surgery on the first postoperative day. The difference in the order of magnitude and the more delayed appearance might in part reflect also the shorter duration of off-pump surgery and lesser severity of insult. In addition, prolonged mechanical ventilation in on-pump surgery results in a further retention of leukocytes in the pulmonary bed. Thus, these factors most likely influence the kinetics of the observed transcriptomic and proteomic response observed in circulating white cells.

Transcripts identified to be induced by CPB in PBMCs included genes involved in cell–cell communication, signaling, and apoptotic demise, confirming altered responsiveness of PBMCs to secondary stimuli, ie, these PBMCs were “primed.” “ Priming,” ie, a different response of a cell, an organ, or a patient to a second insult by virtue of the previous environmental history, is a particularly intriguing concept to explain the well-known increased susceptibility of patients to develop infectious complications and remote organ injury after major cardiac surgery. Regarding the complexity of the priming event, many genes have not previously been associated with on-pump surgery, and thus, their functional role in respect to the better-preserved remote-organ function in off-pump surgery remains speculative and requires further investigation. There is, however, recent evidence that exposure of cells to IFN-α, ie, a transcript found to be induced in PBMCs after on-pump surgery, primes immune cells in the way that IL-10, ie, a prototypical “antiinflammatory” cytokine released on CPB, gains proinflammatory properties.

A recent study by Feezor and colleagues using a similar combined transcriptomic and proteomic approach confirmed that 138 genes of the more than 12,000 genes on the Affymetrix chip used and 7 plasma proteins in patients undergoing thoracic aortic aneurysm repair discriminated between patients developing multiple organ failure and those who did not, and many of the differentially expressed genes were evident even before surgery. It is noteworthy that, like our present observation in their patient collective undergoing major vascular surgery without CPB, circulating leukocytes were also primed but seemingly also were not the source for circulating mediators of inflammation. Thus, a combination of transcriptomic and proteomic assessment of the response to a severe insult such as aneurysm repair or on-pump surgery provides evidence that the circulating PBMCs are “primed,” whereas the inflammatory reaction reflected in the appearance of a multitude of inflammatory mediators seems to occur in another compartment, eg, the ischemic heart or other tissues, such as lung, gut, and liver. In particular, there is evidence to suggest that a localized inflammatory reaction is induced in the reperfused heart that is lacking in off-pump patients. Similarly, a significant proportion of leukocytes is trapped in the lungs and might contribute to the transpulmonary gradient of cytokines observed after CPB. In light of these data, it is intriguing to speculate that white cells overexpressing transcripts for adhesion (eg, L-selectin) and signaling (eg, TLR4) molecules might at least temporarily migrate into the tissue to mount an inflammatory response, depending on tissue-associated paracrine stimulation of the “primed” leukocyte.

MCs by adding proteomic analysis of circulating cytokines, which indeed suggested that a compartmentalized inflammatory response takes place but is not reflected in transcriptomal changes in circulating cells. Alternatively, posttranscriptional mechanisms might also contribute to the negative result regarding changes in steady-state transcripts for circulating cytokines as observed in the present study. Nevertheless, on the basis of the current literature, we demonstrate a potential functional role for differentially regulated genes and those proteins found to be differentially induced, which are consistent with many aspects of the clinical phenotype of these patients. Using this approach, we provide here evidence that release of some cytokines and chemokines (such as IL-8, IL-10, MCP-1, and MIP-1β) is indeed associated with initiation of CPB, whereas others (such as IL-6, G-CSF, and IFN-γ) are reflecting a more generalized stress response. Although these proinflammatory gene products are induced to a similar extent in patients undergoing coronary revascularization with or without CPB, their time course of induction was strikingly different: Whereas they were induced immediately but transiently on CPB, their induction was more delayed, although reaching a similar order of magnitude in
A positive control experiment (Figure 3) confirmed induction of cytokine mRNA, which is typically substantially induced if PBMCs are the source of the cytokine response, although posttranscriptional mechanisms might contribute to increased release of mediators, at least for some mediators, such as chemokines. Nevertheless, these results suggest that moderate increases of transcripts encoding cytokines as reported for CPB patients are below the detection limit of an array experiment and are probably of limited biological significance.

Because the elective patients enrolled in the present study showed signs of some degree of reversible remote tissue injury but did not develop significant multiple organ failure, the observed activation of innate immunity with its proinflammatory and antiinflammatory mediators seems to be controlled or adaptive. Conversely, the “primed” phenotype of PBMCs might contribute to the increased susceptibility of cardiac surgical patients to infectious complications. Thus, additional studies are needed to identify those genes associated with progression of the inflammatory response to overt multiple organ failure or development of infectious complications.

In conclusion, the present data indicate that microarray technology along with multiplexed protein analysis of inflammation-associated proteins is suitable to identify those genes that underlie the attenuated inflammatory response in off-pump as opposed to conventional on-pump surgery. Data obtained with this combined approach suggest that the current paradigm, that CPB is the trigger of a short-lasting, reversible inflammatory reaction affecting circulating cells that have contact with nonphysiological surfaces of the bypass while deliberate avoidance of CPB in off-pump surgery prevents this response, is oversimplified.

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References

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### CLINICAL PERSPECTIVE

Use of cardiopulmonary bypass during open-heart surgery provides controlled operative conditions but induces a vigorous inflammatory response that contributes to postoperative complications involving organ dysfunction. Deliberate avoidance of extracorporeal circulation during surgery has been driven by the hope of decreasing inflammation-related morbidity. Using a concerted approach of transcriptomic and proteomic analyses, we compared the plasma and circulating leukocyte response in patients undergoing open-heart surgery with and without cardiopulmonary bypass. Our data demonstrate that peripheral blood mononuclear cells are not the primary source for these serum cytokines induced during cardiopulmonary bypass. We also found that transcriptional activation in circulating leukocytes affected primarily genes involved in cell–cell adhesion/communication (such as L-selectin or ICAM-2) and signaling (such as IL-1, IL-8, or IL-18 receptors and toll-like receptors 4, 5, and 6), confirming a “primed” phenotype. Interestingly, some mediators responded similarly in off-pump surgery. The finding that contact with the extracorporeal circuit was associated with induction of leukocyte adhesion molecules and signaling factors helps to explain how cardiopulmonary bypass may promote a subsequent tissue-associated inflammatory response. The data further demonstrate that the concept of simple “contact activation” with subsequent release of cytokines by circulating white blood cells is oversimplified. These findings may promote the development of targeted strategies to reduce the inflammatory complications of cardiopulmonary bypass.
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