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Genomic and Proteomic Determinants of Outcome in Patients Undergoing Thoracoabdominal Aortic Aneurysm Repair

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Thoracoabdominal aortic aneurysm repair, with its requisite intraoperative mesenteric ischemia-reperfusion, often results in the development of systemic inflammatory response syndrome, multiorgan dysfunction syndrome (MODS), and death. In the present study, an adverse clinical outcome following thoracoabdominal aortic aneurysm repair was identified by blood leukocyte genomic and plasma proteomic responses. Time-dependent changes in the expression of 146 genes from blood leukocytes were observed ($p < 0.001$). Expression of 138 genes ($p < 0.001$) and the concentration of seven plasma proteins discriminated between patients who developed MODS and those who did not, and many of these differences were evident even before surgery. These findings suggest that changes in blood leukocyte gene expression and plasma protein concentrations can illuminate pathophysiological processes that are subsequently associated with the clinical sequelae of systemic inflammatory response syndrome and MODS. These changes in gene expression and plasma protein concentrations are often observed before surgery, consistent with either a genetic predisposition or pre-existing inflammatory state. The Journal of Immunology, 2004, 172: 7103–7109.

Patients who undergo thoracoabdominal aortic aneurysm (TAAA) repair or mesenteric revascularization for chronic ischemia develop organ failure and die with alarmingly high frequency. Recenwald et al. (1) reported a mortality of 10% following elective TAAA repair, but strikingly, noted that 77% of the patients developed significant postoperative complications. A recent national review found similar mortality and complication rates of 20.3 and 62.2%, respectively, for TAAA repair (2). Reperfusion following intraoperative visceral ischemia associated with TAAA repair is presumed responsible for the release of proinflammatory cytokines and other inflammatory mediators. This activation of innate immunity may lead to the development of a systemic inflammatory response syndrome (SIRS), multisystem organ dysfunction syndrome (MODS), and even death (3, 4).

Svensson et al. (5) first reported that the duration of intraoperative postoperative morbidity following TAAA repair, others including intraoperative blood loss and shock. We subsequently observed that MODS occurred more commonly in patients undergoing TAAA repair whose intraoperative mesenteric ischemia exceeded 40 min (6). Increased circulating levels of TNF-$\alpha$, IL-6, IL-8, IL-10, and shed TNF receptors after TAAA repair were also associated with longer intraoperative mesenteric ischemic times, and the frequency of postoperative organ dysfunction correlated with elevated postoperative plasma concentrations of IL-6 and/or TNF-$\alpha$ (7).

Unfortunately, it is often difficult to identify or predict which patients will have an adverse outcome after acute injuries such as TAAA repair. The advent of microarray technology and multiplex protein analyses has permitted a large-scale survey of the genomic and proteomic responses to surgical and visceral ischemia-reperfusion injury. In the present study, gene expression patterns from peripheral blood leukocytes and circulating plasma protein levels were determined in patients undergoing TAAA repair to explore whether an assessment of genome-wide expression and inflammatory protein production could be used to discriminate between a dichotomous clinical outcome. Such approaches have been used to characterize the malignant phenotype of tumors and their responsiveness to antineoplastic therapies (8–11). The present findings demonstrate that there are differences in leukocyte gene expression and plasma protein concentrations that may identify outcome following visceral ischemia-reperfusion injury. More importantly, these patterns of genome-wide expression and plasma protein concentration are often evident before the surgical trauma and the induction of visceral ischemia-reperfusion injury.

Materials and Methods

Patient selection

Ten patients undergoing repair of thoracoabdominal aortic aneurysms were enrolled. All procedures were performed electively by one of three surgeons at a single institution over 17 mo. The study was approved by the Institutional Review Board at the University of Florida, and each patient provided signed informed consent.

Blood sample collection and RNA isolation

Whole blood was collected into heparinized phlebotomy tubes from each patient after induction of anesthesia, but before the start of surgery (pre-surgery), and at 2 and 24 h after establishment of mesenteric reperfusion. Each sample was immediately centrifuged at $4^\circ$C, 400 × g for 10 min. The
plasma was separated and saved for proteomic analysis (see below), and the buffy coat layer containing the leukocyte populations was recovered for genomic analysis. After contaminating erythrocytes were removed by lysis (buffer EL) (7) (Qiagen, Valencia, CA), total cellular RNA was isolated from the buffy coat using a commercial kit (RNaseasy) (7) (Qiagen). Purity was measured by a spectrophotometer ($A_{260}/A_{280}$ ratio), an ethidium bromide-stained RNA agarose gel, and RT-PCR amplification of a housekeeping gene (Czta superoxide dismutase) (12). Total and differential white blood cell counts were also obtained from an additional blood sample through the diagnostic laboratories at Shands Hospital (Gainesville, FL).

cRNA synthesis and chip hybridization

cRNA was synthesized from 10 μg of total cellular RNA based on the protocol outlined by Affymetrix (Santa Clara, CA), with few modifications as outlined below. cRNA was transcribed in vitro incorporating biotinylated nucleotides using an ENZO BiArray HighYield RNA Transcriptor Labeling Kit (TT) (Enzo Life Sciences, Farmingdale, NY) and hybridized onto Hu95aVer2 oligonucleotide arrays (Affymetrix). The microarrays were hybridized for 16 h at 45°C, stained, and washed according to an Affymetrix protocol (EukGE-WS2v4) using an Affymetrix fluids station, and scanned with an Affymetrix scanner. Fluorescence intensity measurements were calculated and normalized by Microarray Suite, version 5.0 (MAS 5.0; Affymetrix).

Microarray data analysis

After excluding Affymetrix control probe sets, each microarray detected the hybridization intensity of 12,558 probe sets. A total of 5,163 probe sets (41%) was never detected above background intensity levels on any array, and was removed from the dataset. Clustering analysis was performed on a normalized variance of each remaining probe set wherein the mean expression, while allowing for tuning to control the false discovery rate (FDR). The variation in hybridization intensity used to infer expression of the genes represented by the probe sets identified in this manner was clustered using Cluster (Eisen Laboratory, Stanford University) and displayed with TreeView (Eisen Laboratory) (14).

For each time point studied, further analysis was performed on the mean fold change in expression level for each probe set between patients who developed MODS and those who did not. MicroArray Pathway Profiler Finder (MAPPFinder) (Gladdstone Institutes, University of California, San Francisco, CA) (15) identified classes of genes according to the Gene Ontology Consortium (16) that were statistically overrepresented among genes 2-fold up- or down-regulated (MODS vs no MODS) at each time point. MAPPFinder uses a statistical probability to rank the likelihood that the specific gene ontology is represented in the gene list of interest more often than would occur by chance alone.

Proteomic analyses

Plasma was obtained by whole blood centrifugation at 4°C, 400 × g before surgery, and after induction of anesthesia, immediately after the reperfusion, and at 1, 2, 4, 6, 8, 24, 48, and 72 h after reperfusion. Samples were drawn into EDTA-containing tubes, except when the samples corresponded to those designated for genomic analysis (presurgery, and 2 and 24 h after reperfusion). After initial centrifugation of the whole blood, plasma was removed and frozen at 80°C until further analysis. Patient plasma samples were analyzed using the Zymox Protein Profiling Biochip System and the Zymox Human Cytokine Biochip (Hayward, CA). The Human Cytokine Biochip measures 30 different cytokines, chemokines, and growth factors in a multiplexed assay format. The samples were assayed without any sample treatment. Duplicate data were collected, with each assay performed on separate biochips. Quantification of cytokine concentrations was obtained through six-point multianalyte calibration run concurrently with the sample biochips. Median feature intensity was background subtracted after outlier removal, and statistical significance of the signal intensity above background was determined for a 95% confidence interval with a modified Z-factor parameter calculated with 2 SDs (17).

To analyze the differential plasma concentrations of the 30 proteins between patients who developed MODS and those who did not, statistical significance was estimated by a modified $t$ test with permutations of data as described in the SAM software program (13). Because of the limited number of analytes, probability values were reported, instead of FDRs. A conservative significance level of $p < 0.003$ was used, corresponding to probability <0.1 after Bonferroni correction for multiple testing ($p$-B).

Clinical organ failure assessment

Clinical postoperative organ failure was based on the Modified Brussels Organ Failure Score (18). This system uses physiologic parameters in six different organ systems to assess multiorgan dysfunction, and each patient received an organ failure score daily for 14 postoperative days. Statistical analyses on clinical data were performed by Student’s $t$ test or Mann-Whitney rank sum test, where appropriate, and are reported as mean ± SD.

Results

The mean age of the patients was 71.0 ± 6.0 years (range 64–85), and five of the patients were male (50%). Comorbidities included tobacco use (100%), hypertension (70%), chronic obstructive pulmonary disease (40%), coronary artery disease (40%), peripheral vascular occlusive disease (40%), diabetes mellitus (30%), and cerebrovascular occlusive disease (20%). The mean aortic cross clamp time among patients was 49.8 ± 18.5 min, and the estimated intraoperative blood loss was 4510 ± 5180 ml. The mean hospital length of stay was 28.3 ± 17.3 days.

The mean postoperative organ failure score among all 10 patients was 3.20 ± 1.93. The 5 patients who developed multiorgan dysfunction by definition had higher MODS scores than those who did not (5.0 vs 1.4, respectively). Patients who developed MODS also had a longer duration of intraoperative visceral ischemia (62.4 ± 13.3 vs 37.2 ± 15.5 min, $p = 0.025$), higher intraoperative blood loss (6700 ± 6900 ml vs 2300 ± 490 ml, $p = 0.032$), and a longer postoperative hospitalization (42.6 ± 8.9 days vs 14.0 ± 9.1 days, $p < 0.001$). The organ system most often rendered dysfunctional was pulmonary, followed, in order, by the coagulation system, renal system, CNS, cardiac, and hepatic system. Three of the patients with MODS died, giving an overall operative mortality of 30%.

Time-dependent changes in gene expression were present in peripheral blood leukocytes. As shown in Fig. 1, the expression of 152 probe sets (with an FDR of 8 or 5%) representing 146 genes discriminated among presurgery, 2 h, and 24 h after reperfusion (Fig. 1 and Supplemental Table A). Although the separation in gene expression among the time points was not perfect, examination of the dendrogram revealed that the 24-h expression patterns clustered separately (with the exception of one sample) from both the preischemia and 2-h patterns. This temporal expression pattern could be broken down into several categories, depending upon the kinetics of gene expression. The expression of 119 genes after surgery, whereas the expression of 27 genes decreased. The largest category identified 78 genes whose expression increased 24 h after reperfusion injury, followed by 41 genes whose expression increased at 2 h. Several of the genes whose expression increased early after reperfusion injury were expected early activation genes, and genes involved in the innate immune response (Supplemental Table A). They included leukocyte secretory products involved in innate immunity (perforin, granzyme B, TNF-α-induced protein 3), cytokines and their receptors (TNFβ, CXC3), and signal transduction intermediates (STAT4, cytokine-inducible kinase, mitogen-activated protein kinase, mitogen-activated protein kinase, kinase, C/EβPa, C/EβPa). In contrast, genes whose expression decreased after the surgery included a number of MHC class II peptides, and other cytokine receptors (CXC1, IL-2Rβ) chain.

The more relevant question was whether gene expression patterns from patients who developed MODS differed from patterns seen in patients without MODS, and when these patterns were evident. A supervised analysis was performed to distinguish gene expression patterns between patients who did and did not develop MODS. As shown in Fig. 2, the expression of 146 probe sets (with

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* The on-line version of this article contains supplemental material.
an FDR of 8 genes, 5%) representing 138 genes delineated the clinical dichotomous outcome (Fig. 2 and Supplemental Table B). Of these 146 probe sets, 88 (57.5%) were up-regulated in patients who developed MODS, while 58 (42.5%) were down-regulated. Importantly, the differential expression of these associative genes was exhibited in circulating leukocytes at all three time points, even before the surgery. More interestingly, the genes whose expression discriminated outcome (Fig. 2) were not the same genes whose expression changed with time after the surgical injury (Fig. 1); only one probe set was in common (probe set 3397_at, U67369, growth factor independent 1).

Among those 88 probe sets whose expression was up-regulated in patients who subsequently developed MODS were tropomyosin I, TGF-α-binding and -stimulated proteins, α-integrin, matrix metalloproteinase-8, ornithine decarboxylase, and two heat shock proteins (heat shock proteins 70 and 90). Genes down-regulated in patients who developed MODS included several MHC class I proteins (HLA-G- and HLA-B-associated transcript), an apoptosis-associated tyrosine kinase, G-CSF receptor 3, an IFN regulatory factor, L-selectin, and an IL-4-induced transcription factor activator.

We subsequently asked whether the changes in gene expression after surgery or in patients who developed MODS were secondary to changes in the differential leukocyte pattern. Gene expression profiles were based on a constant amount of leukocyte RNA, and variations in the differential count could explain the changes in gene expression. As shown in Table I, however, the total and differential leukocyte count did not change dramatically over the first 24 h, with the exception that the percentage of lymphocytes declined with time. More importantly, the differential leukocyte counts did not significantly differ in the first 24 h when the genomic analyses were performed between patients going on to develop MODS, and those who did not. Therefore, it is unlikely that the differences in expression can be simply explained by differences in the leukocyte pattern.

In addition, it was noted that several of these genetic alterations were present before intraoperative aortic occlusion. Among the genes whose global expression differed between patients who progressed to
develop MODS and those who did not, several genes exhibited significantly higher apparent levels of expression before mesenteric ischemia. As listed in Table II, matrix metalloproteinase-8 (neutrophil collagenase) was nearly 8-fold higher before aortic occlusion in patients who developed MODS. Conversely, expression of several of the Ig H chain peptides was markedly decreased preoperatively.

For each of the time points studied, MAPPFinder was used to identify overrepresentation of gene ontologies among genes that were 2-fold up-regulated or down-regulated in patients who developed MODS compared with those who did not (Table III). Gene ontologies overrepresented before the ischemic injury in patients who subsequently developed MODS included members of the chymotrypsin and trypsin, and xenobiotic metabolism families whose expression was increased. By contrast, genes overrepresented in patients who did not develop MODS included Ag-binding and histocompatibility genes whose expression was consistently down-regulated. Two hours after reperfusion, patients with MODS exhibited overrepresentation of the family of heat shock protein genes, and genes involved in defense response, immune response, cell adhesion, cellular defense, and cell motility. These genes were overexpressed, and many were shown to be persistently up-regulated at 24 h. Surprisingly, decreased expression of the Ag-binding and histocompatibility family of genes was overrepresented at all three time points studied in patients who did not develop MODS (Table III).

The global patterns of plasma protein concentrations similarly yielded divergent responses between patients who developed MODS and those who did not. Examination of the normalized expression levels revealed concentrations of several cytokines in patients who went on to develop MODS in contrast to those who did not (Fig. 3). For example, the mean circulating levels of monocyte chemoattractant protein (MCP)-1 (p-B<0.0001), IFN-γ-inducible protein-10 (IP-10) (p-B<0.0001), monokine induced by IFN-γ (MIG) (p-B<0.0001), IL-10 (p-B = 0.077), IL-6 (p-B = 0.0034), and soluble ICAM-1 (p-B = 0.0038) were all significantly increased, irrespective of time, from patients who developed MODS, and those who did not.

Interestingly, when plasma cytokine concentrations were compared in only the preaortic clamp samples, several analytes were markedly increased in circulating quantity in those patients who progressed to MODS compared with those who did not. For example, soluble CD23 was, on average, 52.4-fold higher among patients who developed MODS. However, due to the small sample size, only IP-10, which was 3.9-fold greater in MODS patients, reach statistical significance (p-B<0.1) preoperatively.

FIGURE 2. A supervised analysis revealed that the expression pattern of 146 probe sets (138 genes) could discriminate between patients who developed MODS (fuchsia blocks) and those who did not (green blocks). A detailed explanation for the dendrogram and cluster analysis is provided in the legend for Fig. 1. Presurgical patterns of gene expression did not cluster together, but rather were equally distributed between patients with and without the subsequent development of MODS. These findings suggest that the divergent patterns of gene expression were already apparent presurgery in this patient population.
of lymphocytes decreased significantly from baseline (markedly elevated or depressed preoperatively in patients who developed MODS compared with those who did not). Among the genes whose expression differentiated between dichotomous clinical outcomes, the apparent expression of several genes was associated with an adverse clinical outcome. Several of these genes are involved in the innate immune response, and reflect activation of leukocyte subpopulations (neutrophil collagenase (matrix metalloproteinase-8), RANTES, pentaxin-related gene, monocyte to macrophage differentiation-associated factor, platelet factor-4, and vascular endothelial growth factor). Others represent a generalized stress response (increased expression of heat shock proteins, and proteins of the ubiquitin-proteosome system). Simultaneously, there was a down-regulation in the expression of several genes involved in the MHC class I response.

Table I. Total and differential leukocyte counts (neutrophils and lymphocytes) obtained preoperatively, and at 2 and 24 h post-surgery, and according to clinical outcome (MODS vs no MODS)

<table>
<thead>
<tr>
<th>By time (hours)</th>
<th>WBC Total</th>
<th>% Neutrophils</th>
<th>% Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.0 ± 4.4</td>
<td>77.3 ± 11.6</td>
<td>14.8 ± 5.7</td>
</tr>
<tr>
<td>2</td>
<td>14.6 ± 9.7</td>
<td>88.5 ± 10.7</td>
<td>8.1 ± 9.6</td>
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<tr>
<td>24</td>
<td>12.8 ± 4.7</td>
<td>88.3 ± 6.9</td>
<td>5.7 ± 4.5</td>
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</table>

By outcome

<table>
<thead>
<tr>
<th>MODS</th>
<th>WBC Total</th>
<th>% Neutrophils</th>
<th>% Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.7 ± 9.0</td>
<td>84.7 ± 14.7</td>
<td>9.9 ± 11.7</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No MODS</th>
<th>WBC Total</th>
<th>% Neutrophils</th>
<th>% Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.6 ± 4.3</td>
<td>85.3 ± 6.8</td>
<td>8.5 ± 5.5</td>
<td></td>
</tr>
</tbody>
</table>

The only significant difference is marked with an asterisk, where the percentage of lymphocytes decreased significantly from baseline ($p < 0.05$).

**Discussion**

SIRS is the clinical manifestation of a nonspecific activation of the immune system commonly observed following multiple trauma, thermal injury, pancreatitis, or visceral ischemia-reperfusion injury. The incidence of MODS and subsequent death with SIRS is alarmingly high, and despite relative success in animal models of systemic inflammation, most current clinical approaches are limited to supportive therapy. Current injury severity scoring systems provide a static assessment of a patient’s physiological condition, but, at present, there are no reliable methods to predict which patients will develop SIRS or MODS. Patients with similar baseline characteristics and nearly identical injuries often have very differing clinical outcomes.

Although the mechanisms by which SIRS progresses to MODS are complex and not fully resolved, inappropriate activation of the innate immune response has been implicated from preclinical and animal studies (3). Unfortunately, clinical trials aimed at inhibiting the inflammatory mediators responsible for the propagation of SIRS have generally failed to improve outcome (19, 20).

Patients undergoing TAAA repair afford unique opportunities to study the SIRS response because the patient population is relatively homogeneous, the injury is reproducible, the timing of the injury is precisely known, and the incidence of MODS is high. In the present study, we investigated whether genome-wide leukocyte gene expression patterns or plasma protein levels, as indicators of the innate immune response, could identify patients at risk of developing MODS.

For genome-wide analyses, we used the Affymetrix U95a oligonucleotide microarray, which simultaneously interrogates 12,558 probe sets. These expression patterns represent a significant fraction of the entire human transcriptome, estimated to be the product of roughly 30,000 genes (21). In addition, we measured simultaneously the plasma concentration of 30 analytes. A supervised analysis identified 146 genes and 7 plasma proteins whose patterns were associated with an adverse clinical outcome. Several of these genes are involved in the innate immune response, and reflect activation of leukocyte subpopulations (neutrophil collagenase (matrix metalloproteinase-8), RANTES, pentaxin-related gene, monocyte to macrophage differentiation-associated factor, platelet factor-4, and vascular endothelial growth factor). Others represent a generalized stress response (increased expression of heat shock proteins, and proteins of the ubiquitin-proteosome system). Simultaneously, there was a down-regulation in the expression of several genes involved in the MHC class I response.

Table II. Among the genes whose expression differentiated between dichotomous clinical outcomes, the apparent expression of several genes was markedly elevated or depressed preoperatively in patients who developed MODS compared with those who did not

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Name</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>681_at</td>
<td>Matrix metalloproteinase-8 (neutrophil collagenase)</td>
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</tr>
<tr>
<td>32821_at</td>
<td>Lipocalin 2 (oncogene 24p3)</td>
<td>7.23</td>
</tr>
<tr>
<td>1491_at</td>
<td>Pentaxin-related gene, rapidly induced by IL-1β</td>
<td>3.41</td>
</tr>
<tr>
<td>266_s_at</td>
<td>CD24 Ag (small cell lung carcinoma cluster 4 Ag)</td>
<td>3.07</td>
</tr>
<tr>
<td>996_g_at</td>
<td>Protein tyrosine phosphatase, receptor type, M</td>
<td>2.97</td>
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<tr>
<td>41618_at</td>
<td>Collagen, type XVII, α1</td>
<td>2.88</td>
</tr>
<tr>
<td>35919_at</td>
<td>Transthyretin 1 (vitamin B12-binding protein, R binder family)</td>
<td>2.41</td>
</tr>
<tr>
<td>36790_at</td>
<td>Tropomyosin 1 α</td>
<td>2.39</td>
</tr>
</tbody>
</table>

**Table II.** Among the genes whose expression differentiated between dichotomous clinical outcomes, the apparent expression of several genes was markedly elevated or depressed preoperatively in patients who developed MODS compared with those who did not

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Name</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>40951_at</td>
<td>Homo sapiens mRNA; cDNA DCFZp564D113</td>
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<tr>
<td>33499_s_at</td>
<td>Ig heavy constant μ</td>
<td>0.29</td>
</tr>
<tr>
<td>33382_at</td>
<td>N-acetylsphingosine amidohydrolase (acid ceramidase)-like</td>
<td>0.38</td>
</tr>
<tr>
<td>40850_at</td>
<td>FK506-binding protein 8 (38 kDa)</td>
<td>0.43</td>
</tr>
<tr>
<td>40282_s_at</td>
<td>D component of complement (adipsin)</td>
<td>0.47</td>
</tr>
<tr>
<td>34105_f_at</td>
<td>Ig heavy constant y3 (G3m marker)</td>
<td>0.48</td>
</tr>
</tbody>
</table>

a The fold change listed below refers to apparent raw expression of patients who developed MODS compared with those who did not preoperatively.
patients may be identified by differences in their patterns of leukocyte activation, gene expression, and plasma protein concentrations.

By surveying 30 known cytokines simultaneously, we observed patterns of circulating concentrations of several of these proteins to be associated with an adverse clinical outcome. In the present study, circulating levels of MCP-1, IL-10, MIG, IL-6, IL-8, IP-10, and sCD23 were all significantly elevated in patients who progressed to MODS. Several of these observations are novel, and
have not been reported previously in patients with SIRS and MODS. Although there have been a number of studies examining the prognostic ability of increased serum IL-6, IL-8, and IL-10 concentrations (7, 24–26), there has been only a limited exploration for many of these other analytes in acute inflammatory conditions. For example, although increased MCP-1 concentrations have been reported in patients with sepsis (27), this is the first documentation that serum MCP-1, IP-10, sCD23, and MIG concentrations are increased in patients at risk of developing MODS and death after surgically induced, ischemia-reperfusion injury. The sCD23 data are particularly interesting because the concentrations of this marker of B cell differentiation have only been reported in patients with cancer or chronic autoimmune or inflammatory diseases, such as type I diabetes (28). Similarly, increases in MIG and IP-10 concentration have not been previously reported in SIRS patients progressing to MODS. It should be noted, however, that these preliminary results from a small number of patients lack the statistical power to predict clinical behavior based on a single analyte or an individual time point. Furthermore, we have not attempted to correlate the genomic and proteomic data in any multivariate analysis because of the small sample sizes. It is interesting, however, that there was very little correlation between the changes in gene expression profiles from the whole blood leukocytes, and the corresponding protein concentrations in the plasma. Although we have focused on the genomic response by circulating leukocytes, our earlier studies have suggested that these cell populations are generally not responsible for the appearance of the immunoinflammatory cytokines in the blood (29). Rather, inflammatory cells in the splanchic bed, and not peripheral blood leukocytes, appear to be the primary source of blood-derived TNF-α and IL-6.

Based on these results, however, it is evident that there are broad patterns of leukocyte gene expression and protein production that change in patients undergoing surgical aortic reconstruction and associated visceral and/or lower torso ischemia-reperfusion. Furthermore, there are also distinct patterns of gene expression and protein production before and following the ischemic injury that are associated with the subsequent development of MODS. In general, a global view of the leukocyte gene expression and plasma protein responses is consistent with the earlier observation that patients who develop MODS following this surgical injury are generally manifesting an exaggerated innate immune and inflammatory response. This conclusion is confirmed by the increased plasma concentration of a number of cytokines involved in the innate immune and inflammatory response, as well as by the over-representation of gene ontologies associated with the inflammatory response. However, equally importantly, the genome-wide expression analyses suggest that the changes in leukocyte gene expression associated with the development of MODS in these patients are much more complex. An adverse outcome is associated with an integrated up-regulation of genes involved in the heat shock response, ubiquitin-proteosome-dependent protein degradation, and the increased expression of extracellular proteins (peptidases), as well as a sustained decrease in the expression of genes involved in the MHC class I response. These studies are of course preliminary, and future investigations will be required to confirm these studies in a prospective manner, and to determine whether the changes in gene expression are merely associative or causative in nature.

References