Traumatic Injury, Early Gene Expression, and Gram-Negative Bacteremia*

Callie M. Thompson, MD1; Chin H. Park, MD1,2; Ronald V. Maier, MD1; Grant E. O’Keefe, MD1

Objectives: Bacteremic trauma victims have a higher risk of death than their nonbacteremic counterparts. The role that altered immunity plays in the development of bacteremia is unknown. Using an existing dataset, we sought to determine if differences in early postinjury immune-related gene expression are associated with subsequent Gram-negative bacteremia.

Design: Retrospective cohort study, a secondary analysis of the Glue Grant database.

Setting: Seven level I trauma centers across the United State.

Subjects: Severely injured blunt trauma patients.

Interventions: None.

Measurements and Main Results: Total leukocyte gene expression was compared between the subjects in whom Gram-negative bacteremia developed and those in whom it did not develop. We observed that Gram-negative bacteremia was an independent risk factor for death (odds ratio, 1.86; p = 0.015). We then compared gene expression at 12 and 96 hours after injury in 10 subjects in whom subsequently Gram-negative bacteremia developed matched to 26 subjects in whom it did not develop. At 12 hours, expression of 64 probes differed more than or equal to 1.5-fold; none represented genes related to innate or adaptive immunity. By 96 hours, 102 probes were differentially expressed with 20 representing 15 innate or adaptive immunity genes, including down-regulation of *IL1B* and up-regulation of *IL1R2*, reflecting suppression of innate immunity in Gram-negative bacteremia subjects. We also observed down-regulation of adaptive immune genes in the Gram-negative bacteremia subjects.

Conclusions: By 96 hours after injury, there are differences in leukocyte gene expression associated with the development of Gram-negative bacteremia, reflecting suppression of both innate and adaptive immunity. Gram-negative bacteremia after trauma is, in part, consequence of host immunity failure and may not be completely preventable by standard infection-control techniques. (Crit Care Med 2014; 42:1397–1405)

Key Words: Gram-negative bacteremia; immunity; trauma

Traumatic injury is the leading cause of death among young people and the fifth leading cause of death overall in the United States (1). The majority of these deaths occur immediately after the injury. Those who survive this initial time period are at risk for developing nosocomial infections. Bacteremia, one of many possible nosocomial infections, is associated with poor outcomes, and its prevalence, particularly due to Gram-negative organisms, is increasing (2–5). We observed that 14% of trauma victims with ventilator-associated pneumonia (VAP) had concomitant bacteremia which was associated with markedly increased length of ICU stays and a 2.5-fold increased risk of death (6).

Immune competence requires the detection, recognition, and destruction of invading microorganisms. The ability of bacteria to access and survive in the bloodstream likely represents a profound failure of the immune response (7). Impaired immune competence after trauma is common. However, it has not been possible to correlate postinjury immune and inflammatory alterations with subsequent infectious complications.

The “Inflammation and the Host Response to Injury” program was a multicenter collaborative research project designed to increase our understanding of the host’s response to injury and the severe systemic inflammatory response that accompanies it. The data and samples collected as part of this program have improved our understanding of the overall response to traumatic injury and the development of subsequent complications. The data have also allowed us to address two objectives regarding bacteremia. First, we sought to confirm whether
bacteremia was associated with a poor prognosis, as we previously observed in trauma patients with VAP. Second, we tested whether early gene expression patterns in patients with bacteremia differed from other severely injured patients.

**MATERIALS AND METHODS**

**Patient Recruitment and Enrollment**

We analyzed data gathered from the Inflammation and the Host Response to Injury research program. The organization details, overall scope, and patient recruitment procedures of the program have been previously described (8). Briefly, 1,819 subjects were enrolled from seven U.S. level I trauma centers from 2003 to 2009. Subjects were enrolled if they had a blunt injury mechanism, had prehospital or emergency department (ED) hypotension (systolic blood pressure [SBP] < 90 mm Hg) or an elevated base deficit (> 6 mEq/L), required blood transfusion within the first 12 hours after injury, and had an Abbreviated Injury Scale (AIS) score of more than 2 for any body region other than the brain. The demographic information that was collected included age, gender, body mass index (BMI), measurements of severity of injury and illness (Injury Severity Score [ISS], Acute Physiology and Chronic Health Evaluation [APACHE] II, and AIS scores), and details of each subject's hospital course including the development of Gram-negative bacteremia (GNB).

This is a secondary analysis of de-identified data and is therefore classified as exempt by the University of Washington Human Subjects Division.

**Statistical Analysis**

Clinical data were evaluated using Stata 12 statistical software (StataCorp LP, College Station, TX). Continuous data, with the exception of the gene expression values, are presented as medians and range and were compared using Mann-Whitney U tests. Categorical data are presented as values and percentages and were compared using chi-square test or Fisher exact test. Actual p values for all comparisons are presented. Both univariate and step-wise logistic regressions were performed to determine the risk of death associated with bacteremia. Variables were included in the final regression model if their individual p value for association with death was less than or equal to 0.1. Graphs were made using Prism (GraphPad Software, La Jolla, CA).

**Propensity Score Matching**

To eliminate some of the inherent heterogeneity in the gene expression values of our cohort, we matched each subject in whom GNB developed to two to three subjects in whom GNB did not develop (non-GNB) using propensity scores for the development of GNB (17). The propensity score for the development of GNB was predicted from a multivariate logistic regression model that included the following variables: age; gender; BMI; ISS; APACHE II scores; AIS scores for the head, face, neck, chest, abdomen, spine, and extremities; lowest SBP and Glasgow Coma Scale from their ED stay; the ED base deficit; maximum blood glucose level in the first 24 hours; the number of units of packed RBCs (PRBCs); and volume of crystalloid received in the first 12 hours after injury. Using nearest neighbor matching, each subject with GNB was matched to two to three subjects without GNB based on their proximity in the database which also represented sequential enrollment in the study. Matching was done using ± 0.05 of the propensity score.

**Gene Expression Data**

The method for the collection of samples and generation of transcriptional profiles has been previously reported (8, 18). Patient samples were applied to Affymetrix U133 plus 2.0 GeneChip platforms (Affymetrix, Santa Clara, CA), and the intensity signal was uploaded to the Trauma Research Database as CEL files. Only the CEL files of subjects with multiple time points and RNA quality more than or equal to 2 were included in the genomic analysis. The CEL files were perfect-matched normalized in dChip (Boston, MA) prior to analysis (19). The signals were then converted and exported as expression values. Settings for the dChip analysis can be found in the supplemental material (Supplemental Digital Content 1, http://links.lww.com/CCM/A861).

**Gene Expression Analysis**

The normalized expression values were adjusted for batch effect using the open access R program (Boston, MA), ComBat.R for samples from time points of 12 and 96 hours postinjury to minimize nonbiological variation (20). ComBat.R processes and settings are detailed in Supplemental material (Supplemental Digital Content 1, http://links.lww.com/CCM/A861). The normalized and batch-corrected expression values for the subjects with GNB and matched subjects without GNB were then imported into GenePattern (Broad Institute, MIT, Cambridge, MA) (21). Using ComparativeMarkerSelection (22–25), the values were analyzed to determine the probe sets that were differentially expressed between the subjects with GNB and those without at each time point (12 hr and 96 hr after injury) using a two-sided t test. The output of the analysis includes a ranking of the probe sets based on the value of the t test and adjusts for multiple hypothesis testing using false discovery rate. Details of the settings used in GenePattern can be found in Supplemental material (Supplemental Digital Content 1, http://links.lww.com/CCM/A861). Then, using the ExtractComparativeMarkerSelection feature in GenePattern, we exported the top 200 ranked probe sets. Using a cutoff of more than or equal to 1.5-fold differential expression, the probe sets representing genes that are known to be involved in either the innate or adaptive immune system were identified and compared with the expression of the same probe sets at the
RESULTS

Characteristics of the Entire Cohort
Data for 1,819 subjects were available and extracted from the database (data abstraction in June 2011). Of those subjects, 224 had an ICU stay of less than 3 days and were excluded from our analysis. The remaining 1,595 subjects were included in our initial analysis.

Patient and injury characteristics and outcomes are included in Table 1 and summarized here. One third of subjects had a severe traumatic brain injury, two thirds had severe chest injury, and nearly half had severe abdominal injury. GNB developed in 8% of subjects and Gram-positive bacteremia developed in 8% of subjects. They had long ICU and hospital length of stays and inhospital mortality of 11%.

Gram-Negative, But Not Gram-Positive, Bacteremia Is Associated With Increased Mortality
GNB developed in 127 subjects and Gram-positive bacteremia occurred in 131. A number of factors were associated with mortality, and these are shown in Table 2. GNB was found to be an independent risk factor for death (odds ratio [OR] 1.86; 95% CI, 1.13–3.08; p = 0.015). However, Gram-positive bacteremia was not. Other factors that were associated with death included age, APACHE II score, and units of PRBCs in first 12 hours. The results of the logistic regression analysis are shown in Table 3.

We then focused on determining whether there were differences in early gene expression in these subjects that could explain a predisposition to subsequent GNB.

Selection of Microarrays by Propensity Score Matching
A total of 121 subjects had total leukocyte microarray data that met sample quality specifications at 12 and 96 hours after injury. Bacteremic subjects differed markedly from the nonbacteremic subjects as summarized in Table 4. Both amount of blood transfusion received and ISS have been associated with increased risk of bacteremia following trauma (26). Additionally, transfusion of PRBCs alters gene expression, typically seen as an up-regulation of inflammatory genes (27). To minimize the impact of potential confounding factors, we used propensity score matching to select patients, and therefore microarrays, that were as closely matched as possible on factors that contribute to the risk of GNB and that might also directly affect gene expression. Propensity scores allowed us to match the 10 subjects with GNB to 26 subjects without (Table 5).

Genome-Wide Expression Analysis
A heatmap of the top 100 differentially expressed at 12 hours probe sets is shown in Figure 1B. Of the top ranked probes with more than or equal to 1.5-fold differential expression, 20 represented 15 genes in the innate or adaptive immune system. The expression data for all 20 probe sets at both 96 and 12 hours after injury can be found in Supplemental Table 1 (Supplemental Digital Content 1, http://links.lww.com/CCM/A861).

A heatmap illustrating the top 100 differentially expressed probe sets is shown in Figure 1B. Of the top ranked probes with more than or equal to 1.5-fold differential expression, 20 represented 15 genes in the innate or adaptive immune system. The expression data for all 20 probe sets at both 96 and 12 hours after injury can be found in Supplemental Table 1 (Supplemental Digital Content 1, http://links.lww.com/CCM/A861).

Analysis of Individual Immune-Related Genes
At the 96-hour time point, 10 inflammatory, two counter-regulatory, and three adaptive immune system genes were differentially expressed. These include interleukin-1β (IL1B) (Fig. 2A) and interleukin-2 receptor β (IL2RB) (Fig. 2B). IL1B expression was similar between groups at 12 hours, but at 96 hours, the subjects in whom GNB developed
had a progressive decrease in gene expression, whereas the expression of IL1B in subjects in whom GNB did not develop remained relatively constant. Expression of IL2RB followed a similar pattern. Granzyme A, chemokine (C-X3-C motif) receptor 1, chemokine (C-C motif) ligand 3-like 3 and 1, T-cell receptor \( \alpha \), killer cell lectin-like receptor subfamily B, member 1, T-cell activation RhoGTPase activating protein, and CD86 molecule expressions were also decreased at 96 hours after injury in the subjects in whom GNB developed.

Counter-regulatory interleukin-1 receptor type II (IL1R2) (Fig. 3A) and CD163 molecule (CD163) (Fig. 3B) were increased at 96 hours after injury in the subjects in whom GNB developed compared with those in whom it did not develop. Specifically, IL1R2 expression was similar between the two groups at 12 hours. At 96 hours, expression remained relatively higher in those destined to develop GNB. Conversely, CD163 expression was similar between the two groups at 12 hours but decreased to a far greater degree by 96 hours in patients without bacteremia.

Human leukocyte antigen (HLA) genes showed decreased expression at 96 hours after injury in the subjects in whom GNB developed (Fig. 4). Concentrating on the two genes highlighted in the figure, for both HLA-DRA and HLA-DMB, expression was similar between the two groups at the 12-hour time point, and at 96 hours, the expression in the subjects in whom GNB developed persisted, whereas the expression in the subjects in whom GNB did not develop showed a progressive increase.

**DISCUSSION**

Our analysis of genome-wide gene expression demonstrates important differences between patients who subsequently developed GNB and those who did not. Up until 12 hours after

<table>
<thead>
<tr>
<th>Variable</th>
<th>Died (n = 181)</th>
<th>Survived (n = 1,414)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ( \geq 56 ) yr</td>
<td>74 (41)</td>
<td>311 (22)</td>
<td>(&lt; 0.0001)</td>
</tr>
<tr>
<td>Male gender</td>
<td>116 (64)</td>
<td>927 (66)</td>
<td>0.7</td>
</tr>
<tr>
<td>Body mass index</td>
<td>30 (25–70)</td>
<td>30 (25–70)</td>
<td>0.23</td>
</tr>
<tr>
<td>Injury Severity Score</td>
<td>34 (29–49)</td>
<td>34 (22–41)</td>
<td>(&lt; 0.0001)</td>
</tr>
<tr>
<td>Acute Physiology and Chronic Health Evaluation II ( \geq 34 )</td>
<td>105 (58)</td>
<td>288 (20)</td>
<td>(&lt; 0.0001)</td>
</tr>
<tr>
<td>Severe head injury</td>
<td>87 (48)</td>
<td>488 (34)</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>Severe chest injury</td>
<td>129 (71)</td>
<td>926 (66)</td>
<td>0.12</td>
</tr>
<tr>
<td>Severe abdominal injury</td>
<td>75 (41)</td>
<td>630 (45)</td>
<td>0.43</td>
</tr>
<tr>
<td>Initial base deficit (mEq/L)</td>
<td>9 (6–13)</td>
<td>8 (6–11)</td>
<td>( 0.005)</td>
</tr>
<tr>
<td>Lowest emergency department systolic blood pressure (mm Hg)</td>
<td>77 (63–90)</td>
<td>84 (73–98)</td>
<td>(&lt; 0.0001)</td>
</tr>
<tr>
<td>&gt; 3 U packed RBCs in first 12 hr</td>
<td>87 (48)</td>
<td>307 (22)</td>
<td>(&lt; 0.0001)</td>
</tr>
<tr>
<td>Total crystalloid in first 12 hr (L)</td>
<td>11 (7–15)</td>
<td>9.6 (7–13)</td>
<td>(0.003)</td>
</tr>
<tr>
<td>Gram-negative bacteremia</td>
<td>27 (15)</td>
<td>100 (7)</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>Gram-positive bacteremia</td>
<td>13 (7)</td>
<td>118 (8)</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Severe head/chest/abdominal injury = body region abbreviated injury score \( \geq 3 \).

Categorical data are presented as count (%) and compared using chi-square test; continuous data are presented as median (interquartile range) and compared with Mann-Whitney U test. Boldface values indicate statistical significance as defined as \( p \leq 0.05 \).

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR for Death</th>
<th>95% CI</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-negative bacteremia</td>
<td>1.86</td>
<td>1.13–3.08</td>
<td>0.015</td>
</tr>
<tr>
<td>Age ( \geq 56 ) yr</td>
<td>2.33</td>
<td>1.4–3.86</td>
<td>0.001</td>
</tr>
<tr>
<td>Acute Physiology and Chronic Health Evaluation II ( \geq 34 )</td>
<td>5.17</td>
<td>2.73–9.79</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>Packed RBCs &gt; 3 units in first 12 hr</td>
<td>2.94</td>
<td>1.58–5.45</td>
<td>0.001</td>
</tr>
</tbody>
</table>

OR = odds ratio.

Gram-positive bacteremia, gender, Injury Severity Score, volume of crystalloid infused in first 12 hr, lowest systolic blood pressure in emergency department, maximum glucose in first 24 hr, initial base deficit, and severe head injury were included in the regression and were not significant risk factors for mortality.

**TABLE 2. Risk Factors for Mortality (\( n = 1,595 \))**

**TABLE 3. Logistic Regression Results, Adjusted Risk of Death**
### TABLE 4. Demographics, Injury Characteristics, and Outcomes by Development of Gram-Negative Bacteremia for Total Cohort ($n = 1,595$)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subjects in Whom GNB Developed ($n = 127$)</th>
<th>Subjects in Whom GNB Did Not Develop ($n = 1,468$)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injury Severity Score</td>
<td>36 (9–66)</td>
<td>34 (1–75)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Acute Physiology and Chronic Health Evaluation II</td>
<td>32 (13–49)</td>
<td>29 (7–59)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Severe abdominal injury</td>
<td>71 (55.9)</td>
<td>634 (43.2)</td>
<td>0.006</td>
</tr>
<tr>
<td>Initial base deficit (mEq/L)</td>
<td>–9.5 (–28.5 to 8)</td>
<td>–8 (–30 to 11)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total packed RBCs transfusion in first 12 hr (U)</td>
<td>2.8 (0–30)</td>
<td>1.5 (0–25)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Total crystalloid in first 12 hr (L)</td>
<td>12.3 (2–47)</td>
<td>9.6 (0–58.1)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Maximum glucose in first 24 hr (mg/dL)</td>
<td>212 (102–669)</td>
<td>191 (75–579)</td>
<td>0.002</td>
</tr>
<tr>
<td>Ventilator days</td>
<td>19 (2–85)</td>
<td>7 (0–135)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>ICU length of stay (d)</td>
<td>22 (4–123)</td>
<td>10 (3–142)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Length of stay (d)</td>
<td>31 (3–239)</td>
<td>20 (3–354)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Mortality</td>
<td>27 (21.3)</td>
<td>154 (10.5)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

GNB = Gram-negative bacteremia, Severe head/chest/abdominal injury = body region abbreviated injury score $\geq 3$.

Categorical data are presented as count (%) and compared using chi-square test. Continuous data are presented as median (range) and compared with Mann-Whitney $U$ test. Age, male gender, body mass index, severe head and chest injury, and lowest emergency department systolic blood pressure; $p$ = not significant.

### TABLE 5. Demographics, Injury Characteristics, and Outcomes by Development of Gram-Negative Bacteremia for Matched Cohort ($n = 36$)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subjects in Whom GNB Developed ($n = 10$)</th>
<th>Subjects in Whom GNB Did Not Develop ($n = 26$)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>275 (19–50)</td>
<td>33 (17–55)</td>
<td>0.57</td>
</tr>
<tr>
<td>Male gender</td>
<td>9 (90)</td>
<td>15 (57.7)</td>
<td>0.06</td>
</tr>
<tr>
<td>Body mass index</td>
<td>28.7 (22.4–49.4)</td>
<td>29.6 (179–46.1)</td>
<td>0.94</td>
</tr>
<tr>
<td>Injury Severity Score</td>
<td>39.5 (22–57)</td>
<td>39.5 (8–75)</td>
<td>0.86</td>
</tr>
<tr>
<td>Acute Physiology and Chronic Health Evaluation II</td>
<td>32.5 (20–41)</td>
<td>30 (23–42)</td>
<td>0.6</td>
</tr>
<tr>
<td>Severe head injury</td>
<td>10 (100)</td>
<td>25 (96)</td>
<td>0.53</td>
</tr>
<tr>
<td>Severe chest injury</td>
<td>7 (70)</td>
<td>16 (61.5)</td>
<td>0.63</td>
</tr>
<tr>
<td>Severe abdominal injury</td>
<td>8 (80)</td>
<td>17 (66.4)</td>
<td>0.39</td>
</tr>
<tr>
<td>Initial base deficit (mEq/L)</td>
<td>–8.65 (–17.6 to –2.1)</td>
<td>–11.25 (–21 to –3.7)</td>
<td>0.27</td>
</tr>
<tr>
<td>Lowest emergency department systolic blood pressure (mm Hg)</td>
<td>80.5 (61–129)</td>
<td>83.5 (0–120)</td>
<td>0.6</td>
</tr>
<tr>
<td>Total packed RBCs transfusion in first 12 hr (U)</td>
<td>15.5 (2–23)</td>
<td>8 (1–18.6)</td>
<td>0.07</td>
</tr>
<tr>
<td>Total crystalloid in first 12 hr (L)</td>
<td>19 (7–47)</td>
<td>15 (6.7–31)</td>
<td>0.24</td>
</tr>
<tr>
<td>Maximum glucose in first 24 hr (mg/dL)</td>
<td>209 (132–355)</td>
<td>180 (128–365)</td>
<td>0.43</td>
</tr>
<tr>
<td>Ventilator days</td>
<td>15.5 (4–48)</td>
<td>12 (2–22)</td>
<td>0.11</td>
</tr>
<tr>
<td>ICU length of stay (d)</td>
<td>20 (6–57)</td>
<td>16.5 (7–37)</td>
<td>0.24</td>
</tr>
<tr>
<td>Length of stay (d)</td>
<td>44 (10–73)</td>
<td>29 (11–93)</td>
<td>0.22</td>
</tr>
<tr>
<td>Mortality</td>
<td>1 (10)</td>
<td>2 (7.7)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

GNB = Gram-negative bacteremia, Severe head/chest/abdominal injury = body region abbreviated injury score $\geq 3$.

Categorical data are presented as count (%) and compared using chi-square test. Continuous data are presented as median (range) and compared with Mann-Whitney $U$ test.
injury, differences were few and unremarkable. However, by 96 hours, the differences were marked and suggest immune suppression. Genes that were down-regulated at 96 hours in the subjects who developed GNB represented genes of both the innate and adaptive immune system. In contrast, counter-regulatory genes of the innate immune system were up-regulated in the subjects destined to develop GNB consistent with an overall picture of suppression of both the innate and adaptive immune system genes at 96 but not before 12 hours. The Inflammation and the Host Response to Injury collaborative recently defined the overall response to traumatic injury and described the phenomenon as a “genomic storm” (28). In the entire study cohort, the duration of altered gene expression correlated with a prolonged and “complicated” recovery, but there were no qualitative differences in the gene expression patterns (28). We, however, do observe differences in inflammatory and immune gene expression in the subgroup of subjects who developed GNB. By matching these bacteremic subjects to similarly severely injured control subjects, we have observed differences in both innate and adaptive immune gene expression that could lead to failure of the host to prevent Gram-negative bacteria from accessing the bloodstream.

Four of the genes identified at 96 hours are important to innate immune responses. First is IL1B (2q14), which encodes interleukin (IL)-1β, which is central to the innate immune response (29). We observed decreased expression of IL1B in the subjects who went on to develop GNB. This is consistent with a study in which investigators observed lower perioperative IL1B expression in patients who subsequently developed postoperative sepsis (30). Second, IL2RB (22q13.1) encodes the β subunit of the IL-2 receptor. It is expressed by lymphocytes and binding with IL-2 leads to T-cell proliferation (31). We observed lower expression of IL1R2 in the subjects in whom GNB developed, consistent with relative immune suppression. Third is IL1R2 (2q12), which encodes the IL-1 receptor type II (IL-1 type II receptor) and functions as a decoy receptor for IL-1β. IL-1 type II receptor prevents processing of the propeptide and blocks the interaction of the mature form of IL-1β with its functional receptor (32). We observed a relative increase in IL1R2 expression which likely contributes to suppressed innate immunity in the bacteremic patients. Finally, CD163 (12p13.3) is expressed by monocytes and macrophages. Binding of such complexes to cells with

![Figure 1. A. Heatmap of top 100 ranked differentially expressed probe sets from within 12 hr after injury. B. Heatmap of top 100 ranked differentially expressed probe sets at 96 hr after injury. GNB = subjects in whom Gram-negative bacteremia developed, non-GNB = subjects in whom Gram-negative bacteremia did not develop.]
CD163 binds to hemoglobin/haptoglobin complexes and increases secretion of IL-10, a known anti-inflammatory cytokine (33). Lower IL1B and IL2RB gene expression and higher IL1R2 and CD163 suggest an overall suppression of innate immunity leading to GNB.

We observed marked decreased expression of all five of the probe sets for HLA genes (chromosome 6) and the CD86 gene in the subjects who developed GNB. Others have observed decreased expression of HLA antigens on monocytes after severe trauma (34, 35). Decreased HLA expression has also been associated with higher infection rates in patients undergoing major operations, and the degree of decreased expression has been associated with increase in mortality from sepsis (36, 37). Our observations and these other reports are consistent with the notion that adaptive immune suppression follows severe traumatic injury and contributes to nosocomial infections and perhaps death.

There are doubtless many factors that contribute to alterations in early gene expression and may therefore ultimately lead to GNB. As shown in Table 4, there were many differences in measures of injury severity, shock severity, and treatments received (crystalloid and blood transfusions) between patients with and without GNB. These risk factors for bacteremia and other infectious outcomes have been reported by others, and each clearly could contribute to the differences in gene expression that we observed at 96 hours after injury. Differences were not yet large or consistent enough to be manifested by the first sampling time point. Despite the effectiveness of propensity matching in reducing the differences between the bacteremic and nonbacteremic subjects, there were still some differences as could be seen in Table 5. Bacteremic patients were more likely male and received more blood in the first 12 hours. Both of these factors may have, in part, contributed to the differences in gene expression and therefore to the development of GNB. Our data can only hint at the potential mechanisms linking the clinical risk factors with gene expression and eventual outcomes.

Our study has important limitations. First, the gene expression profiles were measured from total leukocyte samples, the majority of which are neutrophils, which potentially masks differences in gene expression from minority cell types, such as monocytes. However, the reciprocal increases in CD163 and
IL1R2, both primarily expressed by monocytes, indicate that we were not observing simply a relative reduction in monocyte RNA in subjects who subsequently developed GNB. Second, our analysis was limited by having relatively few bacteremic subjects with available gene expression data. Having more cases may have given us more power to identify other differences in gene expression, which may have resulted in an overall different interpretation of global changes in gene expression. As it is, our limited number of subjects likely resulted in fewer genes meeting the genome-wide threshold for significance. Third, our data and conclusions rely solely on the results of the microarray analyses without any confirmation by individual gene real-time polymerase chain reaction (rtPCR) or other measurements of protein products. However, we have taken a number of steps to ensure that our gene expression results are robust and that they are derived from quality RNA samples that were managed and analyzed correctly. Nevertheless, as we progress further along this path, we will look at individual gene expression with rtPCR. Although we have retrospectively reviewed the clinical and gene expression data, the datasets are robust, complete, and include most of the data needed to address our research question. Importantly, having genome-wide expression data allowed us to assess the global inflammatory state in an unbiased manner.

We believe our observations have important implications for understanding and possibly preventing posttraumatic GNB. For example, recent focus has been on reducing the prevalence of bacteremia and other catheter-related infections by using “bundles” based on guidelines that were established to ensure consistency of technique (38, 39). Doubtless, these interventions are important and have reduced infectious complications in hospitalized patients. However, despite these system-wide approaches, recent reports suggest an increasing prevalence of bacteremia, particularly with Gram-negative organisms (3–5). These reports indicate that although bacteremia can be reduced by these interventions, many occur despite optimized care and likely reflect factors specific to the individual patient, such as alterations in their immune response, as demonstrated here. We believe that without interventions aimed at injury-induced alterations in both innate and adaptive immunity, system-wide interventions will not eliminate GNB. Targeting the use of immunostimulants, such as interferon-γ, in patients with decreased immune gene expression could potentially lead to improved outcomes.

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REFERENCES
