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Persistent Inflammation, Immunosuppression and Catabolism Syndrome after Severe Blunt Trauma

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BACKGROUND

Even with advances in care over the last three decades, severe blunt trauma causes significant long-term morbidity and mortality (1, 2). In the last decade, in-hospital mortality has improved markedly and late multiple organ failure (MOF) deaths are disappearing (3, 4). Regardless, long-term mortality and functional recovery have essentially remained unchanged (2, 5). Severe trauma causes an early systemic inflammatory response syndrome (SIRS) which can result in early MOF in the absence of infection. SIRS has been linked to a compensatory anti-inflammatory response (CARS), reflecting defects in adaptive immunity (6). Recently, observations from multiple sources have led to a single stage model of SIRS/CARS.

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AUTHORSHIP

E.L.V drafted the manuscript. F.A.M, P.A.E, H.V.B, C.L. and L.L.M contributed in the study concept and design. A.B. and T.O.B performed the statistical analysis. C.M.L, H.V.B., and E.L.V. preformed the acquisition of data. T.O.B, L.L.M, P.A.E, E.L.V, C.M.L, F.A.M performed the analysis and interpretation of data. R.U, R.D., A.G.C., L.F.G., D.C.N, A.L.C, L.F.G., J.L., L.L.M., F.A.M. and P.A.E. provided the critical revision of the article.

Xiao et al demonstrated early in the leukocyte transcriptome there is a simultaneous increase in expression of innate immunity genes (i.e. SIRS) and suppression of adaptive immunity genes (i.e. CARS) (7). Furthermore, patients with 'complicated' clinical courses versus uncomplicated patients had an increased magnitude and duration of these genomic changes (i.e. failure to restore homeostasis) (7).

Study of these 'complicated' patients (5, 8) led to the recognition of several clinical patterns in patients who remained critically ill in the ICU. From these observations, we recently proposed a new model of chronic critical illness (CCI) (Figure 1) where the phenotype of 'late MOF' is replaced with a new syndrome termed persistent inflammation, immunosuppression and catabolism syndrome (PICS) (9). These patients generally proceed along a 'complicated' course requiring transfer to long-term acute care facilities (LTACs), where they ultimately experience indolent deaths. Unlike previous paradigms on CCI, this syndrome offers a unique and unifying pathogenic hypothesis that persistent low-level inflammation induces immune suppression and progressive protein catabolism.

Current evidence suggests that trauma patients experience a systemic immunological dysregulation central to organ injury and places them at an increased risk for PICS (10–12). To examine this, we analyzed the clinical data, outcomes and genomic profiles of leukocyte cell populations previously obtained from the "*Inflammation and Host Response to Injury*" Glue Grant (GG) database (TRDB). The goal of this study was to determine whether genomic and clinical data from patients with complicated clinical outcomes after severe blunt trauma have ongoing evidence of PICS, supporting our hypothesis that PICS represents a predominant phenotype of CCI after injury, as well as sepsis.

METHODS

Approval was obtained from the University of Florida Institutional Review Board to analyze deidentified human data obtained from the GG TRDB (13).

Data Source and Study Population

GG data was derived from eight USA designated level 1 trauma centers that enrolled blunt trauma patients in the TRDB from 2001–2011 (14).

The enrolled patients were divided into two distinct cohorts defined as 'complicated' or 'uncomplicated' based on a new metric found to correlate with injury severity and critical illness after trauma, time to recovery from multiple organ injury (TTR). Patients with TTR of >14 days were considered to have complicated clinical courses and those with TTR <5 days were classified as uncomplicated (i.e. rapid recovery). The description of how TTR was calculated can be found in previously published literature (7, 13). Of the 1,989 patients (>16 years), 369 uncomplicated and 785 complicated patients met the pre-determined criteria; 835 were classified as intermediate, not meeting either criterion.

In the initial phase of the GG, 167 of the 1,989 trauma patients enrolled, ages 18–55, agreed to blood sampling for total leukocyte genomics and were matched by age, gender and ethnicity to 37 healthy control subjects (15). Blood sampling for T-cell, monocyte and

neutrophil (PMN) genomic analysis was obtained from a second set of 244 severe trauma patients, ages 16–90, and compared to 21 matched controls. Since we define PICS as patients with persistent inflammation, immunosuppression and catabolism, the 'complicated' patients with TTR in excess of 14 days would most likely include those who meet our PICS criteria (9).

Clinical Outcomes and Laboratory Analysis

Demographics and outcomes recorded included: age, sex, mortality, max Marshall and Denver scores, new injury severity score (NISS), APACHE II, total days on mechanical ventilation (MV) and disposition. Clinical parameters were recorded at admission, worst value over hospital course, and on days 7 and 14 for the following parameters: Marshal score and Denver scores, creatinine, bilirubin, alkaline phosphatase, white blood cell (WBC) count, lymphocyte count, PMN count, platelet count, INR, albumin and PaO2 to FiO2 (P:F) ratio.

Clinical values were compared among the different cohorts grouped in six different analyses: (1) complicated patients values at each time point vs. uncomplicated patients worst values over their hospital course; (2) complicated patients at each time point vs. uncomplicated patients values at admission; (3) uncomplicated patients worst values recorded over their hospital course vs. complicated and intermediate patients values combined at each time point; (4) uncomplicated patients worst recorded values over their hospital course combined with intermediate patients values at each time point vs. complicated patient values at each time point; (5) uncomplicated patients values on admission vs. complicated and intermediate patients values combined at each time point; and finally (6) uncomplicated patients values on admission combined with intermediate patients values at each time point vs. complicated patient values at each time point. Patients without recorded data at the time point analyzed were excluded from the total number of patients for analysis at those time points.

Gene Chip Validation

Four individual chips were used for analysis over the study (Affymetrix HU133+v2, GGh1, GGh2, and GGh3) (16). For the second set of patients principle component analysis (PCA), was performed for overall RMA production between the individual GeneChips™ in an attempt to address the concerns in variability of data production among the GGh chips. PCA is a multivariate statistical technique used for visual representation of the patterns of similarity between variables and observations (17).

Gene Expression Profiles

Blood samples were drawn within 12 hours of injury and at 1, 4, 7, 14, 21, and 28 days post injury. For the first 167 patients, whole blood nucleated cells were isolated, and genome-wide expression analyses of 54,675 probe sets were performed after RNA extraction and hybridization onto Affymetrix U133+v2 GeneChip™ (18, 19). Individual leukocyte subpopulations were isolated either by negative selection (monocytes and T-cells) or by positive selection (PMN) using microfluidics cassettes (18). Genome-wide expression analyses were performed after RNA extraction and hybridization to the GGh GeneChips™

as above. Full detailed descriptions of the protocols and specific methodologies can be found in previously published reports and their supporting text (18).

BRB tools™ and Ingenuity Systems (IPA), were used to identify gene expression differences, compare functional pathways, ontologies and individual gene fold changes between the cohorts and controls. Significant genes were selected by identifying trauma responsive genes in both uncomplicated and complicated patients versus human control subjects (p-value <0.001, f-test). For genes that were represented by multiple probe sets, the probe set with the highest expression value was used for analysis. A distance from reference (DFR) metric (natural log) was calculated for assessing the overall perturbation in gene expression as previously described (20). Leave-one out cross validation was performed to compute the misclassification rate, and a Monte Carlo simulation was conducted to test statistical significance.

Once significant genes were identified, fold changes were calculated between each of the cohorts and control samples. These fold changes in magnitude were significant at p-values of 0.001. Functional pathway analysis in IPA identified pathways that were over or under represented in terms of the observed numbers of genes whose expression significantly changed upon cohort analysis. The Z-score was used to test for significance at a 95% confidence interval (<−2, >2) (21).

Statistical Analysis

Categorical variables were reported as frequencies and percentages. Pearson χ^2 or Fisher's exact tests were used to test independence between categorical variables as appropriate. Normality of distribution was tested using the Kolmogorov-Smirnov test and continuous variables that did not satisfy the normality assumptions were reported as medians (25^{th} and 75^{th} percentiles). Bootstrap method, a nonparametric method in which data is resampled and replaced a large number of times to compute adjusted p-value, was used for adjustments for multiple comparisons.

Mixed model analysis of longitudinal changes in clinical laboratory data was used to account for correlations among repeated measurements for each patient. For each variable, we modeled change over time by including time, recovery class, and their interaction adjusting for abbreviated injury scale (AIS), NISS, age, and sex.

All significance tests were two-sided, with a 0.05 alpha level. Statistical analyses were performed with SAS (v.9.3, Cary, N.C.).

RESULTS

Patient demographics, clinical data and mixed model analysis

Complicated patients were significantly older, with longer days on MV, as well as higher Marshall MOF, NISS, Denver and APACHE II scores, when comparing complicated and intermediate patients combined to uncomplicated patients. This was also true when comparing complicated to the combined cohort of intermediate and uncomplicated patients (Table 1). Complicated patients were noted to have lower lymphocyte counts, P:F ratios, and

albumin levels, as well as higher PMN counts, leukocytosis and creatinine concentrations when compared to both admission and worst values for uncomplicated patients. These changes often persisted to day 14 (Table 2). Results for all cohorts compared can be found in Supplemental Table 1. Mixed model analysis demonstrated that recovery class (complicated, intermediate, uncomplicated), time and their interaction were significant for all clinical variables, except for alkaline phosphatase for which only time was significant. For complicated recoveries, there was a significant change for all variables from admission to day 7, except for the P:F ratio, and from admission to day 14, except for albumin. The difference between admission and worst values were different for Marshall Score, bilirubin, WBC, PMN, lymphocyte count, INR, and creatinine (Table 3).

Microarray Data on Whole Blood Leukocytes

The genome-wide expression patterns for complicated patients were more aberrant from control subjects than those of uncomplicated patients (DFR: 11.8±0.4 vs. 11.6±0.4 respectively, $p<0.001$). Comparison of the transcriptome of the uncomplicated, complicated and control patients on day 7 revealed 30,351 probe sets (14,262 genes) that were significant in differentiating among groups after trauma ($p<0.001$). Also, the overall pattern of gene expression was significantly different, as determined by leave-one-out cross validation (91% to nearest first neighbor). Comparison of complicated to uncomplicated patients on day 7 showed that 415 genes could differentiate between the two ($p<0.001$). Similarly, comparison among complicated, uncomplicated and control cohorts on day 14 demonstrated 26,842 probe sets (13,189 genes) expressed differently among the groups ($p<0.001$) (Supplemental Figure 1a).

Next, we evaluated individual genes whose fold changes from control were significantly different between complicated and uncomplicated patients. We found that complicated patients had an increased magnitude of alterations in specific genes involved in increased inflammation, up regulation of myeloid derived suppressor cells (MDSCs), decreased chemotaxis and defective innate immunity compared to uncomplicated patients on days 7 and 14 (Table 4).

Using Gene Ontology™ and Biocarta™, we found that complicated patients had significant differences in their gene expression patterns from pathways involved in suppression of myeloid cell differentiation, increased inflammation, decreased chemotaxis and defective innate immunity on day 7. Furthermore, multiple comparison analysis demonstrated that complicated patients had significant differences in pathways involved in suppression of adaptive immunity (i.e. regulation of T_{H1} immune response and $CD4^+/CD25^+$ alpha-beta regulation of T-cell differentiation pathways), and increased inflammation (i.e. IL-22 signaling) compared to uncomplicated patients on day 7. On day 14, genome-wide expression illustrated a continued increases in the pathways involved in adaptive immunity suppression (i.e. suppression of myeloid cell differentiation) ($p<0.05$) (Supplemental Table 2).

Evaluation of functional pathways on day 7 revealed over-representation of pathways involved in increased hematopoiesis (T-cell development, differentiation in mononuclear cells, differentiation of myeloid cells), inflammatory response (activation of leukocytes), and

immune cell trafficking (activation of leukocytes) in uncomplicated patients compared to control, as would be expected after traumatic injury. On day 14, uncomplicated patients also illustrated over-representation of pathways involved in hematologic system development (T-cell and blood cell development, differentiation of blood cells and monocytes, and quantity of leukocytes), and hematopoiesis (T-cell differentiation, development of leukocytes and blood cells), immune cell trafficking (activation of lymphocytes). Similar pathways failed to reach significance in complicated patients.

Isolated Leukocyte Subpopulations

PCA and unsupervised cluster analysis of probe sets in control subjects for isolated leukocyte subpopulations demonstrated that their expression was consistent despite the variant of GGh GeneChip™ used (Supplemental Figure 1b) and that each cell line (monocytes, PMNs, and T-cells) exhibited cell-specific gene expression. 4,614 probe sets were significant in differentiating between individual leukocyte subsets ($p<0.001$) (Supplemental Figure 1c).

Evaluation of individual gene fold changes (from controls) showed different expression patterns between complicated and uncomplicated patients in each leukocyte subset. Monocyte and PMN subpopulations from complicated patients on day 7 had significant alterations in the expression of genes involved in decreased chemotaxis, pathogen associated molecular pattern (PAMP) recognition and antigen presentation. In addition, they demonstrated changes associated with increased inflammation and up regulation of MDSC's as compared to controls and often in increased magnitude of change as compared to uncomplicated patients. Several of these changes persisted out to day 14 after injury. T-cells from complicated patients had alterations in genes involved in increased T-reg's, Th1–Th2 skewing and inflammation, as well as, decreased PAMP recognition and chemotaxis on day 7. Gene expression changes involving increased inflammation persisted out to day 14 (Supplemental Table 3).

Gene Ontology™ and Biocarta™ analysis on day 7 in complicated patients (PMN and monocyte subsets) showed up regulation in pro-inflammatory pathways (examples include: IL-1signaling and chronic inflammatory response pathways) down regulation of pathways involved in adaptive immunity (examples include: IL4 and toll-like receptor signaling and antigen processing and presentation pathways) as compared to uncomplicated patients. Similar changes were present on day 14. T-cells from complicated patients on day 7 were noted to have increased expression of pathways involved in modulation of T-cell activity and the inflammatory response (examples include: IL-2 and T-cell receptor signaling and chronic inflammatory response pathways) (Figure 2).

Functional pathway analysis of the leukocyte subpopulations on day 7 revealed under-representations of pathways involved in increased cell line viability, differentiation of leukocytes and blood cells, inflammatory response, hematologic system development, cell movement and immune cell trafficking for PMNs, chemotaxis of phagocytes in monocytes and increased cell viability in T-cells in uncomplicated patients. There was also under-representation (PMNs) of pathways involved in cell death of leukocyte and myeloid cell lines, and homing and chemotaxis of neutrophil pathways. Uncomplicated patients on day

14 had under-representation in pathways involved in proliferation of hematopoietic progenitor cells (PMNs) and apoptosis of the lymphoid system, as well as an over-representation in the cell viability pathway in T-cells, which did not reach similar significance in complicated patient cell lines.

DISCUSSION

The concept of CCI can be found in the literature since the early 1990's under different terms used to describe patients who survived their initial episode of critical illness, but remained dependent on ICU care and not fully recovering (22–24). Recently, it was estimated that there are at least 100,000 of these CCI patients in the US at any one time, incurring a tremendous burden on the already taxed health care system (25). Although in-hospital mortality has decreased, the overall one-year mortality remains unchanged and substantially more patients are being discharged to LTACs. In fact, 41% of 'complicated' patients discharged in the GG went to rehabilitation or LTAC facilities (13).

The underlying immunological and inflammatory response of this new phenotype of CCI is a matter of considerable controversy. Most investigators have focused on prolonged adaptive immune suppression, but immune suppression alone cannot explain the persistent acute phase response, protein catabolism, malnutrition and reduced functional and cognitive abilities.

Genome-wide leukocyte expression analysis can be used to assess simultaneously the inflammatory status and adaptive immune functions at the level of gene regulation. Past studies have strongly suggested that information contained in the leukocyte transcriptome after trauma could be used to identify families of genes involved in inflammation, antigen presentation and T-cell responses as being discriminatory. Thus, we sought clinical and genomic evidence of a persistent inflammatory and immunosuppressive response in leukocytes from patients with different clinical trajectories.

We found that trauma patients who experienced complicated outcomes had overall genome-wide expression patterns which were more aberrant from controls, consistent with previous reports (20). On evaluation, the global genome-wide expression in complicated patients on day seven was significantly increased from uncomplicated patients, indicating that gene expression was still more perturbed in patients who had complicated clinical outcomes. We identified multiple genes whose expressions were significantly different in magnitude and duration in trauma patients with complicated clinical courses compared to those with an uncomplicated clinical trajectory and to healthy subjects. Importantly, changes in gene expression consistent with PICS can be seen as early as seven days post injury in patients with complicated courses.

Gene Ontology™ and Biocarta™ evaluation between cohorts seven days post injury demonstrated that complicated patients had significant changes in pathways involved in increased inflammation and suppression of adaptive immunity, especially those pathways involved in T-cell function and hematopoiesis. Analysis of functional pathways on day seven supported these findings. Patients with *uncomplicated outcomes* were found to have

significant increases in pathways essential to innate and adaptive immunity compared to control, as one would expect after severe injury. Failure of complicated patients to reach similar significance may indicate defects in these functions, leading to depressed innate immunity, despite increased inflammatory responses.

Of note, we found that the changes in genomic expression in complicated trauma patients were reflected by the patients' clinical data. Complicated patients were found to exhibit persistent inflammation supported by elevated WBC counts, immunosuppression with lymphopenia and signs of ongoing protein catabolism (low albumin levels) over their course. In a mixed model analysis adjusted for AIS, NISS, age and sex, having a complicated outcome was independently associated with this leukocytosis, immunosuppression and ongoing protein catabolism over time.

In conclusion, our data supports a novel paradigm that in trauma patients with complicated outcomes; there is increased inflammation, concordant defects of adaptive immunity and signs of host protein catabolism that persist over their hospital course at increased levels. This provides some validation on the genomic and clinical level that trauma patients with complicated outcomes are indeed exhibiting PICS. It is still unclear why certain patients develop PICS, while others seem to recover relatively quickly after injury. Attempts to determine why these differences exist are important for improving outcomes in this expanding CCI population. (3,000 words)

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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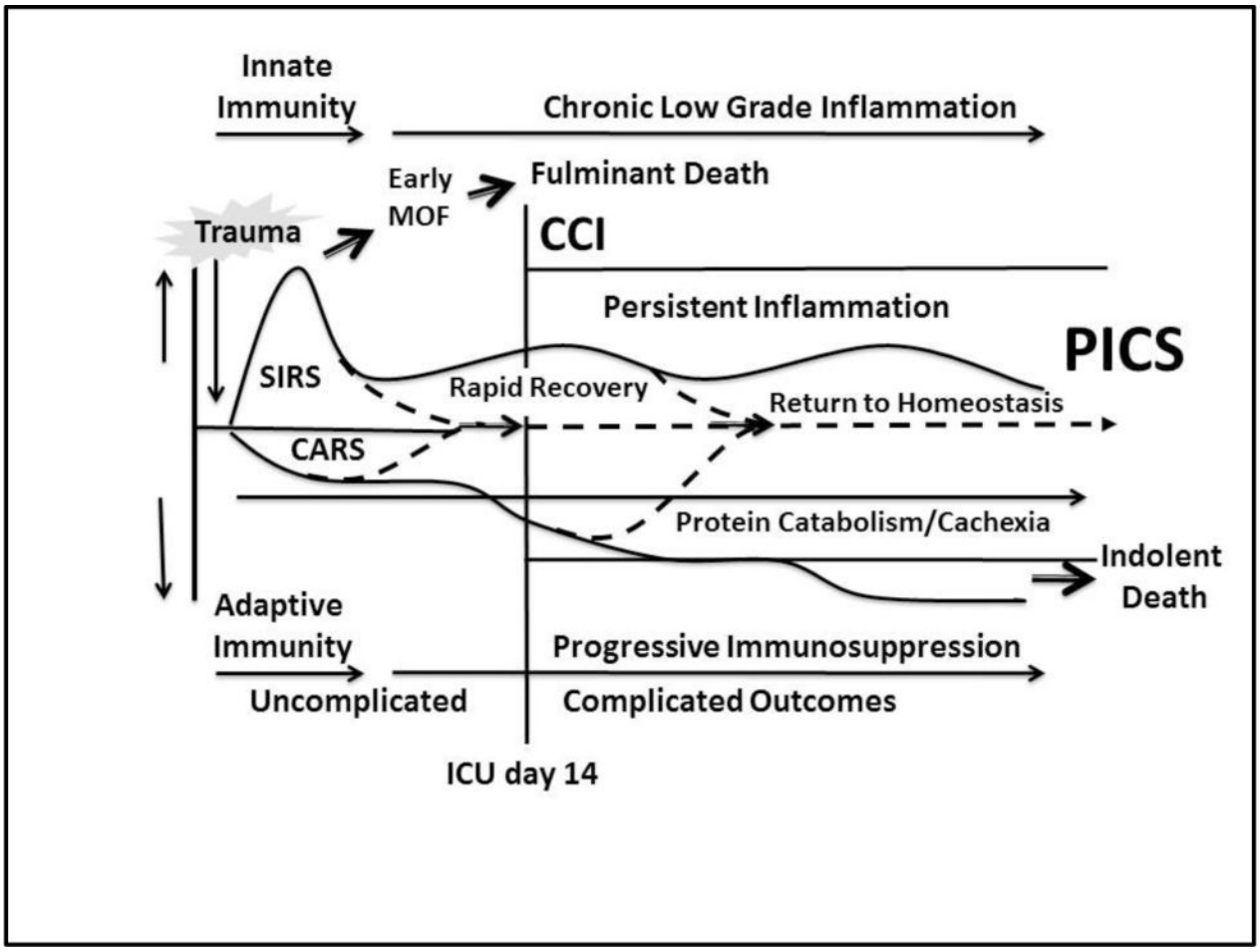

Figure 1.

We propose a new model of the human response after traumatic injury. Trauma causes pro- and anti-inflammation that result in simultaneous SIRS and CARS. Modern ICUs have become much better at recognizing and treating shock early and providing effective evidence based guideline-driven standard operating procedure treatment. This limits the progression into refractory shock and allows fewer patients to express the early multiple organ failure/fulminant death trajectory. Some surviving patients rapidly return to immunologic homeostasis, and experience rapid recover and are discharged from the ICU after a few days. Unfortunately, most have persistent derangement in both innate and adaptive immunity for weeks and progress into a syndrome of chronic critical illness, which in a high percentage of patients (>40%) progresses to PICS. We are presented with the challenge of managing simultaneous chronic dysfunctional inflammation and adaptive immunosuppression, protecting against secondary nosocomial infection, and preventing severe protein catabolism with resultant cachexia, which leads to an indolent death.

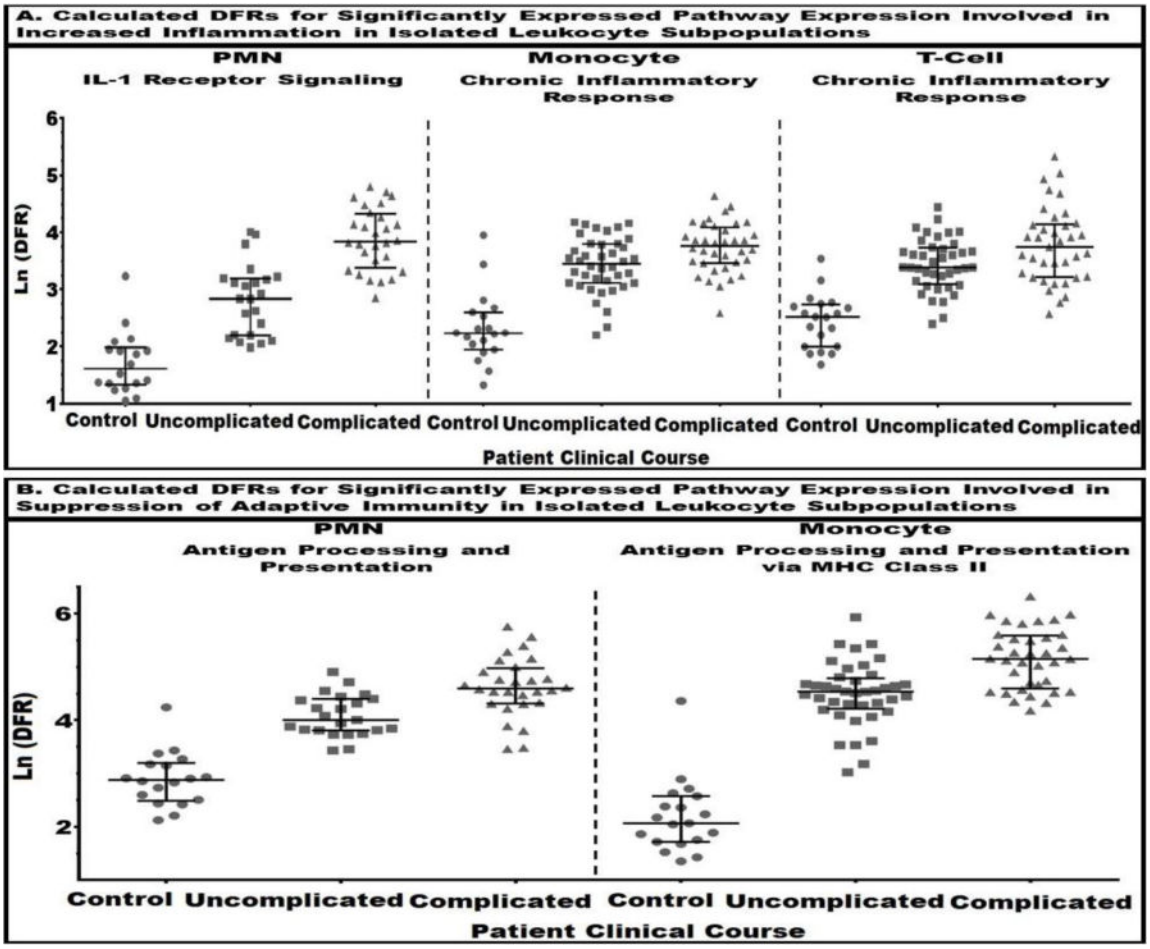

Figure 2.

Heat maps of gene ontology pathways from isolated leukocyte subpopulations were evaluated to determine which pathways appeared to be differently expressed between complicated, uncomplicated and control patients on day 7 after traumatic injury. Difference from reference (DFR) was calculated to determine significance of expression between the cohorts in pathways which were identified to be different. **A.** Scatter plots of the selected canonical pathways involved in increased inflammation on day 7 in neutrophils (PMN), monocyte and T-cell's. The natural log for the DFR scores were calculated for each patient in the complicated, uncomplicated, and control cohorts for IL-1 receptor signaling pathway in PMNs, and the chronic inflammatory response pathway in monocytes and T-cells. Statistical analysis showed the complicated patients had significant up regulation of these pathways compared to uncomplicated patients and controls ($p<0.05$). **B.** Scatter plots of selected gene ontology pathways involved in suppression of adaptive immunity on day 7 in PMN and monocyte's. The natural log of the DFR scores were calculated for each patient in the complicated, uncomplicated, and control cohorts for the antigen processing and presentation pathway in PMNs and the antigen processing and presentation via MHC class II pathway in monocytes. Statistical analysis showed the complicated patients had significant

down regulation of these pathways compared to uncomplicated patients and controls ($p<0.05$).

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Table 1

Demographics and outcomes of complicated and uncomplicated patients were compared. Complicated patients were noted to be significantly older, with increased days on the vent and Apache II, NISS, Max Denver and Max Marshal Scores compared to uncomplicated patients.

Patient Demographics and Outcomes					
	Complicated (N=785)	**Intermediate (N=835)**	**Uncomplicated (N=358)**	**p-value***	**p-value****
Female Gender, n (%)	235 (30%)	292 (35%)	130 (36%)	0.17	0.0128
Survival, n (%)	478 (61%)	826 (99%)	358 (100%)		
Age, median (25th–75th)	46 (30, 61)	40 (25, 54)	35.5 (24, 47)	<0.0001	<0.0001
Max Marshall Score, median (25th–75th)	9 (7, 11)	8 (6, 9)	5 (3, 6)	<0.0001	<0.0001
Max Denver Score, median (25th–75th)	3 (1, 5)	1 (0, 2)	0 (0, 1)	<0.0001	<0.0001
Apache II Score, median (25th–75th)	33 (29, 37)	28 (24, 32)	23 (17, 27)	<0.0001	<0.0001
NISS, median (25th–75th)	43 (34, 57)	34 (27, 48)	27 (22, 34)	<0.0001	<0.0001
Days on Ventilator, median (25th–75th)	15 (5, 22)	6 (4, 10)	2 (0, 2)	<0.0001	<0.0001

* p-value represents complicated patients and intermediate patients compared to uncomplicated.

** p-value represents complicated patients compared to uncomplicated patients and intermediate patients.

Table 2

Table showing selected clinical data in complicated patients on admission and on days 7 and 14, as well as the admission and worst values recorded over the hospital course in uncomplicated patients.

Patient Clinical Data					
Admission					
Variable	**Complicated (N=785)**	**Uncomplicated Worst Values (N=358)**	**Uncomplicated Admission Values (N=358)**	**p[1]**	**p[2]**
Denver Score	3 (1, 5)	0 (0, 1)	0 (0, 1)	*	*
Marshall Score	9 (7, 11)	2.4 (1.6, 3.3)	5 (3, 6)	*	*
White Count (1000/ul)	14.5 (9.8, 20.2)	12.9 (9.6, 16.2)	15.5 (10.9, 22)	*	NS
Neutrophil Count (1000/ml)	7.2 (4.5, 10.8)	0.9 (0.7, 1.2)	9.98 (7.1, 13.1)	*	*
Lymphocyte Count (1000/ml)	0.7 (0.5, 1)	11.1 (8.4, 14.1)	1.3 (0.8, 1.7)	*	*
PaO2:FiO2 Ratio	164 (113, 225)	303 (210, 363)	339 (261, 409)	*	*
Albumin (g/dl)	1.9 (1.6, 2.3)	2.3 (2, 2.6)	–	*	NS
Creatinine (mg/dl)	1 (0.8, 1.32)	0.8 (0.7, 0.9)	0.9 (0.7, 1)	*	*
Day 7					
Denver Score	2 (1, 3)	–	–	*	*
Marshall Score	8 (7, 10)	–	–	*	*
White Count (1000/ul)	15.6 (11.8, 20.3)	–	–	*	NS
Neutrophil Count (1000/ml)	11 (8.11, 13.57)	–	–	*	NS
Lymphocyte Count (1000/ml)	1.2 (0.8, 1.64)	–	–	*	NS
PaO2:FiO2 Ratio	163 (118, 215)	–	–	*	*
Albumin (g/dl)	1.7 (1.4, 2.1)	–	–	*	NS
Creatinine (mg/dl)	1 (1, 1)	–	–	*	*
Day 14					
Denver Score	1 (0, 2)	–	–	*	*
Marshall Score	6 (5, 8)	–	–	*	*
White Count (1000/ul)	16.2 (12.7, 20.8)	–	–	*	NS
Neutrophil Count (1000/ml)	10.7 (8.8, 14.9)	–	–	*	NS
Lymphocyte Count (1000/ml)	1.21 (0.77, 1.77)	–	–	*	NS

Patient Clinical Data					
Admission					
Variable	**Complicated (N=785)**	**Uncomplicated Worst Values (N=358)**	**Uncomplicated Admission Values (N=358)**	**p^1**	**p^2**
Alkaline Phosphatase (per unit)	163 (121, 238)	–	–	*	*
PaO2:FiO2 Ratio	193 (136, 244)	–	–	*	*
Albumin (g/dl)	1.7 (1.4, 2.1)	–	–	*	*
Creatinine (mg/dl)	0.8 (0.6, 1.2)	–	–	*	*

* represents $p<0.05$, NS represents non-significant with $p>0.05$.; p1 = complicated patients values at each time point vs. uncomplicated patients worst values over their hospital course; p2 = complicated patients values at each time point vs. uncomplicated patients values at admission.

Table 3

Table showing mixed model analysis of changes in clinical variables over time adjusted for age, sex, new injury severity score, abbreviated injury scale, recovery class and time interaction.

Association Between Changes in Clinical Variables Over Time and Recovery Class				
	Complicated (N=785)	**Intermediate (N=835)**	**Uncomplicated (N=358)**	**Significant Effects**
Marshall Score				
Admission	9 (7, 11)	8 (6, 9)	5 (3, 6)	Recovery class, time, recovery class*time, sex, NISS
Day 7	8 (7, 10)	4 (0, 6)		
Day 14	6 (5, 8)	0 (0, 2)	2.37 (1.61, 3.32)*	
Denver Score				
Admission	3 (1, 5)	1 (0, 2)	0 (0, 1)	Recovery class, time, recovery class*time, sex, NISS
Day 7	2 (1, 3)	0 (0, 1)		
Day 14	1 (0, 2)	0 (0, 0)	0 (0, 1)*	
Bilirubin (mg/dl)				
Admission	1.2 (0.8, 2.4)	1 (1, 2)	2 (1, 2)	Recovery class, time, recovery class*time, sex
Day 7	3 (1, 7)	2 (1, 3)		
Day 14	3 (1, 6)	1 (0.6, 1.3)	1 (0.8, 1.1)*	
White Count (1000/ul)				
Admission	14.5 (9.8, 20.2)	15.9 (11.3, 21.7)	15.5 (10.9, 22)	Recovery class, time, recovery class*time, age, NISS
Day 7	15.6 (11.75, 20.26)	13.3 (10.3, 17.3)		
Day 14	16.23 (12.7, 20.8)	13.8 (10.9, 18.2)	12.9 (9.6, 16.2)*	
Neutrophil Count (1000/ml)				
Admission	7.21 (4.53, 10.8)	9.58 (7.76, 14.01)	9.98 (7.1, 13.06)	Recovery class, time, recovery class*time, age
Day 7	11 (8.11, 13.57)	9.6 (7.1, 12.9)		
Day 14	10.68 (8.81, 14.9)	9.5 (7.2, 13.5)	0.88 (0.69, 1.21)*	
Lymphocyte Count (1000/ml)				
Admission	0.7 (0.46, 1)	0.83 (0.6, 1.1)	1.25 (0.75, 1.68)	Recovery class, time
Day 7	1.2 (0.8, 1.64)	1.3 (0.96, 1.81)		
Day 14	1.21 (0.77, 1.77)	1.6 (1.2, 2.2)	11.1 (8.43, 14.1)*	
Platelet Count (1000/mm3)				
Admission	85 (67, 107)	92 (76, 114)	130 (109, 160)	Recovery class, time, recovery class*time, age, NISS
Day 7	158 (101, 211)	190 (148, 243)		
Day 14	439 (294, 601)	650 (438, 862)	128 (109, 158)*	
Alkaline Phosphatase (per unit)				
Admission	54 (45, 72)	52 (41, 65)	50.5 (44, 57)	Time
Day 7	88 (65, 122)	88 (67, 122)		

Association Between Changes in Clinical Variables Over Time and Recovery Class

	Complicated (N=785)	Intermediate (N=835)	Uncomplicated (N=358)	Significant Effects
Day 14	163 (121, 238)	134.5 (97, 173)	54.5 (48, 75.5)*	
INR				
Admission	1.3 (1.1, 1.5)	1.3 (1.1, 1.4)	1.2 (1.05, 1.3)	Recovery class, time, recovery class*time
Day 7	1.2 (1.1, 1.3)	1.18 (1.1, 1.2)		
Day 14	1.2 (1.1, 1.3)	1.1 (1.1, 1.2)	1.2 (1, 1.3)*	
PaO2:FiO2 Ratio				
Admission	164 (113, 225)	310 (248, 390)	338.5 (260.5, 409)	Recovery class, time, recovery class*time, age, sex
Day 7	163 (118, 215)	223.5 (181, 286)		
Day 14	193 (136, 244)	411 (339, 438)	303 (210, 363)*	
Albumin (g/dl)				
Admission	1.9 (1.6, 2.3)	2.1 (1.7, 2.4)	–	Recovery class, time, recovery class*time, age, sex
Day 7	1.7 (1.4, 2.1)	1.8 (1.6, 2.2)		
Day 14	1.7 (1.4, 2.1)	2.1 (1.7, 2.5)	2.3 (2, 2.6)*	
Creatinine (mg/dl)				
Admission	1 (0.8, 1.32)	1 (1, 1)	0.9 (0.7, 1)	Recovery class, time, recovery class*time, age, sex
Day 7	1 (1, 1)	0.7 (0.6, 0.895)		
Day 14	0.8 (0.6, 1.2)	0.6 (0.5, 0.7)	0.75 (0.655, 0.92)*	

*Using worst values over stay. Abbreviations: NISS, new injury severity score; AIS, abbreviated injury score.

Table 4

Genes of Interest over Time

Table showing the fold changes of selected genes from complicated and uncomplicated patients at day 7 and day 14 after severe blunt traumatic injury that were significant in fold change as compared to healthy human controls ($p<0.001$) and in increased magnitude compared to uncomplicated patients.

Day 7			Day 14		
Gene	**Complicated Patients**	**Uncomplicated Patients**	**Gene**	**Complicated Patients**	**Uncomplicated Patients**
Chemotaxis					
CXCL6	**−1.7**	**−1.6**	*CXCR3*	**−1.8**	**−1.5**
CXCL10	**−2.1**	**−1.6**	*CXCL10*	**−1.7**	**−1.2**
CCL4	**−1.8**	**−1.5**	*CCL5*	**−2**	**−1.3**
CCL5	**−3.1**	**−2**			
IL8	**−4.6**	**−3.6**			
Inflammation Related Peptides/Proteins					
HP	**21.5**	**12**	*HP*	**17.4**	**10.2**
MMP8	**57.9**	**19.7**	*MMP8*	**53.1**	**33.7**
CD177	**66.3**	**25.9**	*CD177*	**20.5**	**8.3**
			IL1B	**1.6**	**1.5**
Pathogen-associated Molecular Pattern (PAMP) Detection					
TLR7	**−2.9**	**−2.1**	*TLR7*	**−2.5**	**−1.6**
TLR3	**−1.2**	**−1.2**	*CD14*	**3**	**2.9**
Immune Related Genes/Antigen Presentation/Co-Stimulatory Molecules					
HLA-DMA	**−2.1**	**−1.2**	*HLA-DMA*	**−1.9**	**1**
HLA-DMB	**−3.5**	**−1.9**	*HLA-DMB*	**−2.8**	**−1.5**
HLA-DOA	**−2.3**	**−1.9**	*HLA-DOA*	**−2.2**	**−1.9**
HLA-DOB	**−2.1**	**−1.8**	*HLA-DOB*	**−2.1**	**−1.8**
HLA-DPA1	**−4.5**	**−2.4**	*HLA-DPA1*	**−3.5**	**−1.6**
HLA-DQA1	**−4.9**	**−2.7**	*HLA-DQA1*	**−3.7**	**−1.3**
HLA-DQB1	**−5**	**−2.5**	*HLA-DQB1*	**−2.6**	**−1.5**
HLA-DRA	**−2.1**	**−1.1**	*HLA-DRA*	**−1.9**	**1.1**

Day 7			Day 14		
Gene	**Complicated Patients**	**Uncomplicated Patients**	**Gene**	**Complicated Patients**	**Uncomplicated Patients**
CD83	**−1.8**	**−1.5**			
CD84	**1.2**	**1.3**	*CD84*	**−2.1**	**−1.8**
CD69	**−3.1**	**−3**			
Up Regulation of Myeloid Derived Suppressor Cells (MDSCs)					
CSF2	**1.2**	**1.2**	*IL1R2*	**6.9**	**2.8**
ARG1	**20.9**	**14.7**	*ARG1*	**18.7**	**12**
IL4R	**3.9**	**3.3**	*IL4R*	**2.8**	**2**