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The Glue Grant experience: characterizing the post injury genomic response

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The Inflammationand Host Response to Injury, Large Scale Collaborative Research Program

Abstract

Despite ongoing improvements in resuscitation, care, and outcomes, traumatic injury remains a significant health care and economic burden. The causes are multifactorial, but our approach to the clinical management of these patients remains limited by our current understanding of the pathobiology of the disease. A multicenter, multidisciplinary program known as the “Inflammation and the Host Response to Injury” Large Scale Collaborative Research Program was created by the National Institute of General Medical Sciences (NIGMS, U54 GM062119–10) in 2001 in a 10-year effort to address some of these issues. Its primary goal is to describe the human genomic response to severe trauma and burns, and to examine changes in gene expression in the context of different clinical outcomes. The Program has not only successfully implemented clinical care guidelines for managing the severe trauma patient based on the best available evidence to minimize iatrogenic variability, but it has also examined the genome-wide, immune-inflammatory response in total and isolated blood leukocyte populations. This review will address current milestones as well as future directions for the Program.

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Keywords

Polytrauma; Protocols; Critical care; Inflammation

Introduction

Although decades of improvements have reduced the morbidity and mortality associated with severe trauma, it remains a significant health and economic burden globally. Estimates have placed the economic toll at over $200 billion a year in the U.S. alone, and although mortality during the “golden hour” has improved with better and faster delivery of emergent care, patients still succumb to complications and mortality associated with the prolonged hospital recovery phase [1–4]. In addition, although results from clinical studies are available and have often been integrated into trauma care algorithms, the standardization of clinical practice for the critically ill is variably followed and remains a significant contributor to the iatrogenic detriment of these patients [5–7].

A major unexplained challenge in the care of the critically injured is that biologics designed to treat the recognized underlying inflammatory and immunological aberrations in critically ill patients have been largely unsuccessful. This is, in part, due to the fact that our understanding of the complex pathobiology in severe injury is still poorly elucidated, as well as the basis of the heterogeneity in the patient response. Some of the limitations are inherent to focusing on a single protein and/or pathway that may be deranged during injury. Although it is true that these preclinical and clinical studies have greatly improved our understanding of how these mediators and/or pathways may individually regulate features of critical illness such as multisystem organ failure, treated individually, they have not yielded tangible improvements in patient outcomes.

To address some of these obstacles, the “Inflammation and Host Response to Injury” Large Scale Collaborative Research Program was created in 2001 as a multidisciplinary clinical investigation consortium composed of ten academic Level 1 trauma and burn centers across the United States. The goal of the Program was to provide a comprehensive assessment of the genomic and proteomic response in blood leukocyte populations from patients who have experienced severe trauma, either through severe blunt or burn injury. Furthermore, the Program sought to describe the genomic and proteomic response among patients with different clinical trajectories and outcomes. The Program is “discovery science” in its purest form, with no pre-existing hypotheses or dogmas to validate. Rather, the Program was designed to simply describe the human response to injury at a level far greater than had been previously possible, by focusing on the transcriptome and proteome of blood leukocyte populations.

It was predetermined that any multicenter investigation into the host response to severe trauma would require the standardization of patient management protocols, in order to minimize iatrogenic-induced variation in genomics and proteomics secondary to differences in treatment. Although not an original goal of the Program, the standardization of best practices was determined to be a requisite prior to conducting the genomic and proteomic analyses. Therefore, a comprehensive series of protocols were developed based on published

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literature established evidence-based medicine and expert consensus and uniformly applied at each of the participating centers.

The art of protocolized medicine

Despite having derived a consensus on clinical algorithms to treat critically injured patients, one of the most significant challenges to the optimal clinical management of these patients remains the implementation of evidence-based guidelines to treat the critically ill. Certainly, though physicians may agree on these guidelines, there have been several studies that have documented the limited translation of these algorithms into actual practice. Rubenfeld has suggested three possible factors as a cause of this gap between guidelines and implementation: knowledge, attitude, and/or behavioral barriers [7]. Increasingly, single and multicenter studies are being conducted to examine the effect of implementing protocols on outcomes. Gao et al. were one of the first groups to demonstrate an association between compliance with protocols based on the Sepsis Bundle and improved clinical outcome [8, 9]. Similarly, a recent study from Spain has demonstrated that by instituting the established Surviving Sepsis Campaign guidelines in 59 medical-surgical intensive care units (ICUs), a decrease in hospital mortality was observed [5]. Importantly, while absolute changes in protocol utilization compliance rates were small, their increased utilization produced significant improvements in outcome for this patient population. Therefore, while these barriers are often multifactorial and complex, they are increasingly demonstrated to improve survival.

In fact, as physicians, we are obliged to implement the best available therapies and guidelines, as well as to constantly reassess the compliance and outcomes in our patients to push the standard of care higher. As mentioned above, in order to minimize the potential impact and effect of variability in clinical management on the pathophysiologic response of severely injured trauma patients at the genomic level, the Program not only implemented but audited quarterly through a series of onsite visits and thorough chart reviews a bundle of guidelines at each of the participating centers. These protocols included guidelines for deep vein thrombosis prophylaxis, enteral feeding, transfusion indications, mechanical ventilation, glycemic control, resuscitation endpoints, and the management of complications such as ventilator-associated pneumonia [10–16]. The protocols were based on the best available evidence and agreed upon by leading experts in trauma, inflammation, and sepsis, and allowed for the comprehensive control of known clinical variables. These protocols were subsequently published as a series of papers in the Journal of Trauma for the general clinical research community to adapt for future interventional clinical trials in the critically ill. Certainly, as noted in the study from Ferrer et al., despite achieving some improvement in compliance initially, after 1 year, the compliance with the resuscitation bundle of the Surviving Sepsis Campaign guidelines returned to baseline [5], and, therefore, constant vigilance is needed if the long-term acceptance and translation of these guidelines to ingrained behavior for clinical practice is desired. In our Program, long-term adherence to guidelines was monitored by on-site visits from Program staff who reviewed the medical records. Constant surveillance resulted in continual improvements of variable magnitude in compliance with the individual protocols.

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Glue Grant implementation of evidence-based clinical care guidelines

During the course of the Glue Grant Program, long-term adherence to guidelines was monitored by on-site visits from Program staff who reviewed the medical records. Constant surveillance resulted in marked improvements in some but not all of the compliance criteria. We noted improvements in tidal volumes in patients with ARDS/ALI, a decrease in inappropriate venothromboembolism prophylaxis, and the use of bronchiolar lavage for the diagnosis of ventilator-associated pneumonia (Annals of Surgery, in press). In addition, the Program's overall mortality decreased over the study period from 22 to 11% in the last 2 years (Fig. 1) (Annals of Surgery, in press). In effect, by meeting the consortium's goals, the Program has been able to demonstrate an association between guideline compliance/auditing with improvements in clinical outcome at not one center, but multiple Level 1 academic trauma centers across the U.S. In addition, these data set the stage for the prospective genomic/proteomic study and provide an excellent clinical platform that is unmatched for studying the complex pathophysiology that occurs following severe blunt injury.

Current approaches and understanding of the pathophysiology in traumatic injury

One of the most widely accepted paradigms for the post injury/septic response was proposed by Roger Bone in the 1990s [17]. His SIRS/CARS theory was based on pre-clinical work in endotoxemia, inflammation, and sepsis, and described the established clinical phenotype, demonstrating an early induction of a systemic inflammatory response syndrome (SIRS) followed by less well characterized, immunosuppressive, compensatory, anti-inflammatory response syndrome (CARS) [17]. Though much of the work is centered in sepsis and not traumatic injury, the two pathologies are often intertwined both clinically and pathologically, as severely injured patients experience both a SIRS response early following injury that, in some cases, leads to sepsis and/or multisystem organ failure, the leading cause of mortality and morbidity in this population. However, the argument as to the etiology of these syndromes either as a direct result of the injury or secondary to attempted endogenous homeostasis has been in scientific debate for over two decades.

Traumatic injury causes the systemic release of both damage-associated molecular pattern molecules (DAMPs) and, with the breach of epithelial surfaces, pathogen-associated molecular pattern molecules (PAMPs) [18]. The interaction of these DAMPs and PAMPs with toll-like receptors (TLRs) leads to the activation of innate immunity that, in turn, elaborates a diverse set of chemokines and cytokines [18]. These findings have been demonstrated in animal models of endotoxemia, trauma, burn, and polymicrobial sepsis [18–20]. Many of these studies have gone on to subsequently block the activity of single proteins or cytokines that are released in association with the pathologic challenge and demonstrate reduced severity of disease and enhanced survival [21].

One of the first studies to demonstrate that the blockade of a cytokine in sepsis/septic shock was able to improve outcome was demonstrated by Tracey et al [22]. Subsequently, in the late 1990s and early 2000s, the focus of interventional treatments was to suppress an exaggerated inflammatory or SIRS response. Baboons administered anti-tumor necrosis

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factor alpha (TNF-α) were protected against live *Escherichia coli* administration and subsequent SIRS compared to those animals administered control antibody [22]. However, attempts to block TNF-α in patients with sepsis failed to demonstrate any clinical benefit and, in fact, worsened mortality at high doses in some septic shock patients [23]. Similarly the blockade of another cytokine that is closely associated with inflammation and sepsis, interleukin 1β, in animal models appeared to improve survival, but when translated into clinical trials, had no effect [24, 25]. Alternative approaches focused on addressing the CARS immunosuppressive response to trauma and sepsis, by administering IFNγ or GM-CSF, but also without significant clinical benefit [26, 27].

These studies highlight the problematic approach of: (1) translating preclinical studies using models that may be inappropriately matched to clinical pathologies and (2) focusing on single protein/cytokine mediators as the cause of organ injury. Although these investigations were and are critical to elucidate the immunologic and physiologic processes that are activated during injury/sepsis, and demonstrate the complexity of treating critically ill patients, it is clear that we still have not completely elucidated the disease process and its etiology. In fact, we would argue that ongoing efforts with mono-therapies following severe injury are doomed to failure for two reasons: (1) the host response to severe trauma is multifactoral and single therapies are unlikely to be successful in a large proportion of these diverse, genomically unique patients, and, thus, (2) therapeutic interventions must be closely linked to diagnostics that can identify specific immunological abnormalities in specific patients amenable to a therapeutic intervention. Drug trials that treat all patients indiscriminately with powerful biologicals are likely to be beneficial to few and either unhelpful or harmful to many.

Characterizing the post injury response in the Glue Grant

Our Program chose to take a different, more comprehensive approach. The primary goal of the Program was to document both the genomic and proteomic response to endotoxin administration, burn injury, and traumatic injury [28–30]. Also, by investigating these three insults, the Program could potentially identify commonalities and disparities between each of the inflammatory insults. In addition, since many animal models of trauma, shock, and sepsis use a variety of inflammatory challenges as a corollary to the human pathologies present during severe blunt traumatic injury, the study might also be able to capture whether preclinical studies were reflective of the conditions which they are purported to study. This is an important concept because, as described above, the translation of animal results into the clinic has met limited success. Finally, by looking at the genome-wide and proteomic response to severe injury in patients with successful versus adverse outcomes, it might be possible to identify patterns of gene expression or proteomics associated with various clinical trajectories.

The first challenge before the Program was to determine if the analytical and organizational strategies proposed would be valid in the setting of disease or, this case, namely, trauma [28]. Interestingly, although we identified some small “analytical noise” and intersubject variance in the genome-wide expression of either healthy or traumatically injured individuals, the significant transcriptomic changes induced by trauma within the first 24 h

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were far greater and more dramatic [28] (Fig. 2). As shown in the first panel, the variation in the total circulating leukocyte ("buffy coat") gene expression between healthy subjects and trauma patients was remarkably similar, suggesting that trauma does not increase the variance in gene expression. Rather, trauma appears to produce a significant reprioritization of the leukocyte transcriptome that is remarkably similar among a large number of different individuals. Together, these features make it relatively easy to identify large numbers of genes differentially expressed following trauma, as the genomic response would "cluster" tightly and separate based on study group characteristics (Fig. 2b). This initial study validated and provided a platform for the proposed approach to examine the genomic changes associated with endotoxin, burn injury, or traumatic injury.

Another seminal study from the group was an investigation of the genome-wide response to endotoxin in healthy volunteers in whole blood leukocytes over a 24 h post infusion period. Aside from the expected upregulation of the innate immune machinery thought to be responsible for the induction of the SIRS response, a substantial dysregulation in the genes associated with mitochondrial bioenergetics was also noted [30]. These genomic changes represented a dramatic reprioritization of the human leukocyte genome in response to a small dose of endotoxin [30]. This report was an unprecedented account of human genomic changes following a prototypical inflammatory event (Fig. 3). The genomic changes demonstrate the dramatic impact of endotoxin on gene expression and the rapid return to baseline as seen clinically in this acute phase model of inflammation.

In another early investigation from the Program, Laudanski et al. examined the genome-wide expression in both whole blood leukocytes, as well as isolated T cell and monocyte subsets from severely injured trauma patients with multisystem organ failure and healthy matched controls [31]. Importantly, transcriptomic analyses of these cell subsets versus whole blood revealed differences between the total blood leukocyte expression in comparison to T cell or monocyte gene expression in trauma patients [31]. In the T cell genome-wide analyses from trauma patients, many proapoptotic genes/pathways were upregulated that were not seen in the total leukocyte analyses. In addition, the enriched T cell analyses also demonstrated an increase in immunoinhibitory signaling molecules (SOCS1/3, SHP-1, and CTLA-4) that were also not observed in the whole blood leukocyte analyses [31]. These data correlated closely with preclinical and clinical studies, showing that injury and severe sepsis induces widespread T cell apoptosis and adaptive immune energy [32–37].

Though these findings were significant, changes in genomic expression do not always translate to changes in protein levels. In the same study, Laudanski et al. also performed a functional validation of several of the genes altered in the T cell analyses and found a similar decrease in the protein levels of CD3, CD4, CD28, and CXCR3, as well as an increase in CD152, PD-1, and CD86 in T cells [31]. In addition, decreased expression of CD86 and HLADR in monocyte genomic analyses were also validated via protein expression [31]. Importantly, these studies provided an investigational foundation for the larger, more comprehensive prospective study of genomic and proteomic changes proposed by the Program.

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Looking prospectively: genomic characterization of severely injured patients in the Glue Grant

Between 2003 and 2009, over 2,300 trauma subjects were enrolled into the epidemiological component of the study, and more than 450 subjects had blood sampling for total or enriched blood leukocyte subpopulation gene expression analyses (Table 1). Enrolled patients were then treated according to the established set of protocols (SOPs) as described above and blood was taken at 12 h, 1, 4, 7, 14, 21, and 28 days post traumatic insult. Similar to the results seen in the endotoxin studies, the evidence suggests a dramatic reprioritization of the entire leukocyte genome ([75%), including T cells, monocytes, and neutrophils, following trauma with long-lasting perturbations in the transcriptome extending beyond the 28-day study period (manuscript in review). This was evidenced by the failure of many gene expression profiles to return to a baseline as observed in healthy non-traumatized patients. Interestingly, although the expression of many proinflammatory mediators rapidly increased within the first 12 h of injury, simultaneously, the expression of many anti-inflammatory genes did as well, as did the depression of adaptive immune related genes (MHCI, II, etc.) by antigen-presenting cells, as well as T cell-responsive genes. These data have profound implications in the understanding of the pathology of trauma as it relates to preclinical studies and the model proposed by Roger Bone. For example, although many of the mediators thought to be part of the CARS response (IL-4 and IL-10) are, indeed, present at later stages of disease, the gene expression of these proteins is rapidly increased and significantly expressed within 12 h of injury. This implies that the commonly accepted paradigm is incorrect and there is no sequential SIRS then CARS, but, rather, a simultaneous invocation of the two responses at the genomic level. However, since the anti-inflammatory response is more prolonged and the initial inflammatory response so massive, the net phenotypic result produces an early inflammatory clinical phenotype followed by a delayed anti-inflammatory phenotype.

Development of a prognostic signature

Another potential application of these genome-wide expression analyses is the ability to predict uncomplicated versus complicated outcome early in severely traumatized patients in order to be able to goal direct therapy. In a study conducted by Warren et al., a difference from reference score (DFR) was determined based on the overall average changes in the genomic expression profile of the total blood leukocytes obtained from severe blunt trauma patients (within 12 h of injury) and healthy volunteers [38]. This DFR score was then compared with the APACHE score, Injury Severity Score (ISS), and outcome to determine an association of higher DFR scores with poorer outcome (ICU length of stay, maximum Denver score, etc.) [38]. The findings clearly showed that, contained in the genomic response data from 12-h post injury, was information that could be used to predict outcome even better than when patients were stratified for severity of injury by the ISS and APACHE score. As shown in Table 2, the predictive information was also contained in several subsets of the entire genome, and focusing on the expression of several ontologies of genes, such as cytokines and coagulation proteins, gave a similar predictive ability compared to the overall genomic alterations. Though this study was based on the whole blood leukocyte genome,

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future analysis will focus on leukocyte subsets and particular genes that differ between those patients who have a complicated outcome versus those who do not. Attempts by other groups are also ongoing to associate clinical outcomes with specific gene signatures. For example, Biberthaler et al. have examined the genomic profiles of monocytes from patients post traumatic injury and have identified some association between clinical parameters and canonical signaling pathways, such as multisystem organ failure with cellular development, cell death, and ephrin signaling pathways [39–41]. However, much more work needs to be done in order to prospectively validate and to further understand what the biological implications of these genomic findings are and whether they can be used as a prognostic signature for poor outcome in these severely injured patients. Hopefully, small gene sets or even specific genes will be identified as predictors of outcome and allow the selection of patients that will benefit from specific therapeutic interventions.

Bedside rapid point-of-care device to determine the prognosis of trauma patients: is it possible?

A critical remaining challenge is the ability to process and analyze genomic changes as a point-of-care and small-volume analyte, preferably while restricting the process to select subsets of cell populations. Within the multidisciplinary Program are material engineers who are focused on novel rapid cell separation devices that could be used to isolate T cells, monocytes, or granulocytes for cell population genomic analyses. Kotz et al. recently demonstrated the use of a microfluidics device that can capture neutrophils from peripheral blood in 5 min using a small volume (\150 μL) [12, 42–44] (Fig. 4). This platform has been reformatted so that T cells, monocytes, and neutrophils can be captured separately and simultaneously from a small volume of peripheral blood. This technology has also been adapted for application at the bedside. Since the genomic response using whole blood leukocytes may “hide” sub-population responses and the ability to identify critical function/biology with pertinent individual leukocyte sub-populations, which may subsequently provide a more useful prognostic score based on the response of a specific leukocyte population, peripheral blood from a separate large, prospectively selected group of severely injured patients has been collected and enriched for T cells, monocytes, and neutrophil major subsets using these microfluidic cassettes. Genome-wide analyses of these arrays are currently being conducted.

Conclusions/future directions

The economic and health care burdens of traumatic injury and critical illness remain significant. Efforts to decrease complications and improve the quality of patient care are ongoing. It is clear that the path to improve on our current standard of care for these patients lies in both physician acceptance of the constant need to evolve and the widening of our translational research focus to more accurately capture the pathology of the disease. The development and widespread implementation of high-throughput analyses have permitted the application of these exciting technologies to the challenges of recovery from severe injury.

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The Inflammation and Host Response to Injury Program has attempted to do this by focusing not only on capturing genomic and proteomic data from severely injured patients but also by instituting guidelines based on the best available evidence at each of the participating centers (Fig. 5). By doing this, the Program established two goals simultaneously: (1) the standardization of care for trauma and critically ill patients and (2) the ability to comprehensively describe the genomic and proteomic perturbations that occur within these patients with minimal iatrogenic clinical/therapeutic variability (Fig. 5). Although more thorough analyses are currently underway to further understand the genomic responses to severe trauma in individual cell subsets, one of the key endpoints for the program would be a rapid bedside assay that would yield a prognostic signature sensitive and specific enough to determine which trauma patients would have a relatively uncomplicated outcome versus those who were at higher risk for a complicated outcome (Fig. 5). By using genomics to target both individuals and therapies, the Program will move the clinical field forward towards truly personalized medicine.

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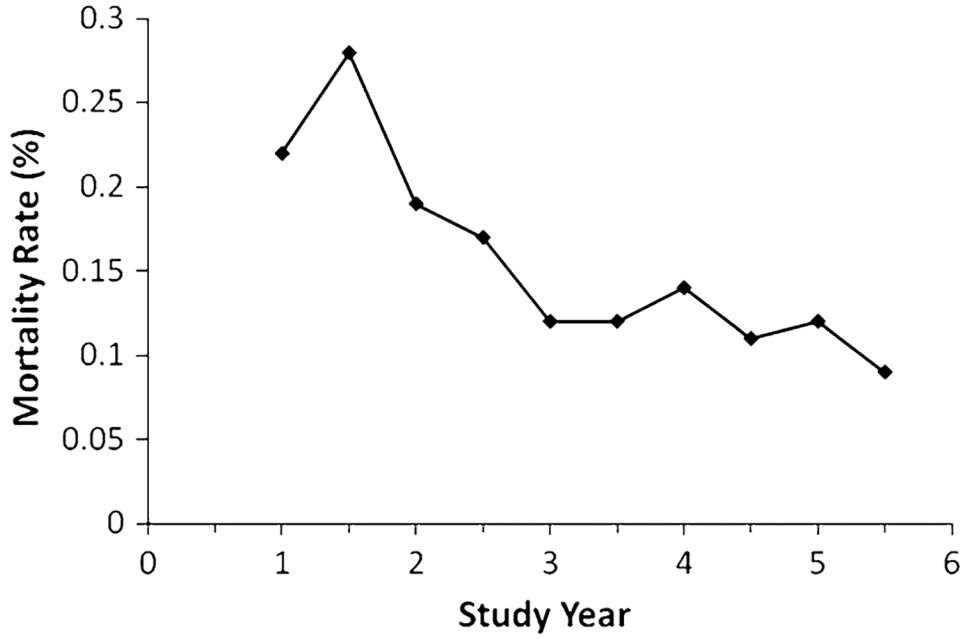

Fig. 1.
The Program mortality over the study period [45]

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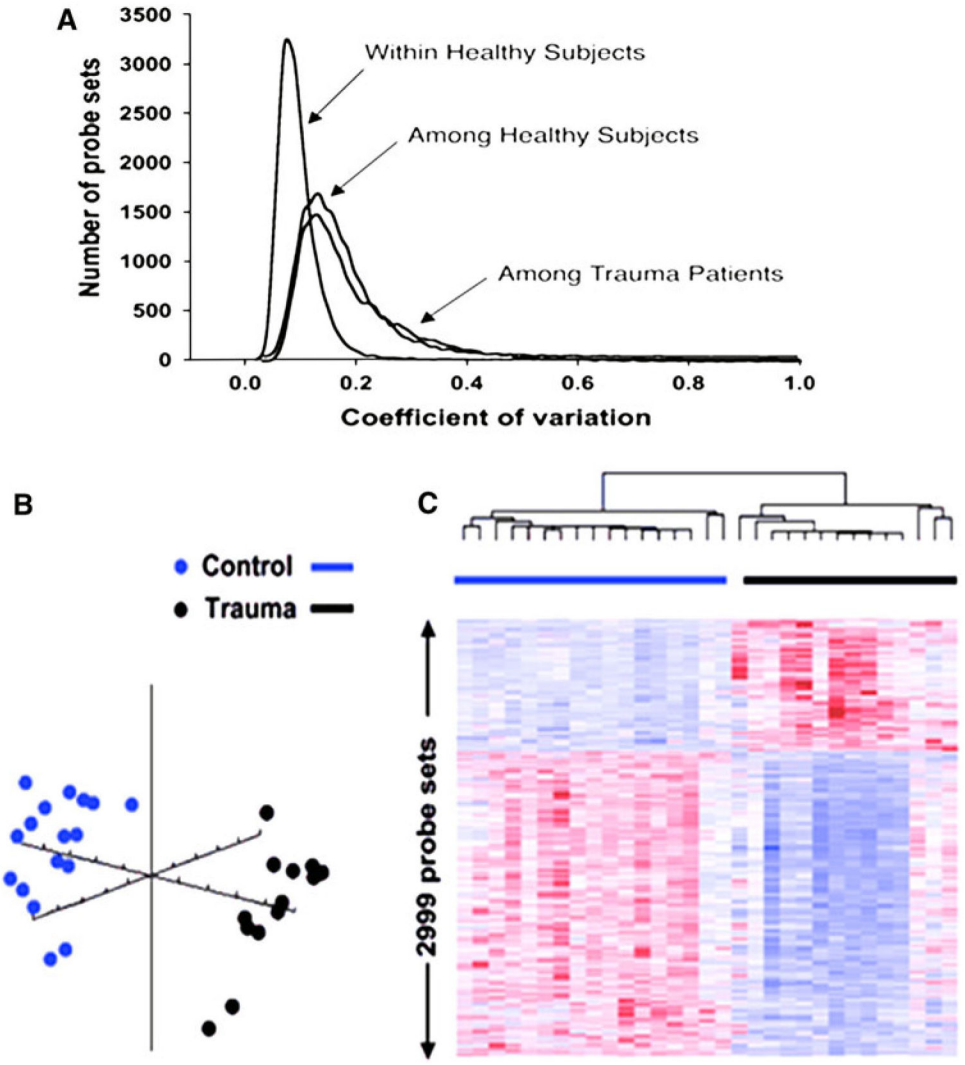

Fig. 2.

As a validation and test of the genomic array being used for the larger prospective study, the intersubject variation and intracohort variation (healthy controls or traumatically injured patients) had to be determined. a Similar levels of transcriptomic variation among healthy subjects versus among trauma patients, but more than the variance within healthy subjects. Principle component (b) and hierarchical cluster (c) analyses on leukocyte gene expression from 14 trauma and 17 healthy subjects demonstrating that the genomic signatures of the individuals within either healthy controls or trauma patients “cluster” close as a group but can be separated based on cohort characteristics (control vs. injured patients) (figure adapted from Cobb et al. [28])

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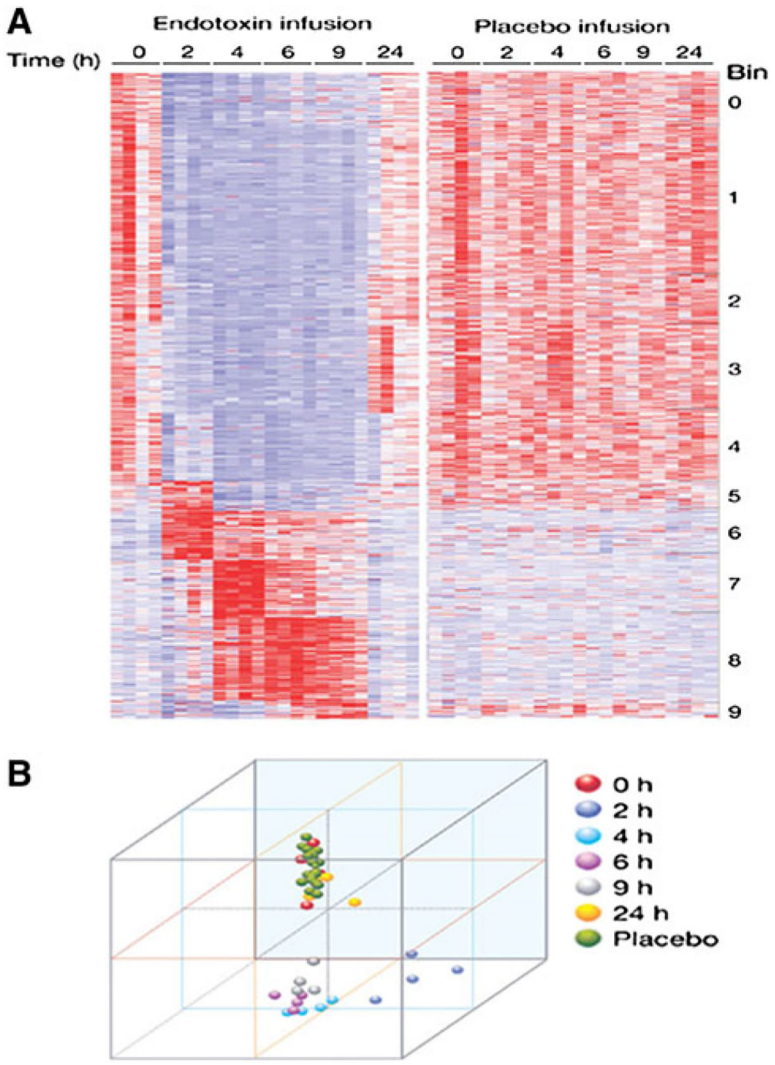

Fig. 3.

Total leukocyte genomic expression from eight healthy volunteers at 0, 2, 4, 6, 9, and 24 h after endotoxin administration ($n = 4$) or vehicle ($n = 4$) and subjected to K means hierarchical clustering to group genes with similar genomic and temporal expression (a) and principal component analysis to demonstrate similarities in the genomic expression of individuals at each time point, as well as to show the differences in transcriptome characteristics in those individuals administered endotoxin from baseline (b). c A hypothetical inflammatory cell was constructed from the 292 representative genes involved in inflammation and innate immunity that demonstrate the composite genomic changes over 24 h (*top*) or the temporal genomic inflammatory network changes at each time point (*bottom*) (figure adapted from Calvano et al. [30])

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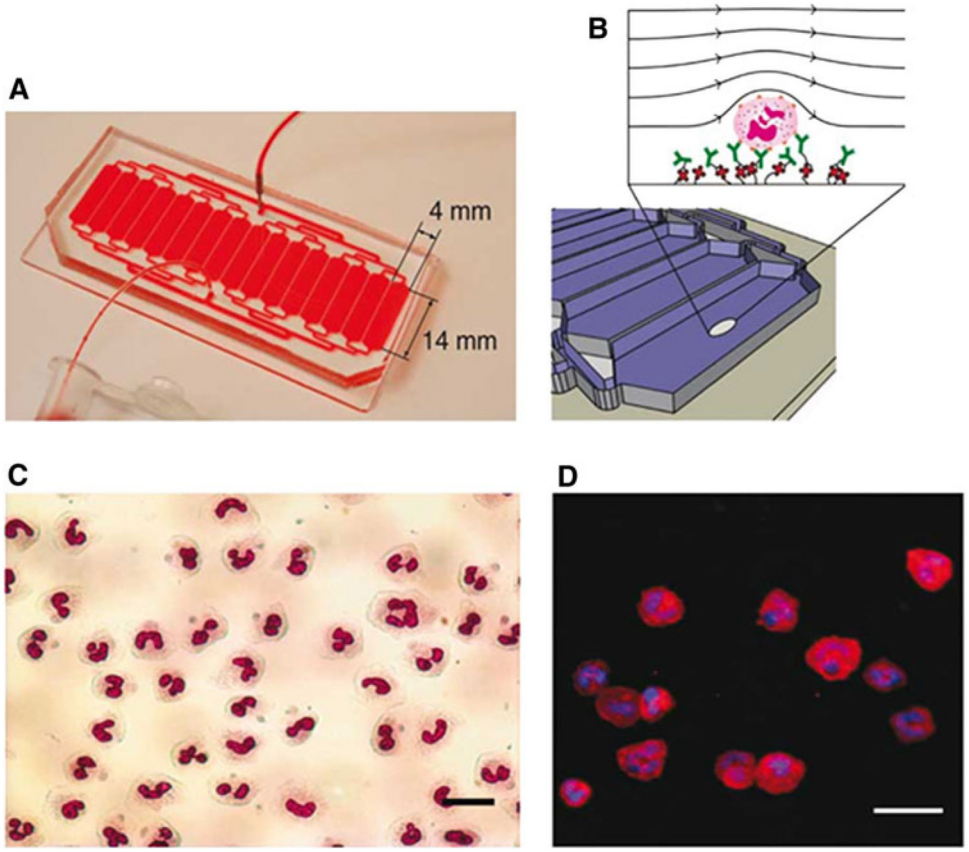

Fig. 4.

Microfluidic chip design demonstrating small size (a) and the chip surface depiction(b) with biotinylated CD66b antibodies (*green*) bound to neutravidin molecules (*red*) linked to the surface of the device. Demonstration of the microfluidic chip cell yield purity by Wright–Giemsa (c) or immunofluorescence using neutrophil cell surface markers (d) (*scale bar* 25 *lm*) (figure adapted from Kotz K et al. [42])

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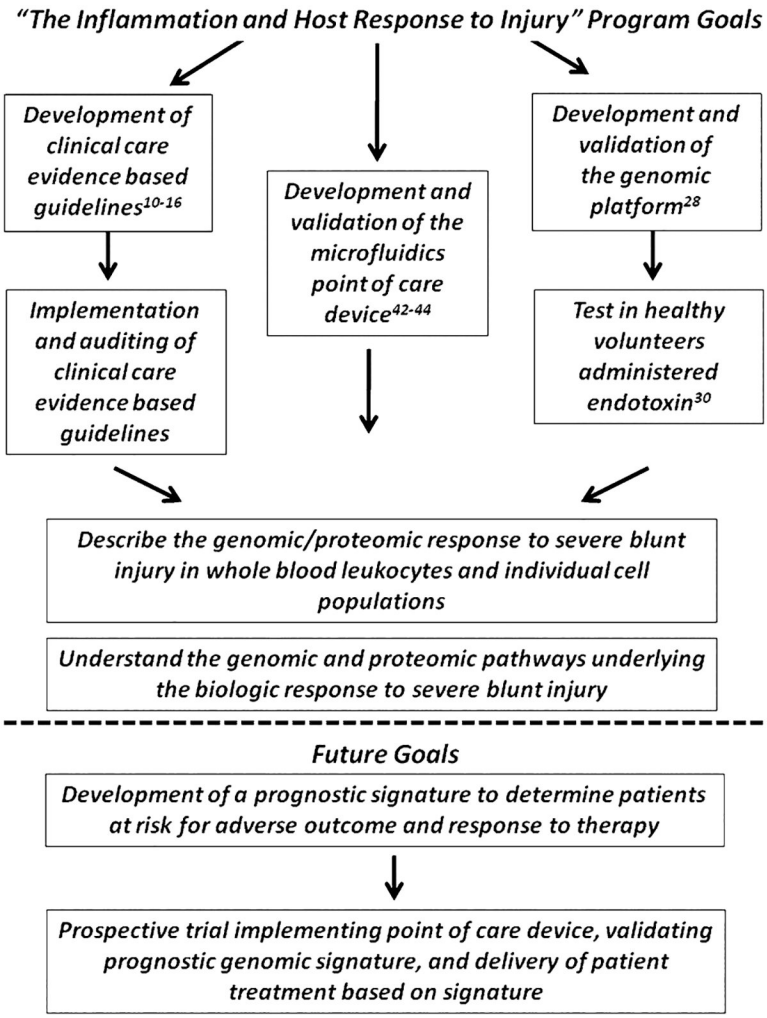

Fig. 5.
The "Inflammation and Host Response to Injury" Glue Grant Program goals

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Table 1

Glue Grant inclusion criteria

Inclusion criteria
Greater than 16 years of age
Evidence of shock defined as:
Base deficit ≥ 6 meq/L or
SBP < 90 mm Hg
Blood transfusion within 6 h of injury

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Table 2

Spearman correlation (*p* values) between the difference from reference score (DFR) calculated for the entire genome and for gene ontologies participating in the inflammatory response with injury severity and the development of organ failure (adapted from [38])

Gene ontologies	Probe sets	Severity of physiologic abnormality based on the APACHE score	Severity of injury based on the ISS score	Degree of organ failure based on the Marshall score	Degree of organ failure after controling for the score APACHE and ISS
Entire genome	50,874	0.2944 (0.0002)	0.2664 (0.0007)	0.3009 (0.0001)	0.1875 (0.0191)
Antioxidant	494	0.3212 (<0.0001)	0.1018 (0.2062)	0.2460 (0.0018)	0.1523 (0.0576)
Chemokine	887	0.2954 (0.0002)	0.2547 (0.0012)	0.3444 (<0.0001)	0.2403 (0.0025)
Coagulation	392	0.3164 (<0.0001)	0.1899 (0.0169)	0.3381 (<0.0001)	0.2403 (0.0025)
Cytokines	2,400	0.3245 (<0.0001)	0.1691 (0.0337)	0.3357 (<0.0001)	0.2393 (0.0026)
Heat shock	263	0.1914 (0.0160)	0.1994 (0.0120)	0.2043 (0.0100)	0.1218 (0.1299)
HMGB1	130	0.1315 (0.0995)	0.1362 (0.0880)	0.1914 (0.0160)	0.1393 (0.0828)
TLR (toll-like receptors)	17	−0.0554 (0.4381)	−0.0087 (0.9134)	0.0450 (0.5745)	0.0693 (0.3900)