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Genomic responses in mouse models poorly mimic human inflammatory diseases

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A cornerstone of modern biomedical research is the use of mouse models to explore basic pathophysiological mechanisms, evaluate new therapeutic approaches, and make go or no-go decisions to carry new drug candidates forward into clinical trials. Systematic studies evaluating how well murine models mimic human inflammatory diseases are nonexistent. Here, we show that, although acute inflammatory stresses from different etiologies result in highly similar genomic responses in humans, the responses in corresponding mouse models correlate poorly with the human conditions and also, one another. Among genes changed significantly in humans, the murine orthologs are close to random in matching their human counterparts (e.g., R^2 between 0.0 and 0.1). In addition to improvements in the current animal model systems, our study supports higher priority for translational medical research to focus on the more complex human conditions rather than relying on mouse models to study human inflammatory diseases.

human disease | translational medicine | inflammation | immune response | injury

Murine models have been extensively used in recent decades to identify and test drug candidates for subsequent human trials (1–3). However, few of these human trials have shown success (4–7). The success rate is even worse for those trials in the field of inflammation, a condition present in many human diseases. To date, there have been nearly 150 clinical trials testing candidate agents intended to block the inflammatory response in critically ill patients, and every one of these trials failed (8–11). Despite commentaries that question the merit of an overreliance of animal systems to model human immunology (3, 12, 13), in the absence of systematic evidence, investigators and public regulators assume that results from animal research reflect human disease. To date, there have been no studies to systematically evaluate, on a molecular basis, how well the murine clinical models mimic human inflammatory diseases in patients.

The Inflammation and Host Response to Injury, Large Scale Collaborative Research Program has completed multiple studies on the genomic responses to systemic inflammation in patients and human volunteers as well as murine models (14–18). These datasets include genome-wide expression analysis on white blood cells

obtained from serial blood draws in 167 patients up to 28 d after severe blunt trauma (15), 244 patients up to 1 y after burn injury, and 4 healthy humans for 24 h after administration of low-dose bacterial endotoxin (14) and expression analysis on analogous samples from well-established mouse models of trauma, burns, and endotoxemia (16 treated and 16 controls per model) (16–18). In humans, severe inflammatory stress produces a genomic storm affecting all major cellular functions and pathways (15) and therefore, provided sufficient perturbations to allow comparisons between the genes in the human conditions and their orthologs in the murine models.

In this article, we report on a systematic comparison of the genomic response between human inflammatory diseases and murine models. First, we compared the correlations of gene expression changes with trauma, burns, and endotoxemia between human subjects and corresponding mouse models. Second, we characterized and compared the temporal gene response patterns seen in these human conditions and models. Third, we also identified the major signaling pathways significantly regulated in the inflammatory response to human injuries and compared them with the human *in vivo* endotoxemia model and three murine models. Fourth, we

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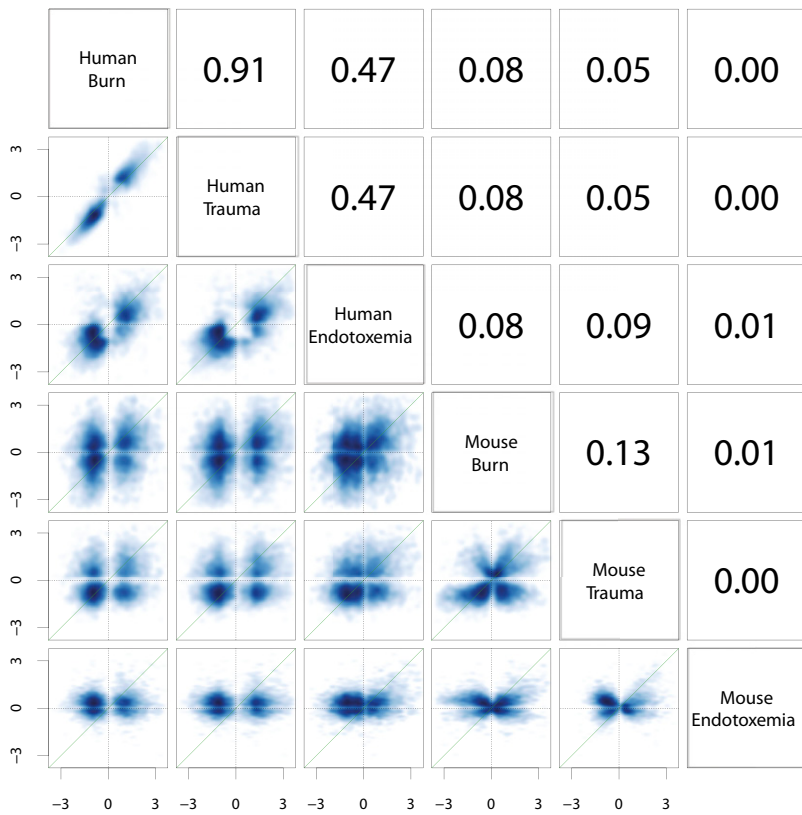


Fig. 1. Correlations of the gene changes among human burns, trauma, and endotoxin and the corresponding mouse models. Scatter plots and Pearson correlations (R^2) of the log twofold changes of 4,918 human genes responsive to trauma, burns, or endotoxemia (FDR < 0.001; fold change ≥ 2) and their murine orthologs in the murine models. As shown in the upper left, the genomic responses to human trauma and burns are highly correlated ($R^2 = 0.91$). In contrast, as shown in the lower right, the murine models correlate poorly with each other ($R^2 = 0.00-0.13$) and almost randomly with the corresponding human conditions ($R^2 = 0.00-0.09$). Similar results were seen with rank correlation (SI Appendix, Fig. S1).

sought and evaluated representative patient and murine studies of several additional acute inflammatory diseases. These results show that the genomic responses to different acute inflammatory stresses

are highly similar in humans, but these responses are not reproduced in the current mouse models. New approaches need be explored to improve the ways that human diseases are studied.

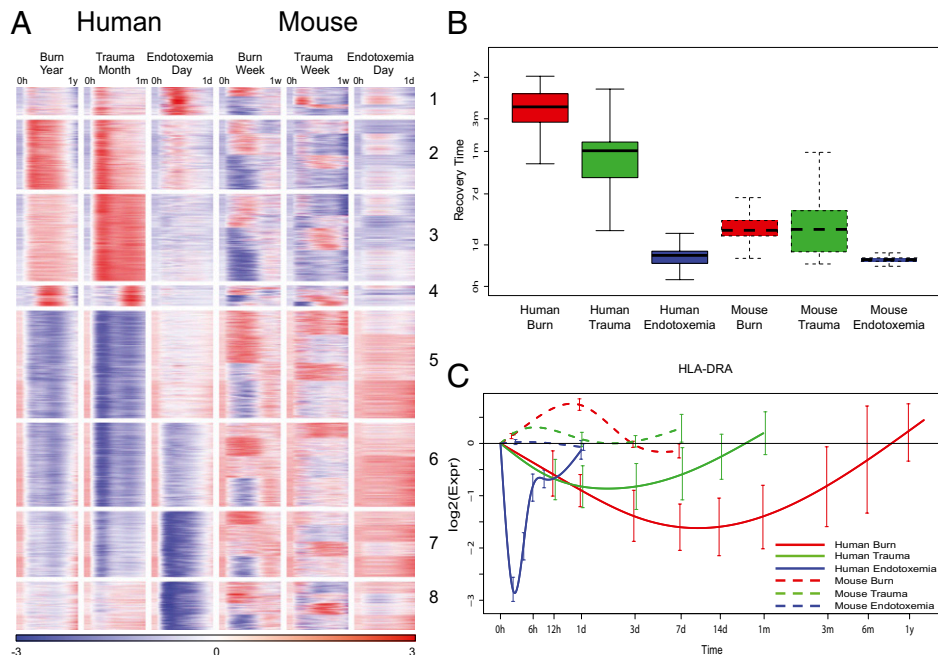


Fig. 2. Comparisons of the time-course gene changes for human burns, trauma, and endotoxin and the murine models. (A) K-means clustering of 4,918 genes responsive to human systemic inflammation over time. Time intervals for the human conditions: up to 1 y (burns), 1 mo (trauma), and 1 d (endotoxemia); time intervals for mouse models: 1 wk (trauma and burns) and 1 d (endotoxemia). (B) Box plots of recovery times of gene changes in the human and mouse conditions. (C) Log₂ expression changes vs. time of HLA-DRA as an example where genes changed significantly over a long time in human injuries but minimally in the murine models.

Results

Correlation of Gene Changes to Trauma, Burns, and Endotoxemia Between Human Subjects and Murine Models. Significantly changed genes were identified for each dataset; 5,544 genes were identified as significant [false discovery rate (FDR) < 0.001 and fold change ≥ 2] (*Materials and Methods*) between patients and healthy subjects (4,389 genes in trauma, 3,250 genes in burns, and 2,251 genes in endotoxemia). Among these 5,554 genes significantly changed in human conditions, 4,918 genes had mouse orthologs assayed, which were included in the subsequent analysis.

The maximum fold changes of gene expression were calculated in log scale between patients and healthy subjects for each dataset of human burn, trauma, and endotoxemia and between treated and control groups for the corresponding murine models. Between two datasets, the agreements of the maximum gene fold changes (Pearson correlation and rank correlation between the two datasets) as well as the directionality of the changes (the percentages of genes changed to the same direction between the two datasets) were compared.

Among the 4,918 genes, correlations of the maximum gene changes (Pearson correlation, R^2) (Fig. 1) and percentages of genes changed in the same direction (*SI Appendix, Fig. S1*) in all six conditions indicate that there was very high similarity in gene response between human trauma and human burns ($R^2 = 0.91$, 97%), and moderate similarity between human endotoxemia and human injuries ($R^2 = 0.47$, 88%). In contrast, there was very low correlation of expression changes between human genes in any of the conditions and their mouse orthologs in the mouse models ($R^2 \leq 0.09$, ~47–63%). By random chance, 50% of the genes between two uncorrelated conditions are expected to change in the same direction. Furthermore, there was poor correlation of the mouse gene orthologs with each other among the three murine models ($R^2 \leq 0.13$, ~39%–63%). Similar results were seen when the rank correlation was calculated as in *SI Appendix, Fig. S1*. We further examined subsets of 4,918 genes that showed an even greater magnitude of changes (*SI Appendix, Fig. S2*) and observed a modest increase of correlation between murine models and human conditions. For example, the correlations

increase to $R^2 = 0.11$ – 0.28 among the top 105 genes with the greatest magnitude of changes (fold change ≥ 6).

To probe further and address the possibility of bias against the murine models by requiring murine orthologs to correlate with human genes responsive in the human conditions, we examined the reverse (human orthologs corresponding to the responsive mouse genes) (*SI Appendix, Fig. S3*). High correlation was maintained in the human orthologs ($R^2 = 0.88$), whereas there were only minimal increases in correlations among mouse genes ($R^2 \leq 0.22$). To address the potential confounding feature of different sample sizes between the studies, we randomly resampled (*SI Appendix, Fig. S4A*) comparable small numbers of humans and mice and showed that the correlations remained high between human injuries ($R^2 = 0.85$). In addition, with comparable sample sizes, the human conditions introduced a more profound genomic response (more than or equal to three times more genes) than the corresponding mouse models (*SI Appendix, Fig. S4B*). However, intragroup variances remained comparable (*SI Appendix, Table S1*).

Comparison of the Temporal Response Patterns Between the Human Conditions and Murine Models. An inherent assumption in the use of murine models to mimic human systemic inflammation is that the time course of injury and repair between the species is similar. Decisions about timing of sample acquisition, endpoints for analysis, or dosing of drugs are dependent on this assumption.

We queried gene changes in the human conditions and mouse models over different time spans (days, weeks, months, and years). We then analyzed the time-course pattern of the expression of each gene by clustering (Fig. 2A) and further characterized by response time (time for the gene change to reach one-half of its maximum value) and recovery time (time for the gene to decrease to one-half its maximum value) (Fig. 2B and *SI Appendix, Fig. S5*). Although in all conditions, the gene response time occurred within the first 6–12 h, the recovery time differed dramatically. Genomic disturbances recovered in the murine models within hours to 4 d but lasted for 1–6 mo or more in patients. For example, HLA-DRA changed over a long time period in human injuries but only minimally in the murine models (Fig. 2C). In addition, although

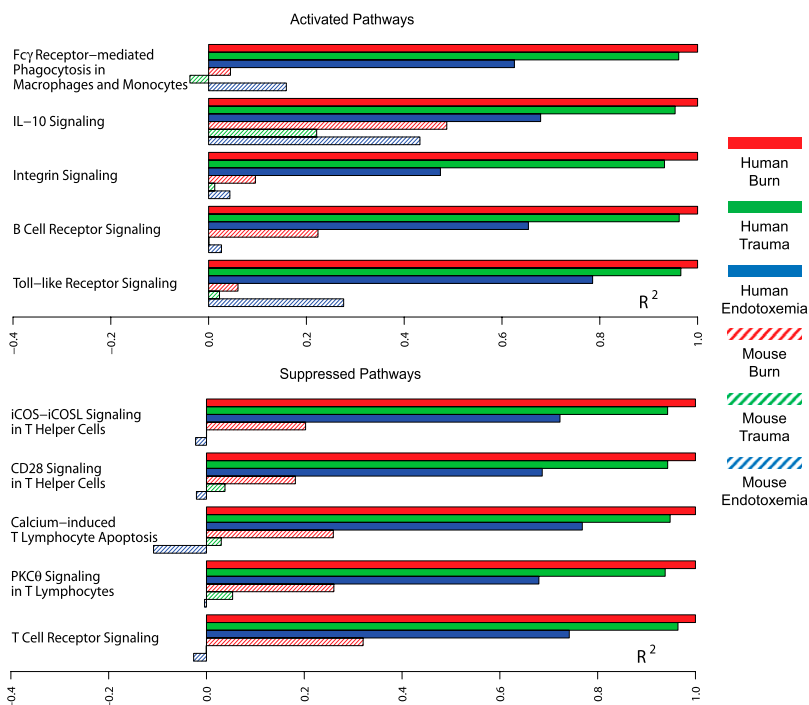


Fig. 3. Pathway comparisons between the human burns, trauma, and endotoxin and mouse models. Shown are bar graphs of Pearson correlations (R^2) for the five most activated and suppressed pathways between the four model systems (human endotoxemia and the three murine models) vs. human trauma and burns. Negative correlations are shown as negative numbers ($-R^2$). Human burn is shown as the reference. In every pathway, human endotoxemia had much higher similarity to human injury than mouse models.

genes within each cluster changed in same directions over the time course between human conditions (SI Appendix, Fig. S6), there was great variability between the three murine models, even within the clusters, suggesting that mouse responses differed not only from the human conditions but also from one another (SI Appendix, Fig. S7).

Comparison of the Significantly Regulated Pathways Between the Human Conditions and Murine Models. We identified the major signaling pathways significantly regulated in human injuries and compared them with a human endotoxemia model and three murine models. In human injuries, most of the pathways up-regulated were related to innate immune response, and those pathways down-regulated were related to adaptive immunity. Fig. 3 shows the correlations of human burn injury with the three mouse model systems and human trauma for the five most activated and most suppressed pathways. The correlations depended on the individual pathway compared (SI Appendix, Table S2). For example, the mouse endotoxin model, which is often used as a proxy for inflammation in humans, had a maximum correlation of 0.43 for IL-10 signaling, but it had no or negative correlations for all five of the most down-regulated pathways. In every pathway, human endotoxemia had much higher similarity to human injury than seen in the mouse models.

Additional examination of the top 40 pathways shows that the median correlations and percentages of genes changed in the same direction were 0.16 and 67% for mouse burns, 0.03 and 60% for mouse trauma, and 0.00 and 52% for mouse endotoxemia. Therefore, individual gene activation in the human conditions was not necessarily predicted by the ortholog in the corresponding mouse model in either direction or magnitude [e.g., the toll-like receptor (TLR) pathways] (SI Appendix, Fig. S8).

Comparison of the Genomic Responses in Several Additional Acute Inflammatory Diseases and Mouse Models. We were surprised by the poor correlation between the genomic responses in the mouse models and those responses in human injuries, especially given the

Table 1. Studies available in GEO on severe acute inflammatory diseases in human and murine models

Disease	GEO accession	R^2	Percent
Human			
Burns (as reference)	GSE37069	1.00	100
Trauma	GSE36809	0.91	97
Endotoxemia (test)	GSE3284	0.47	88
Endotoxemia (verification)	GSE3284	0.59	90
ARDS	GSE10474	0.55	84
Sepsis	GSE13904	0.76	93
Sepsis	GSE9960	0.64	87
Sepsis	GSE13015	0.61	86
Sepsis	GSE28750	0.63	85
Acute Infection	GSE6269	0.50	83
Mouse			
Burns	GSE7404	0.08	60
Trauma	GSE7404	0.05	61
Endotoxemia	GSE7404	0.00	47
Endotoxemia	GSE5663	0.00	50
ARDS	GSE19030	-0.01	48
Sepsis (CLP)	GSE5663	0.03	53
Sepsis (CLP-Mild)	GSE5663	0.02	52
Sepsis	GSE19668	0.05	58
Sepsis	GSE26472	0.02	55
Infection	GSE20524	0.08	61

R^2 represents Pearson correlation. Negative correlations are shown as $-R^2$. Percent represents the percentages of genes changed to the same direction between the two datasets. CLP, cecal ligation and puncture.

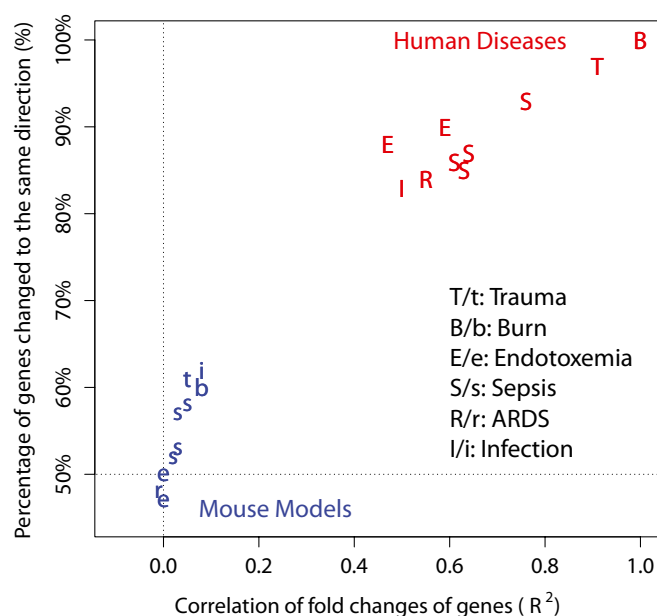


Fig. 4. Comparison of the genomic response to severe acute inflammation from different etiologies in human and murine models. GEO was queried for studies in the white blood cells of additional severe acute inflammatory diseases (sepsis, ARDS, and infections) in human and mouse. The resulting datasets are listed in Table 1. Shown are correlations (R^2 ; x axis) and directionality (%) of gene response from the resulting multiple published datasets in GEO compared with human burn injury.

worldwide prevalence of the use of mice to model human inflammation. We, therefore, sought and evaluated additional patient and corresponding mouse model studies from Gene Expression Omnibus (GEO) (19) for several other severe acute inflammatory diseases [sepsis, acute respiratory distress syndrome (ARDS), and infections] as listed in Table 1.

The fold change of each gene measured was calculated between patients and controls in a human study or between treated group and control group in a murine model study. The gene response in each dataset was then compared with the 5,554 genes significantly changed in human trauma, burns, and endotoxemia. Correlations and directionality of gene response were calculated using these common genes between each additional dataset and the burn injury that was used as the reference.

As shown in Fig. 4 and Table 1, the genomic responses in patients correlated well with each other, whereas this response was mimicked poorly by the mouse models. The same relationship held for the directionality of the gene response. For example, in humans, correlations of 0.91 (trauma), 0.55 (ARDS), 0.54–0.79 (sepsis), and 0.50 (infection) were observed with percentage of the genes changing in the same direction of 97%, 84%, 86–92%, and 83%, respectively. For murine orthologs, there were correlations between 0 and 0.08, with 47–61% of genes changing in the same direction—random chance would predict 50%. In addition, published data with radiation injury (20) showed similar results (SI Appendix, Fig. S9).

Discussion

Studying disease in patients is much more complex than studying model systems. In the trauma patients that we studied, patient heterogeneity existed in the most relevant characteristics, including demographics, such as age [years, median (M) = 33, middle quartiles (MQ) = 25–44] and sex (male = 63%), severity of injury as indicated by maximum Abbreviated Injury Scale (M = 4, MQ = 3–5), Injury Severity Score (M = 33, MQ = 22–41), and worst base deficit (M = -9.1, MQ = -12.0 to -6.4), variable

amounts of blood received (total transfusion in milliliters; $M = 1,900$, $MQ = 1,050\text{--}3,000$), and patient clinical outcomes as indicated by survival (96%), multiorgan failure (maximum Marshall score; $M = 5$, $MQ = 3\text{--}7$), time to recovery (days; $M = 7$, $MQ = 4\text{--}15$), nosocomial infections (54.5%), and hospital length of stay (days; $M = 21$, $MQ = 12\text{--}32$) (15, 21). Similarly, in the burn patient population, significant variations existed in the extent of burn injuries, for example, in terms of total body surface area of burns (TBSA; $M = 53\%$, $MQ = 32\text{--}74\%$), percentages of flame burns (86%) and inhalation injury (52%), and survival (85%). In addition, patients received a veritable pharmacopeia of drugs that can affect their pathophysiological and genomic response. However, despite of these heterogeneities, we observed highly consistent genomic response in patients between trauma and burns in contrast to the lack of correlations in the murine models.

Critically ill patients with different underlying acute injuries have similar-appearing physiological reactions, a condition known by consensus definition as Systemic Inflammatory Response Syndrome (22, 23). An unproven hypothesis central to the pursuit of drug targets for this syndrome has been that the molecular mechanisms underlying this syndrome are similar regardless of initiating etiology. The very high correlation in response between trauma, burns, and endotoxemia in humans strongly supports this hypothesis and suggests that such an approach may be possible.

There are multiple considerations to our finding that transcriptional response in mouse models reflects human diseases so poorly, including the evolutionary distance between mice and humans, the complexity of the human disease, the inbred nature of the mouse model, and often, the use of single mechanistic models (3, 12, 13, 24). In addition, differences in cellular composition between mouse and human tissues (24, 25) can contribute to the differences seen in the molecular response. Additionally, the different temporal spans of recovery from disease between patients and mouse models are an inherent problem in the use of mouse models. Late events related to the clinical care of the patients (such as fluids, drugs, surgery, and life support) likely alter genomic responses that are not captured in murine models.

The evolution of the immune system for any species is, at least in part, a direct consequence of the microbe-exerted selection pressure for that species. Recent articles have highlighted tradeoffs that species make to balance often opposing evolutionary strategies for resistance vs. tolerance or resilience to infection (26, 27). Relative to the human response, mice are highly resilient to inflammatory challenge (28, 29). For example, the lethal dose of endotoxin is 5–25 mg/kg for most strains of mice, whereas a dose that is 1,000,000-fold less (30 ng/kg) has been reported to cause shock in humans (30). The extreme sensitivity of humans relative to mice to massive inflammation may result in genomic responses that reach an upper threshold in each human disease, whereas the resilience of mice may prevent maximum responses and lead to greater heterogeneity. This attenuated murine response also suggests that a higher level of injury in mouse models might result in a transcriptome response that better mimics the response seen in the patients. For example, a limitation of the current study is that, in the burn patients, the TBSA of burns ($TBSA > 20\%$, $M = 53\%$, $MQ = 32\text{--}74\%$) is, on average, higher than the TBSA of the murine model ($TBSA = 25\%$). However, currently, there is no existing murine model with intensive care support that would allow consistent survival of mice with substantially greater than 25% TBSA burn injury.

As a cornerstone of modern biomedical research, the use of mouse models has dominated scientific literature (3, 13). The prevailing assumption—that molecular results from current mouse models developed to mimic human diseases translate directly to human conditions—is challenged by our study. Because virtually every drug and drug candidate functions at the molecular level, one practical approach forward is to raise the bar by requiring molecular detail in the animal model studies indicating whether

the model mimics or fails to mimic the molecular behavior of key genes, key pathways, or the genome-wide level thought to be important for the relevant human disease.

New approaches could be explored to improve current models. For example, by first requiring comprehensive genomic descriptions in patient studies to define the human disease, the disease-altered pathways could be used as a guide to develop the animal model. The quality of the animal model could then be determined by how well it reproduces the human disease on a molecular basis rather than simply phenotype. In addition, the development of synthetic human models by *in vitro* reconstitution of disease-related cell types or tissues (31) might similarly improve current disease models. Furthermore, new genomic information, such as the availability of personal genomes (32) or exomes (33) to capture the disease heterogeneity directly from patients or systematic interpretation of genome-wide signatures in human diseases (34, 35), will likely complement or short circuit the need for mouse models in disease discovery and drug development. Notwithstanding, our study supports higher priority to focus on the more complex human conditions rather than relying on mouse models to study human inflammatory diseases.

Materials and Methods

Patient Enrollment and Sampling. The Inflammation and the Host Response to Injury, Large Scale Collaborative Research Program was funded to study the early inflammatory response to serious injuries. Between 2003 and 2009, 1,637 patients were enrolled at one of seven US Level I trauma centers if they experienced a blunt injury associated with prehospital or emergency department systolic hypotension (<90 mmHg) or an elevated base deficit (>6 mEq/L), blood transfusion requirement ≤ 12 h, and had an Abbreviated Injury Scale score > 2 for any body region, exclusive of the brain. Of these patients, serial blood samples were obtained from 167 patients for genomic analysis. The first blood sample was taken within 12 h of the injury and 1, 4, 7, 14, 21, and 28 d after injury. Study subjects were treated under the guidance of standard operating procedures developed, implemented, and audited across all participating centers to minimize treatment. Details of the study design are described in ref. 15. Similarly, between 2000 and 2009, 244 burn patients were enrolled at one of four burn centers if they were admitted to a participating burn center within 96 h after injury, had burns over at least 20% of the TBSA, and required at least one excision and grafting procedure. After admission, study subjects were treated under the guidance of standard operating procedures developed, implemented, and audited across all participating centers to minimize treatment variation. Demographics, clinical outcomes, and complications occurring in the intensive care unit and within 28 d posttrauma and up to 1 y postburns were recorded and described in ref. 15, and they are available at <http://TRDB.bluegrant.org>. In addition, 35 healthy control subjects (16–55 y) were recruited between 2004 and 2007. Two control subjects were studied two times approximately 2 y apart, yielding 37 samples. The Institutional Review Board of each participating center approved the study.

Human Endotoxemia Model. Eight healthy male and female subjects between 18 and 40 y of age provided written informed consent. Subjects were *i.v.* administered either National Institutes of Health Clinical Center Reference Endotoxin *Escherichia coli* 0113 (CC-RE-Lot 2) at a dose of 2 ng/kg body weight ($n = 4$, one female and three males) or 0.9% sodium chloride ($n = 4$, one female and three males) over a 5-min period. Blood samples were collected before endotoxin infusion (0 h) and 2, 4, 6, 9, and 24 h after infusion.

Murine Injury Models. Male C57BL/6J mice were purchased from Jackson Laboratory. The study was approved by the Institutional Animal Use and Care Committees of each participating institution. The mice were acclimated for at least 1 wk before use in these experiments at 8 wk of age. Details of the protocols have been described previously. Groups of 12 mice undergoing the injury protocols were given inhalation anesthesia with isoflurane and then subjected to 25% TBSA scald burn or trauma/hemorrhage (T/H). After burn injury, mice were resuscitated with 1 mL normal saline injected *i.p.* T/H consisted of laparotomy followed by withdrawal of sufficient blood from an arterial line to decrease and maintain mean arterial blood pressure at 35 mmHg for 90 min, after which times these mice were resuscitated with Ringer lactate (four times the shed blood volume). Groups of 12 mice also underwent sham burn or sham T/H procedures under anesthesia. In separate experiments, groups of six injured or sham mice were anesthetized with isoflurane

and exsanguinated by cardiac puncture at 2 h, 1 d, 3 d, or 7 d after injury. Mice receiving LPS, 10 ng *E. coli* 0113, the same endotoxin applied to the human study, in 200 μ L PBS or saline control by i.p. injection were not anesthetized. The amount of LPS was chosen to generate similar cytokine induction patterns as seen in a human LPS study (17).

Gene Expression Profiling. Total blood leukocytes were isolated according to protocols published previously. Total cellular RNA was extracted and hybridized onto an Affymetrix HU133 Plus 2.0 GeneChip according to the manufacturer's recommendations. Gene expression data have been deposited in GEO under the accession numbers listed in Table 1.

Analysis of Time-Course Gene Expression Data. The trajectory of longitudinal expression of each gene was obtained by a cubic spline function, and the mean expression of controls was considered as the base line expression. The maximum deviation of the trajectory line from the base line was referred to as the maximum fold change or simply, the fold change of the gene between patients and healthy controls. For each gene, the time required for the gene to change to one-half of its maximum fold change was defined as its response time, and the time required for the gene to return back to one-half after it reached maximum point was defined as its recovery time. The significance of the longitudinal gene expression change was estimated using EDGE (Extraction of Differential Gene Expression) by 1,000 random permutations. Significant genes were selected by FDR < 0.001 and fold change ≥ 2 for each dataset; 5,544 genes were identified as significant between patients and healthy subjects (4,389 genes in trauma, 3,250 genes in burns, and 2,251 genes in endotoxemia).

Human–Mouse Orthologs. Entrez genes (20,273) were assayed on Affymetrix human HU133 plus v2 arrays, among which 16,646 genes had mouse orthologs according to the Mouse Genome Database (17), and 15,686 genes

were assayed on Affymetrix mouse MOE430 arrays. Among 5,554 genes significantly changed in human conditions, 4,918 genes had mouse orthologs assayed on the mouse arrays, which were included in the subsequent analysis.

Comparison of Gene Response Between Datasets. The maximum fold changes of gene expression were measured in log scale between patients and healthy subjects for each dataset of human burn, trauma, and endotoxemia and between treated and control groups for the corresponding murine models. Between two datasets, the agreements of the maximum gene fold changes (R^2) as well as directionality of the changes (%) were compared. R^2 represents the square of Pearson correlation. Similar results were seen when the rank correlation was calculated as in *SI Appendix, Fig. S1*. Percent represents the percentages of genes changed to the same direction between the two datasets.

Representative patient and corresponding mouse model studies were identified from GEO for several additional severe acute inflammatory diseases (sepsis, ARDS, and infections) (Fig. 4 and Table 1). The fold change of each gene measured was calculated between patients and controls in a human study or between treated and control groups in a murine model study, and for a time-course dataset (GSE20524), the maximum fold change was calculated. The gene response in each dataset was then compared with the 5,554 genes significantly changed in human trauma, burns, and endotoxemia.

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