The Glue Grant: Inflammation and the Host
Response to Injury

Methods in Bioengineering

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Harvard Medical School

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Shriners Burn Hospital – Boston

Chief, Burn Service
Massachusetts General Hospital
Burden of Trauma

- Hundreds of thousands of Americans die each year and over 2.6 million are hospitalized from trauma, sepsis, and burns at a societal cost estimated at over $260 billion.

- Morbidity and mortality rates of those surviving initial resuscitation have not improved.

- Clinical trials in trauma have been industry-supported.

- Benefits from traditional research approaches have been limited.
SIRS and CARS in Burn Patients

- Initial resuscitation followed by the pro-inflammatory response (SIRS)
  - Proportional to severity of the injury
  - Can lead to early MODS

- After a period of relative clinical stability, a compensatory anti-inflammatory response (CARS)
  - Suppressed immunity
  - Diminished resistance to infection
  - May be discharged uneventfully or develop late MODS
Our Core Structure

- PORC (R. Maier, D. Herndon)
- Genomics (R. Davis)
- PACB (L. Moldawer)
- CAM (D. Schoenfeld)
- DIC (R. Tibshirani, D. Donaho, W. Wong, J. Storey, W. Xiao,)
- Proteomics (R. Smith, C. Miller-Graziano)
- IDDC
- Administration
Program Highlights

- U54 program at MGH - currently in 5th year
- 22 performance sites
- Genome-wide expression profiling in humans and partial profiling in mice
- Phenotype-genotype link to clinical database of patients with trauma, sepsis and/or burns
HOST RESPONSE TO INJURY
Strategy to Improve Biological Understanding

Patient
Whole Blood

Overall Response
Innate Inflammatory Response
Regulatory Cell Response

T Cells
MO
Neutrophils

Buffy Coat

0 Years

Genomics
High-throughput Proteomics
Functional Proteomics

Biinformatics
Data interpretation

BIOLOGICAL UNDERSTANDING
Current Accomplishments

Biological Accomplishments

• Demonstrated genome-wide changes in gene exp. after injury (~10,000 genes) in buffy coat
• Limited number of genes (~1,000) predicted a differential outcome towards MODS
• Identified over 3,500 distinct proteins in plasma with ~600 proteins over the first 7 days
• Demonstrated tissue-specific genome-wide expression with contrasts and commonalities to buffy coat
• Identified novel functional modules based on initial analysis of leukocyte subpopulations
Leukocyte RNA Isolation By Buffy Coat and Lysis Techniques

- “Buffy coat” isolation of RNA from leukocyte-enriched population
- Centrifuge to obtain interface between RBC and plasma
- Elimination of residual RBC with lysing solution (Buffer EL, Qiagen, Inc.)
- Standard RNA isolation
- “Lysis method” involves mixing whole blood first with lysis buffer (ACK Buffer) to remove RBCs
- Centrifuge to pellet unlysed WBCs.
- Wash pelleted cells, and standard RNA isolation
• 943* probe sets discriminated between SEB stimulated and unstimulated whole blood with Buffy coat

• 303* probe sets discriminated between SEB stimulated and unstimulated whole blood with PAXgene™

• 254 probe sets in common
• Blood sample was obtained from a single subject, and total RNA isolated by either PAXgene™ or Buffy coat methods. cRNA was generated from 10 μg of starting material using Affymetrix protocols, and detected using a 2% agarose gel and an Agilent 2100 system.

Physiol Genomics
19:247-54, 2004
G004 GenMAPP Analysis

Apoptosis

Cytotoxic T cell
- FAS-Ligand
  - TRAIL Homotrimeric Complex
    - TRAIL
    - TNF alpha
      - TNF receptor 1 (TNFR1)
      - TNF receptor 2 (TNFR2)

FADD
- RIP
  - FAS
  - Pro Caspase-8
    - TRADD
      - TRAF1
        - MEKK1
          - JNK

IAP1
IAP2
IAP3
- NIK
  - IKK
    - NF-kappa B
      - NF-kB p105
      - NF-kB p65

Caspase-8
- Caspase 9/APAF-1 heterodimer
  - Cytochrome C
    - BCL-xL
    - BAK
    - BAX

Nucleus
- NF-kB p65

Apoptosis

Expression Dataset
Gene Color: Set both, PAI, or BC
Genes significant at p < 0.001.

Legend
- Difference seen with either method
- Difference seen with PAI only
- Difference seen with Buffy Coat only
- No criteria met
- Not found

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Last modified: 3/1/03
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Physiol Genomics
19:247-54, 2004
<table>
<thead>
<tr>
<th>Description</th>
<th>Pearson Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>from cRNA hybridization (n=4)</td>
<td>0.997 ± 0.001</td>
</tr>
<tr>
<td>from RNA starting material (n=4)</td>
<td>0.994 ± 0.002</td>
</tr>
<tr>
<td>Leukocyte gene expression from same healthy subject over 24 hrs (n=4 subjects, 4-6 time points, per subject)</td>
<td>0.991 ± 0.003</td>
</tr>
<tr>
<td>Leukocyte gene expression from individual healthy subjects (n=17)</td>
<td>0.955 ± 0.017</td>
</tr>
<tr>
<td>from individual leukocyte populations in different healthy subjects (n=6)</td>
<td></td>
</tr>
<tr>
<td>comparing different cell types from same healthy subjects (n=6)</td>
<td></td>
</tr>
<tr>
<td>MO vs T cells</td>
<td>0.862 ± 0.016</td>
</tr>
<tr>
<td>T cells vs BC</td>
<td>0.888 ± 0.023</td>
</tr>
<tr>
<td>MO vs BC</td>
<td>0.929 ± 0.013</td>
</tr>
<tr>
<td>Leukocyte gene expression from individual trauma patients (n=14)</td>
<td>0.913 ± 0.037</td>
</tr>
</tbody>
</table>

Healthy Subjects

Trauma Patients

**A.** Pearson Correlation Coefficient

Among Individual Healthy Subjects: 0.955 ± 0.017
Among Individual Trauma Patients: 0.913 ± 0.037
Between Healthy Subjects and Trauma Patients: 0.888 ± 0.037

**B.**

- Healthy Subjects
- Trauma Patients

**C.**

- Healthy Subjects
- Trauma Patients
A. Pearson Correlation Coefficient

<table>
<thead>
<tr>
<th>Subject</th>
<th>Coefficient</th>
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<tbody>
<tr>
<td>Subject #1</td>
<td>0.990 ± 0.006</td>
</tr>
<tr>
<td>Subject #2</td>
<td>0.990 ± 0.002</td>
</tr>
<tr>
<td>Subject #3</td>
<td>0.993 ± 0.002</td>
</tr>
<tr>
<td>Subject #4</td>
<td>0.993 ± 0.002</td>
</tr>
</tbody>
</table>

B. Coefficient of variation

C. Coefficient of Variation

<table>
<thead>
<tr>
<th></th>
<th>Replicate Healthy Subjects</th>
<th>Individual Healthy Subjects</th>
<th>Individual Trauma Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ± S.D.</strong></td>
<td>0.093±0.0003</td>
<td>0.182±0.0006</td>
<td>0.207±0.001</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>0.086</td>
<td>0.162</td>
<td>0.167</td>
</tr>
<tr>
<td><strong>90%</strong></td>
<td>0.134</td>
<td>0.275</td>
<td>0.359</td>
</tr>
<tr>
<td><strong>10%</strong></td>
<td>0.057</td>
<td>0.103</td>
<td>0.100</td>
</tr>
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</table>

A. Pearson Correlation Coefficient

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Correlation Coefficient</th>
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<tr>
<td>Lysis vs Monocytes</td>
<td>0.929 ± 0.013</td>
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<td>Lysis vs T cells</td>
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<td>Monocytes vs T cells</td>
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B. Graph showing data points for Lysis, Monocytes, and T cells.

C. Heatmap showing correlation data with Lysis, Monocytes, and T cells.
Human LPS Model

Gram-negative bacteria

LPS

LBP

TLR-4

Cell

Cytokine expression

NF-κB Signaling

TNF

TNFR

TRADD
TRAFs

TRAF6

NIK

IKK

IκBα

Degradation

Ub

IKK

α/β/γ

p50

p65

Nuclear

NF-κB

IL-1

MyD88

IRAK

AcP

IL-1R

Unknown UV Sensor

LPS

Toll-Like Receptors

LBP

Cytokine expression
CR1 Blood Collection Schema

LPS infusion

Hrs -1 0 2 4 6 9 24

Genomic
PAXgene 10mls
Buffy Coat 20mls
Lysis 20 ml

At these two time points:
PAXgene 20mls
Buffy Coat 40mls
Lysis 40 ml

CBC
WBC/Diff
Purple tube, 3 ml

Tompkins, Nature, 2005
A virtual cell of innate immunity
time point 24h
Global Innate Immunity Network

- 0. RELA genes
- 1. HLA-D
- 2. TUB-A
- 3. POLR-II
- 4. NDUFS
- 5. ATP-V
- 6. CCT
- 7. PSM
- 8. RPS/RPL
Current Accomplishments

Infrastructure Development

- Guidelines for early management of severe trauma and burn patients
- Analytical protocols for cell isolation
- Web-based fully relational database
- Novel computational and bioinformational tools
- Novel approaches for high throughput proteomics
- Microfluidics for the isolation of enriched leukocyte populations

www.gluegrant.org
Erythrocyte Lysis

Lysis buffers: Ammonium Chloride, Deionized Water

“Bulk” Lysis
$\tau \sim 15$ to $20$ min

Microfluidic Lysis
$\tau \sim 1$ sec
Differentials Following Microfluidic Lysis

Microfluidic lysis retains normal subcellular populations

- Total Leukocytes
- CD3+ Lymphocytes
- CD19+ B Lymphocytes
- CD66b+ Granulocytes
- CD36+ Monocytes

Bar graph showing the comparison between Whole Blood (BD FACSlyse) and Microfluidic Lysis.
Flow Cytometry

Bulk lysis results in significant cell loss.

Sethu et al., Analytical Chemistry (in press)
### Table 1. Expression of Cell Surface Activation Markers + SEB Stimulation

<table>
<thead>
<tr>
<th></th>
<th>(a) microfluidic lysis (%)</th>
<th>(b) whole blood (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no stimulation</td>
<td>2-h SEB stimulation</td>
</tr>
<tr>
<td>T helper cells CD4 CD69&lt;sup&gt;high&lt;/sup&gt;</td>
<td>1.56 ± 0.54</td>
<td>9.27 ± 5.75&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>monocytes CD36&lt;sup&gt;+&lt;/sup&gt; CD11b/Mac-1&lt;sup&gt;high&lt;/sup&gt;</td>
<td>4.55 ± 3.60</td>
<td>73.69 ± 6.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>monocytes CD36&lt;sup&gt;+&lt;/sup&gt; CD18&lt;sup&gt;high&lt;/sup&gt;</td>
<td>0.99 ± 0.48</td>
<td>37.81 ± 17.52&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>monocytes CD36&lt;sup&gt;+&lt;/sup&gt; HLA-DR&lt;sup&gt;high&lt;/sup&gt;</td>
<td>5.48 ± 2.72</td>
<td>20.44 ± 13.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>granulocytes CD66b&lt;sup&gt;+&lt;/sup&gt; CD11b/Mac-1&lt;sup&gt;high&lt;/sup&gt;</td>
<td>3.05 ± 3.91</td>
<td>80.50 ± 6.89&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>granulocytes CD66b&lt;sup&gt;+&lt;/sup&gt; CD18&lt;sup&gt;high&lt;/sup&gt;</td>
<td>1.29 ± 0.72</td>
<td>68.11 ± 22.71&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Summary of expression levels of cell surface activation markers on leukocytes isolated using the microfluidics cassette versus whole blood (FACSlyse) lysis are presented. Early activation markers were used to score in vivo activation of CD 4<sup>+</sup> T lymphocytes, CD 36<sup>+</sup> monocytes, and CD 66b<sup>+</sup> granulocytes. Data are presented as percentages ± SD for sample size n = 5. Asterisks denote samples where SEB stimulation causes a significant increase (p < 0.05) in expression of the respective activation marker. <sup>b</sup> p < 0.05. No stimulation versus 2-h SEB stimulation.
INFLAMMATION AND THE HOST RESPONSE TO INJURY

Inflammation and the Host Response to Injury is a research program supported by the National Institute of General Medical Sciences (NIGMS), a component of the National Institutes of Health. This collaborative program aims to uncover the biological reasons why patients can have dramatically different outcomes after suffering a traumatic injury.

It is the first large-scale interdisciplinary program to attempt to solve the life-threatening problem of inflammation following major trauma or burn. Inflammation and the Host Response to Injury brings together major medical and research institutions, and researchers in the fields of surgery, genomics, proteomics, biostatistics, bioinformatics, computational biology, and genetics to focus on the microbiology of inflammation.

More...

ATTACKING INJURY, ONE MOLECULE AT A TIME
by Alison Davis

Most of us are lucky enough never to have suffered a major injury. Thrown into the steering wheel of a car... trapped by the door of a crashed vehicle... falling three flights off a ladder.

For those who do experience horrific accidents like these, paramedics can often keep severely hurt people alive through the first minutes to hours after the injury, and fortunately, many of these patients recover.

But some patients who make it to the hospital and appear to be getting better will suddenly, and unexpectedly, take an unfortunate fork in the road.

More...

IN THE NEWS

Welcome to the new Glue Grant web site

Our site been completely redesigned with additional content

Glue Grant Quarterly Meeting

The next regular quarterly meeting of the Program will be held at

Public forum at Shock Society meeting in June

The annual meeting of the Shock Society is a place for interactions
Website: www.Gluegrant.org

- Public access to educational information

- Consortium member access to
  - Clinical, analytical, and animal model protocols
  - Data
  - Results
  - Publications
  - Experimental methods

- Participating investigator to GLIMS, GMDS, Clinical Databases and protocols under development
Genetics and Molecular Medicine

The impact of genomics and computing on healthcare will accelerate and progress over the next 10 years.

Phase I > 5 Years
- Dramatic expansion in diagnostic tests for existing disease
- ID of discrete molecular pathologies in major diseases (right Rx: right disease)

Phase II > 5-10 Years
- Increasing no. of new Rx derived by rational analysis of molecular basis of disease
- ID of pharmacogenetic markers for patient responses to Rx (pharmacogenetics)
- New imaging probes for dynamic assessment of body functions