



Analysis of the Inflammatory and Immune Response to Injury by Quantitative Blood Leukocyte Proteomics for Severe Trauma Patients

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Overview

- Blood leukocyte subpopulations including T-cells, monocytes, and neutrophils (PMN) represent unique cell types for studying inflammatory and immune response to injury.
- Microscale sample processing protocol & quantitative approach have been established for high sensitivity analysis of <100K cells.
- Initial shotgun proteomics efforts established extensive databases of peptide identifications for the three cell types with a combined coverage of ~40% of the human proteome.
- Quantitative analyses of 211 monocyte samples from 41 trauma patients revealed interesting time-dependent patterns of proteomic changes.
- Additional data is currently being collected on all three cell types with the aim of identifying potential classifiers for clinical outcomes.

Introduction

- The host response to injury is a collection of pathophysiological processes that depend critically upon the regulation of the innate immune system.
- High-throughput quantitative proteomic analysis of blood leukocyte subpopulations from clinical patients is aimed at discovering novel protein classifiers for clinical outcomes, as well as at obtaining new insights into the host response to injury.
- The challenges of clinical leukocyte proteomics mainly include the sensitivity required for microscale samples, the throughput needed to analyze a large number of samples, and the accuracy of relative quantitation.
- Patient sample complexity and heterogeneity provide another level of challenges for informatics data analysis and interpretation.

Methods

Experimental

Human leukocyte samples

T-cell, monocyte, and PMN were isolated from whole blood using 8-mL CPT™ cell preparation tubes. The samples were reviewed by the IRB boards within the U54 Glue Grant for human subject research in accordance with federal regulations.

Sample preparation

- The individual cell pellet samples were processed using a trifluoroethanol (TFE)-based protocol with trypsin within a single tube without the need for clean-up.
- Pooled samples were fractionated into 25 fractions by strong cation exchange (SCX) chromatography (PolySulfoethyl A 200 mm x 2.1 mm column) to establish initial databases for each cell types.
- For quantitative analyses, an ¹⁸O-labeled reference peptide sample was generated by post-digestion trypsin-catalyzed ¹⁶O/¹⁸O exchange reaction. The labeled reference is then spiked into each clinical sample to enable ¹⁶O/¹⁸O based quantitation

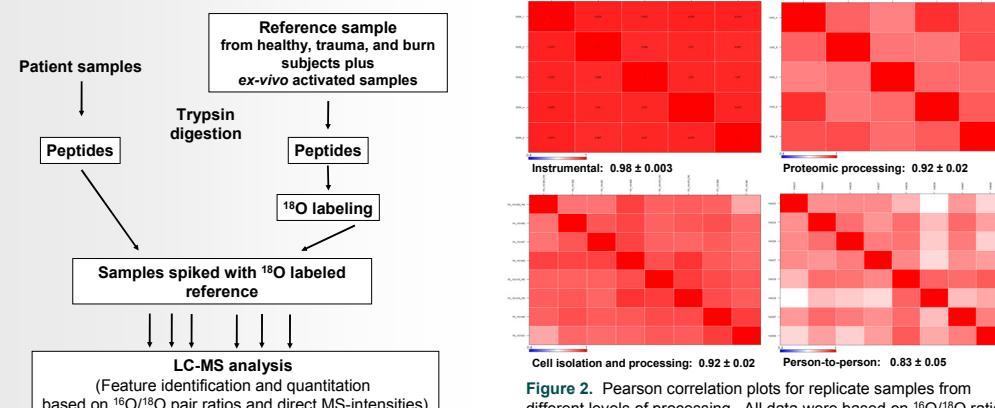
LC-MS/(MS) analysis

All samples analyzed on an LTQ-Orbitrap instrument equipped with automated 4-column LC system. Column: 75 µm id x 65 cm capillary packed with 3 µm Jupiter C18.

Data Analysis

Peptides from LC-MS/MS data identified by SEQUEST database searching. LC-MS data were analyzed using in-house software tools Decon2LS and VIPER, and peptides were identified using the accurate mass and time tag strategy. The large-scale quantitative data generated are then analyzed with a downstream tool called DAxTE for normalization, data visualization, and statistical analyses^[1].

Dual-quantitation approach for large-scale quantitative clinical proteomics



Enabling analyses of small clinical blood leukocyte samples for candidate biomarker discovery

Results

Leukocyte proteome coverage

Table 1. The proteome coverage for T-cell, monocyte, and neutrophil (PMN) as profiled by 2D-LC-MS/MS

	T-cell		Monocyte		PMN		
	Control	Ex vivo (CD5, CD28)	Control	Ex vivo (LPS, SEB)	Control	Ex vivo (LPS, IL6)	Trauma
Proteins	4328	5266	3704	5091	4953	4459	5362
Total Proteins	6060		6059		7422		
Total peptides	33,481		37,606		43,768		

Micro-scale analysis and reproducibility evaluation

Goal: To achieve reproducible and quantitative analysis of 100K or less leukocyte cells (1-10 µg total protein/sample)

Table 2. Summary of different levels of variations being evaluated

Levels of variations	Experiment
Instrumental	Replicate analyses of the same sample on LC-MS
Proteomic processing	Replicate proteomic processing and LC-MS
Cell isolation and processing	Replicate cell isolation, processing, and LC-MS
Person-to-person	Samples from different healthy volunteers

Variations evaluated by Pearson correlation

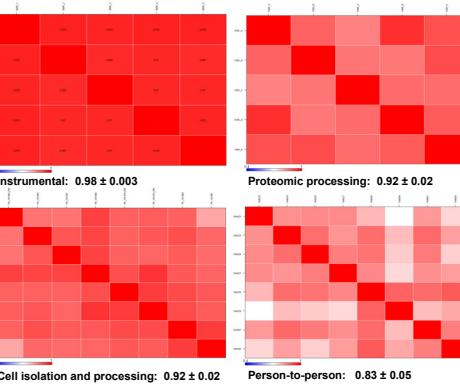


Figure 2. Pearson correlation plots for replicate samples from different levels of processing. All data were based on ¹⁶O/¹⁸O ratios with a common labeled reference sample.

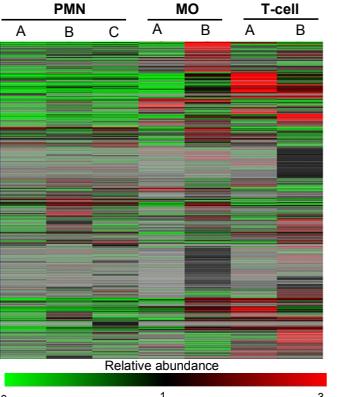


Figure 1. Heatmap of relative protein abundances for human PMN, monocytes, and T-cells. Protein spectral count data was converted to relative scale by dividing the average spectrum count across all conditions. Proteins showing significant changes found in several functional categories, including inflammatory disease and immunological disease; a number of protein patterns agree with previous functional proteomic studies.

Quantitative analysis of 211 trauma monocyte samples, collected at different time points post-trauma

Proteins showing significant post-trauma abundance changes

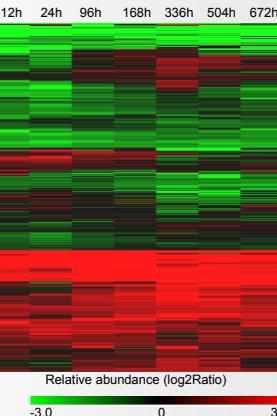


Figure 4. Heatmap of 347 proteins that show significant time-dependent changes. A total of 1509 proteins were identified with quantitative information and proteins with significant changes were identified by ANOVA test with false discovery rate of <5% based on Q-value.

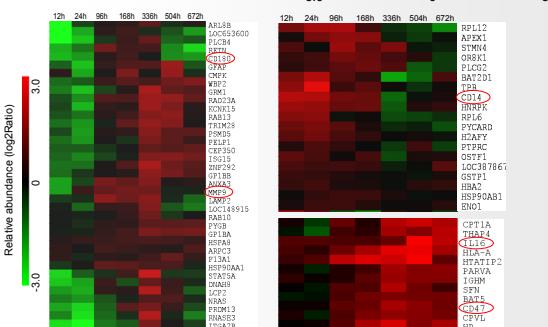


Figure 5. Examples of protein clusters.

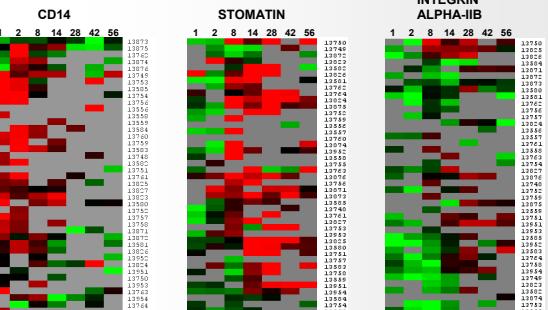


Figure 6. Heatmaps of individual proteins illustrating patient heterogeneity. Gray areas indicate either missing time point or data.

Conclusions

- A high throughput quantitative proteomics methodology has been established for microscale blood leukocyte sample analyses
- Extensive coverage of leukocyte subpopulation proteomes achieved, representing a major resource for the human blood proteome
- Relatively good technical reproducibility for leukocyte proteome analyses demonstrated via dual-quantitation approach (~15% CV for labeling approach; ~25% CV for label-free approach)
- Initial analysis of 211 trauma monocyte samples showed promising results with interesting dynamic protein abundance patterns
- Proteins showing significant abundance changes found in several functional categories, including inflammatory disease and immunological disease; a number of protein patterns agree with previous functional proteomic studies
- Additional T-cell and monocyte samples being analyzed to identify novel protein biomarkers predictive of patient outcomes

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