IDENTIFICATION OF IMMUNE RESPONSE SIGNATURES UTILIZING INTEGRATED CYTOMIC AND PROTEOMIC TECHNIQUES

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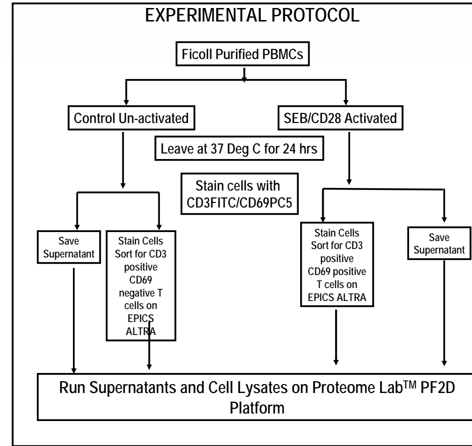
ABSTRACT

The identification of signatures associated with disease could impact every aspect of patient care from screening to treatment. To arrive at such signatures a multipronged approach needs to be pursued. Studies that have so far been carried out in isolation for e.g. gene expression, protein synthesis, etc. need to be integrated to understand the complex responses that translate to patterns. In the current study the authors have attempted such an evaluation by integrating well known techniques of cell sorting with protein fractionation to identify signatures of immune cellular activation.
Peripheral blood mononuclear cells were subjected to restricted polyclonal stimulation with staphylococcal enterotoxin B (SEB) or left untreated. Cells were then labeled to identify T cells and isolated using flow-based sorting techniques. Lysates of unsorted activated and non-activated PBMCs as well as sorted T cells were fractionated by two-dimensional gel-free liquid chromatography. Intact proteins were separated by their isoelectric points in the first-dimension and further separated by hydrophobicity on a second-dimension. Using powerful differential display software a high resolution protein profile of the complex mixtures was obtained.
Qualitative and quantitative differences in protein profiles in activated and non-activated cells were identified using a “proteomic only” strategy resulting in immune response signatures. Further integration of cell sorting techniques with proteomics refined the profile by enabling a finer mapping of the T cell signatures. The liquid-phase fractions of proteins in the intact state, permits direct characterization of the differentially-expressed proteins. Further combinations of genomic, proteomic and cytomic profiling will accomplish a unified evaluation of signatures relevant to this response and other research models.

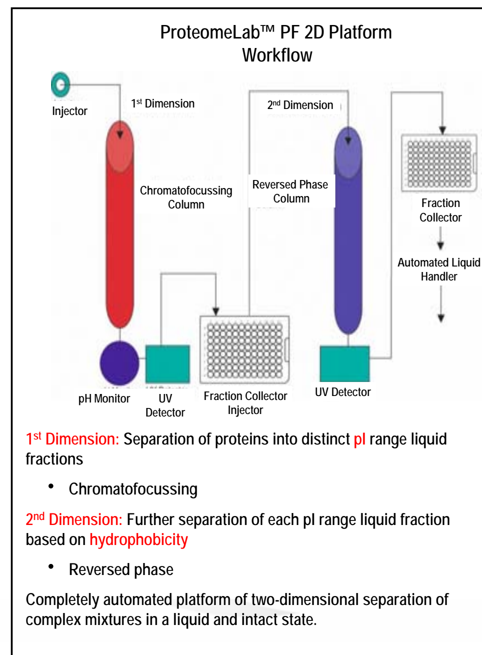
AIM

Identify “proteome profiles” of PBMC activation in cellular and secreted fractions using integrated proteomic and cytomic techniques.

EXPERIMENTAL PROTOCOL



ProteomeLab™ PF 2D Platform Workflow



1st Dimension: Separation of proteins into distinct pI range liquid fractions

- Chromatofocussing

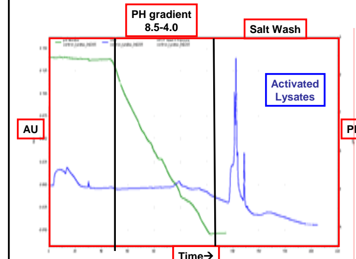
2nd Dimension: Further separation of each pI range liquid fraction based on hydrophobicity

- Reversed phase

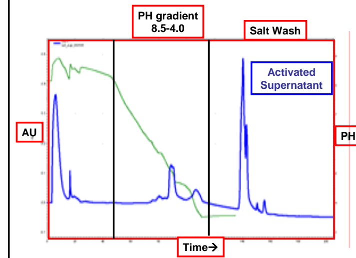
Completely automated platform of two-dimensional separation of complex mixtures in a liquid and intact state.

RESULTS

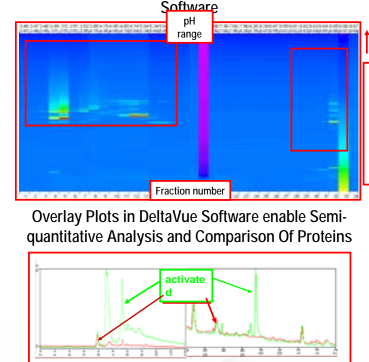
1st Dimension (pI) Profile of Activated-CD3 positive CD69 positive Cell Lysates Using 32Karat Software



1st Dimension Profiles of Activated Supernatants Using 32Karat Software

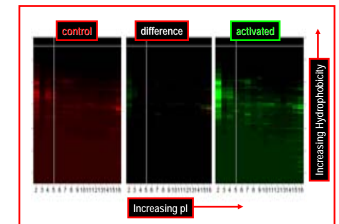


Complete profile of Proteins Secreted in Activated PBMC Cultures Visualized Using ProteoVue Software

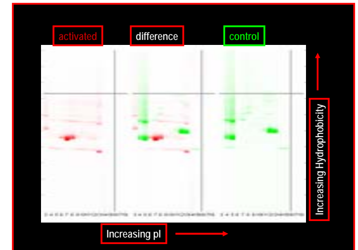


Overlay Plots in DeltaVue Software enable Semi-quantitative Analysis and Comparison Of Proteins

Comparison of Control CD3 positive-CD69 negative and Activated CD3 positive-CD69 positive Cell Lysates Using DeltaVue-Differential Display Software



Comparison of 2-dimensional Fractionated Control and Activated Supernatants Using DeltaVue-Differential Display Software



OBSERVATIONS AND CONCLUSIONS

Sorted cells representing control and activated T cells as well as corresponding supernatants exhibit differences in protein profiles as seen on ProteoVue and DeltaVue Plots

- Qualitative and quantitative difference of protein profiles in control CD69 negative and activated CD69 positive T cells are easily observed
- Qualitative and quantitative difference of protein profiles in control and activated culture supernatants are easily observed
- Some protein profiles remain unchanged in the control and activated cell lysates and supernatants
- Majority of the proteins in both control and activated cultures are eluted at lower pH ranges in the pH gradient

Proteins of interest that are differentially expressed in activated cell lysates and supernatants are easily fractionated from complex mixtures in a gel-free and intact state using the above described combination of flow-based cytomic and 2-dimensional proteomic techniques enabling the identification of biomarkers of activation

The proteins of interest can be further identified using other techniques such as MS, peptide mapping, western blots, ELISAs etc

Further integration of genomics, proteomics and cytomics techniques can enable a holistic interrogation of biomarkers of cellular activation

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