PROTEOMIC STRATEGIES TO ANALYZE CELL-FREE FRACTIONS FROM ACTIVATED PERIPHERAL BLOOD MONONUCLEAR CULTURES

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ABSTRACT

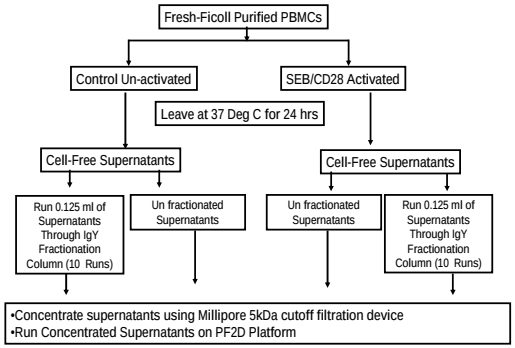
The use of Proteomic strategies in the discovery process is imperative since post-transcriptional modification can produce dramatic changes in protein levels and activity that are invisible to DNA arrays. The introduction of new and improved proteomics solutions with increased sensitivity, specificity and ease of use has been integral in facilitating this process. The current study has evaluated signatures of immune response in cell-free fractions of control and activated peripheral blood nuclear cell cultures using proteomic combinations designed to improve sensitivity of detection and ease of use.

Peripheral blood mononuclear cells cultured in medium containing human AB serum were subjected to activation for 24hrs using Staphylococcal enterotoxin B. Cell-free fractions from the activated and control cells were fractionated by two-dimensional chromatography in the liquid and intact phase. To improve the sensitivity of detection of protein signatures, the secreted components were also subjected to a fractionation strategy using IgY antibodies to deplete the most abundant proteins in human serum and then analyzed by two-dimensional liquid chromatography. Intact proteins were separated by their isoelectric points in the first dimension and further separated by hydrophobicity on a second-dimension. The net result was the generation of high-resolution protein profile of the complex mixture. Qualitative and quantitative differences in protein profiles in activated and non-activated cell free fractions could be easily identified using powerful software. The use of the IgY fractionation technique to deplete the abundant proteins in serum containing growth medium enhanced the sensitivity of the differential analysis. The gel- free and intact nature of the fractions of interest allows for further interrogation and identification of the differentially expressed proteins to elucidate an activation signature in supernatants. Thus the combination of proteomic techniques enables a more refined and targeted profiling and analysis of complex events associated with an immune response

AIM

- Evaluate the proteome profile of activated PBMC supernatants
- Finer mapping of secreted proteome signatures using strategies to deplete the most abundant proteins in culture medium containing serum.

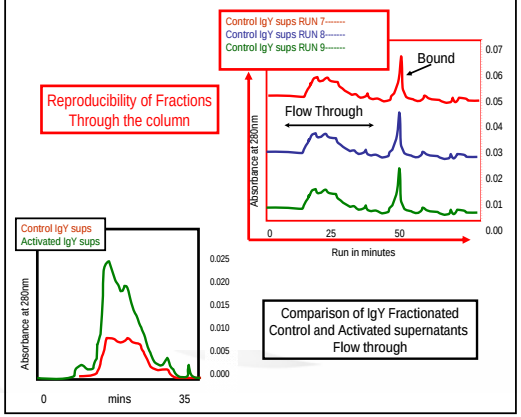
EXPERIMENTAL PROTOCOL



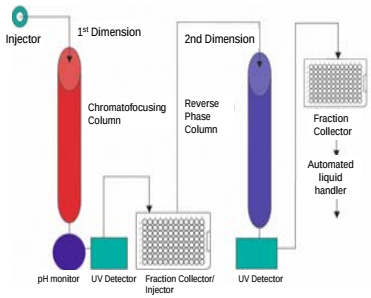
Proteins Depleted Using IgY 12 Primate Affinity Column*

HAS	IgA
Orosomucoid	IgM
Alpha 2-Macroglobulin	HDL (apo A-I)
IgG	HDL (apo A-II)
Fibrinogen	Alpha 1-ATT
Transferrin	Haptoglobin

*ProteomeLab IgY LC10 Kit A24355-Beckman Coulter Inc.



ProteomeLab™ PF 2D Platform Workflow



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

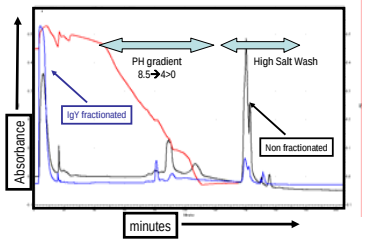
- Chromatofocussing

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

- Reversed phase

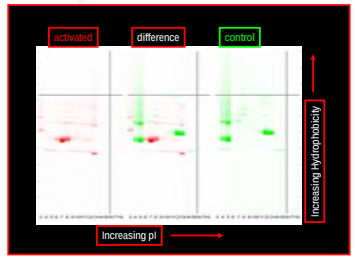
Completely automated platform of two-dimensional separation of complex mixtures in a liquid and intact state.

First Dimension Profile of IgY Fractionated Supernatants Compared to Non-Fractionated Supernatants

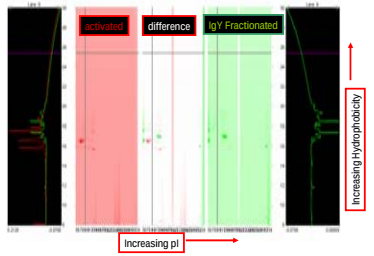


Complete Proteome Profile of Activated Supernatants

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



Comparison of Activated and IgY Fractionated Activated Supernatants Using DeltaVue-Differential Display Software



OBSERVATIONS AND CONCLUSIONS

- Complete proteome profiles of secreted components from control and activated PBMCs are easily obtained and evaluated using the PF2D gel-free separation system.
- Qualitative and quantitative differences and similarities of protein profiles in control and activated supernatants are easily observed
- The IgY fractionation procedure enables a highly reproducible separation of the most abundant proteins in human serum by affinity chromatography.
- The Igy fractionated supernatant enables the identification of proteins of lower concentration that are possibly "masked" by the higher concentration serum proteins
- The IgY fractionation procedure enables the identification of cell secreted components that are associated with high abundant proteins in serum
- 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 supernatants are easily fractionated from complex mixtures in a gel-free and intact state using the above described 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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