The Two-Dimensional Protein Fractionation (PF 2D) Platform Facilitates a Comprehensive Mapping of Protein Expression Profiles in Activated and Non-Activated Peripheral Blood Mononuclear Cells

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Abstract

Analyzing the protein expression profiles associated with the activation of an immune response is an important step in understanding the detailed mechanisms of the response. However, using currently available techniques, it has been difficult to analyze in totality, the complex series of protein expression profiles and interactions associated with activation of an immune response.

Proteome™ Lab PF 2D is a novel platform that enables the comprehensive mapping and differential analysis of proteins and peptides in complex mixtures. The technology utilizes HPLC as the basis for the multi-dimensional separation of proteins in the liquid phase in a completely automated format. The net result is the generation of high resolution and distinct protein profiles of the complex mixture based on pI and hydrophobicity that can be effortlessly viewed using ProteoVue software. Additionally, differentially expressed protein profiles in samples can be also easily identified using DeltaVue software. The gel-free liquid nature of the system allows for easy isolation of the proteins of interest in the native, intact state that can be further analyzed using conventional techniques of ELISA, Western blot and Mass Spectrometry techniques.

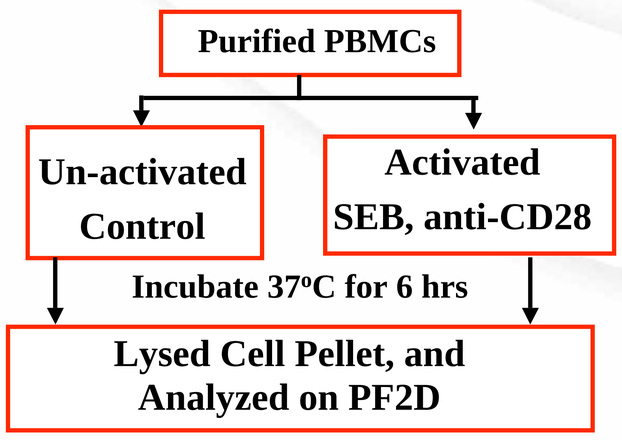
To evaluate changes in protein profiles associated with the activation of an immune response, peripheral blood mononuclear cells were stimulated with the superantigen, Staphylococcus enterotoxin B (SEB) and anti-CD28 or left un-treated for 6 hours. The cell lysates were then analyzed using the PF2D platform. Using the ProteoVue and DeltaVue software, distinct differences in protein expression in activated and non-activated samples could be easily identified. The PF2D platform has applications in the fields of drug discovery, drug and vaccine-based clinical trials, identification of disease-related markers as well as in basic research to identify proteins of interest.

Aim

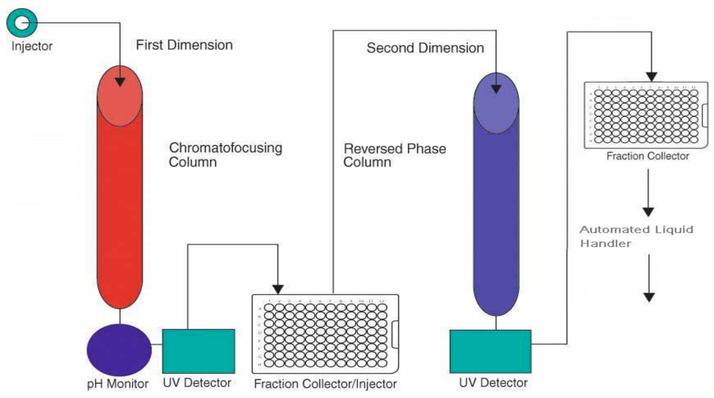
Use the Proteome™ Lab PF 2D Platform to analyze changes in protein expression associated with an immune response

Model System

Stimulate the T-cell lymphocyte sub-population using Staphylococcal Enterotoxin B (SEB) and anti-CD28



Proteome™ Lab PF 2D Platform Workflow



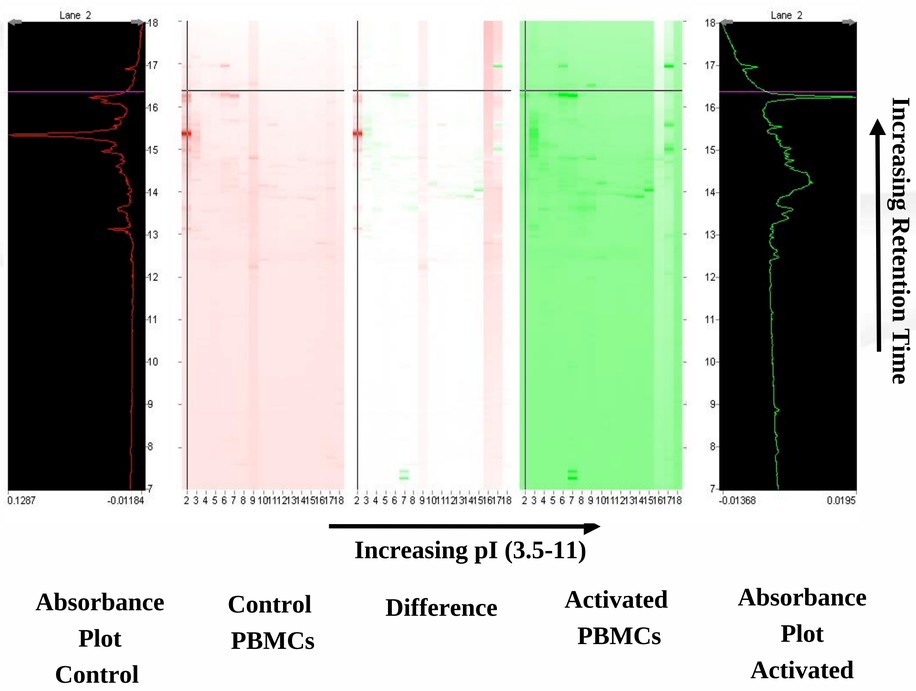
1st Dimension: Separation of proteins into distinct pI range liquid fractions

– HPLC Chromatofocussing

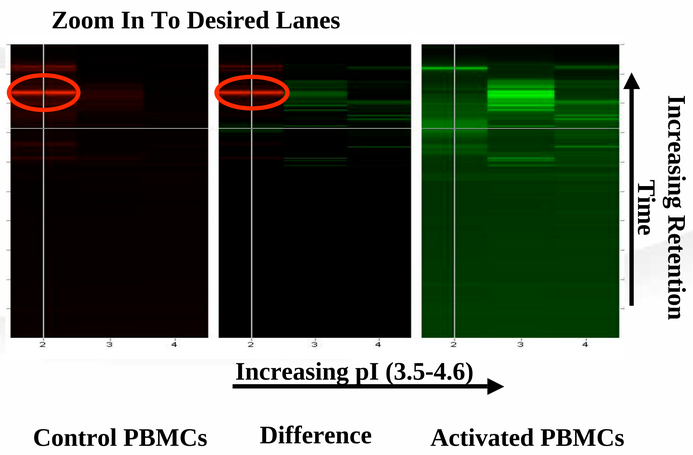
2nd Dimension: Further separation of each pI range liquid fraction based on hydrophobicity

– RP NPS (Reverse Phase Non Porous Silica) of pI range fractions

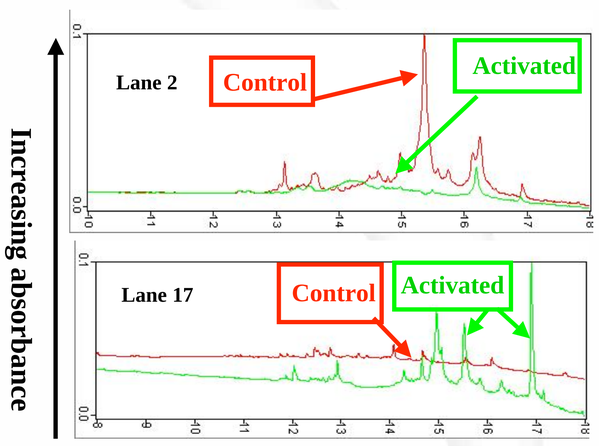
Complete Protein Profile of PBMCs
Differential Display Using DeltaVue-Software



Partial Protein Profile of PBMCs
Using DeltaVue-Differential Display Software



Differences In Control And Activated PBMCs
Overlay Plots Using DeltaVue Software



Observations and Conclusions

- Control and activated PBMCs exhibit distinct protein profiles as seen on the ProteoVue and DeltaVue Plots
  - Appearance and disappearance of protein profiles in control and activated PBMCs
  - Increased and decreased expression of proteins in activated compared to control PBMCs and vice versa
  - Some protein profiles remain unchanged
  - Activated samples produce more basic proteins while depleting the most acidic proteins
- Proteins of interest can be identified easily and isolated from complex cell lysates in gel-free, native state
- Proteins of interest could be analyzed further using ELISAs, Westerns, and Mass Spectrometry
- Completely automated method with very user-friendly software packages.

Applications of the Proteome™ Lab PF 2D Platform

This system is an important tool for use in the identification of disease related biomarkers, which might serve as good therapy targets, or as indirect markers for therapy monitoring.

The following areas will benefit most from this technology:

1. Basic and advanced research
2. Clinical research
3. Discovery, validation, and development of Immunotherapeutics