A Novel Modeling System for Analysis of High Dimensional data: Definition of T-cell Effector and Memory Subsets Using GemStone™ Software

Inokuma MS, Trotter J*, Hanruterberger BC*, Munson ME*, Herbert DJ*, Bray CM*, Ghanekar SA*, Maimo VC*, and Bagwell CB

BD Biosciences, 2350 Qume Dr., San Jose, CA 95131; BD Biosciences, 11077 N. Torrey Pines Rd. La Jolla, CA 92037; Verity Software House, 45A Augusta Rd. Topsham, ME 04086

Abstract

Introduction

The differentiation of human T-cells has been the focus of intense research in recent years, but remains elusive due to the complexity of T-cell subsets, phenotypic heterogeneity, and the need for methods that distinguish entire populations of cells. The use of color flow cytometry has provided insight into the broad spectrum of T-cell subsets identified in the human population. However, the proliferation of cell surface markers and the need for high-dimensional data analysis has hindered the process of accurately identifying T-cell subsets. These limitations in technology have hindered the proliferation of T-cell subsets identified in vivo and in vitro, making the identification of T-cell subsets and their functional capacities more difficult.

To address this challenge from a new direction, we used the GemStone™ software to analyze anti-tumor immunity and other T-cell subsets. GemStone™ software is a powerful tool for analyzing high-dimensional flow cytometry data. It allows for the interrogation and presentation of high dimensional flow cytometry data. The development of this system allows for a more sophisticated analysis of T-cell subsets, which can be associated with memory phenotypes.

1. Simplification of multicolor data analysis: Dot plots versus GemStone models

1a. Standard 2-dimensional dot plot analysis of 9-color data of both the CD4 and CD8 T-cells, resulting in 4 distinct subpopulations represented in 21 dot plots.

1b. Progression of T-cell ontlogy is represented in 2 parameter dot plots.

2. GemStone models are reproducible between different users and between healthy donors

2a. Different users generate similar GemStone models

2b. Data from healthy donors result in similar models

3. Analysis of 38 correlated phenotypic markers on CD8 T-cells

3a. User A

3b. User B

3c. User C

3d. User D

3e. User E

4. Cytokine expression in activated CD8+ T-cells can be associated with memory phenotypes

4a. Pre-stimulated peripheral blood from a known CMV responsive donor was stimulated with CMV peptide pools (9600/1, with 2000/lot). Total RNA was isolated with the BD LSR II flow cytometer. The samples were analysed using the PEG Display and Probability State Modeling System in GemStone.

Summary

GemStone models present high dimensional data in a biologically relevant, easy to interpret format.

• Generation of models is consistent between multiple users and across healthy donors

• When 38 phenotypic markers were combined into one model, four major CD8+ T-cell subsets can be identified in the data from four healthy donors.

References


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