Program number 284

Visualization of CD4 T-cell memory and effector differentiation using GemStone

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Abstract

The progression of memory and effector differentiation of CD4 T-cells is driven by an array of transcription factors in response to external and internal signals. These in turn direct the expression of soluble factors and surface markers for interaction with other immune cells. Several CD4+ effector subsets have been identified, based on function and expression of markers, but there is much debate over the number of subsets, the function, and the stages of development. This is further complicated by the heterogeneity and plasticity inherent in the CD4 T-cell populations. In this study, we use new features in GemStone, paired with 10-color flow cytometry data, to determine phenotypic classification of CD4 T-cells The new AutoAnalysis feature was used to generate CD4 T-cell models and to correlate 42 phenotypic markers. The models show subtleties of phenotypic expression that have not been previously described, for example, the three expression levels of CD28 and heterogeneity in CD27 expression. The models also demonstrate that the expression patterns of such markers as CCR7 and CD45RA differ significantly from CD8 T-cells. Additionally, CD4 T-cell subsets, including Th1, Th2, and Treg will be investigated using branched models. Information from these models can be used to better understand the role of CD4 T-cell subsets in adaptive immune responses and to map and identify changes in disease states.

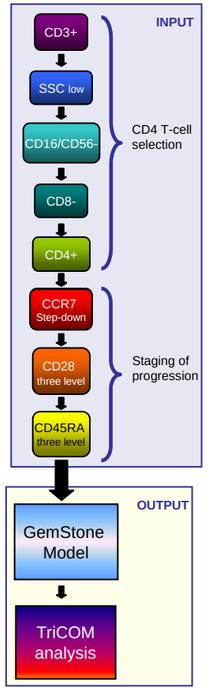
Introduction

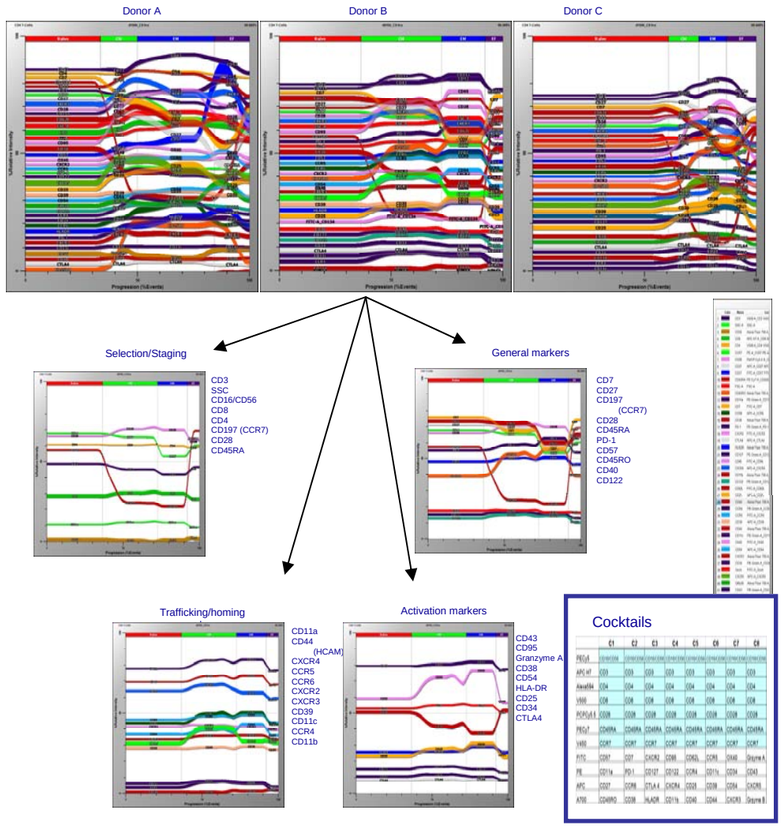
The differentiation of human T-cells has been the focus of intense discussion in defining the lineage relationship of effector and memory cells, and more recently, in understanding the heterogeneity of these populations. The large pool of available surface and intracellular phenotyping markers, the inherent complexity of the *in vivo* systems, and the lack of comprehensive tools has resulted in much debate. While the CD8 T-cell compartment is slightly more characterized[1], the heterogeneity and plasticity of the CD4 T-cells have presented challenges in defining the effector and memory stages[2].

To approach this challenge from a new perspective, we used GemStone software, a novel approach for the analysis of multiparameter flow data by using a Probability State Model (PSM) to identify and quantify subsets. A PSM is used to classify events into populations by probability, based on a model defined by some basic biological information. The phenotyping markers in the assays are used to create a set of parameter profiles for a cell type subset. A parameter profile then uses a set of control points to define how the subset transitions over the state index axis[3].

1. Analysis of progression in CD4 T-cells of healthy donors

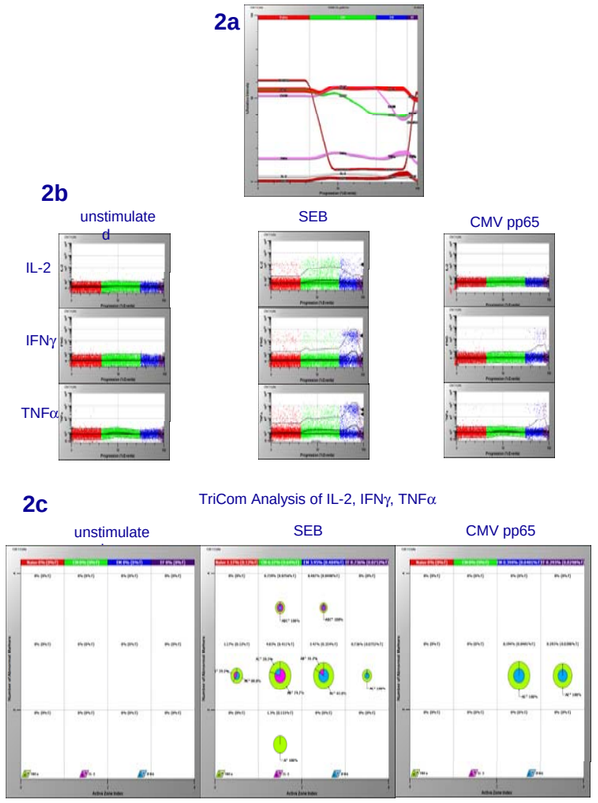
Semi-automated analysis using a prebuilt model





Healthy donor peripheral blood stained with eight 11-color panels was acquired with a Special Order BD™ LSR II flow cytometer. A CD4 T-cell template document was generated in Gemstone. The template was designed to select the populations of interest using CD16/CD56, CD3, CD4, CD8, and SSC and to define differentiation stages using CD28, CCR7 (CD197) and CD45RA. The remaining markers were allowed to fall in place in reference to the progression established by the three model markers. By having a scaffold of common markers (in blue), eight cocktails can be combined into one parameter overlay display so that all the markers can be correlated to one another.

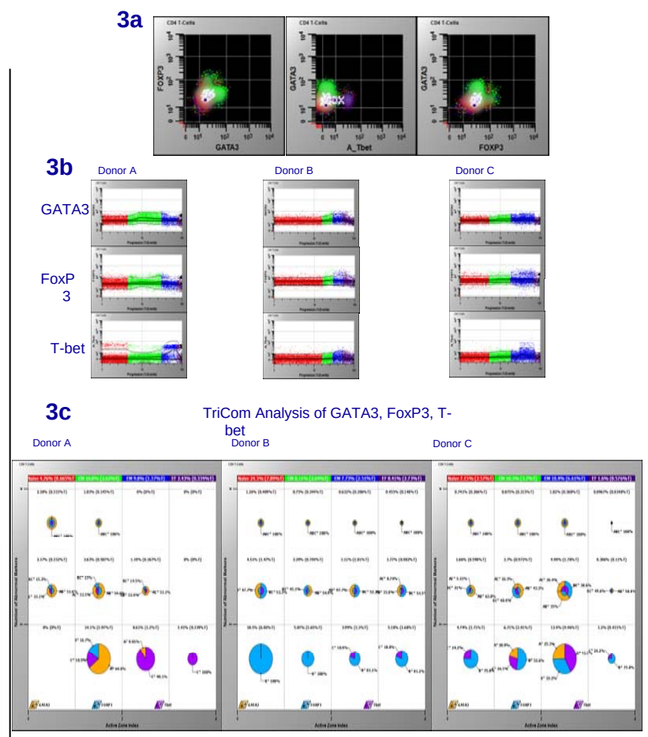
2. Analysis of polyfunctional CD4 T-cells



Peripheral blood from a known CMV sero-positive donor was stimulated with CMV peptide pools of pp65 or with superantigen B (SEB), a positive control, in the presence of Brefeldin A for 6 hrs, then stained with a 11-color cocktail. A CD4 T-cell GemStone template was used to establish progression of T-cell differentiation. (2a). Parameter profiles (2b) show the expression of individual cytokines in the context of CD4 T-cell differentiation. The TriCom displays (2c) are a visualization of the polyfunctionality of the T-cells. The X-axis depicts the four stages of CD4 antigen-experienced progression. The bottom row depicts the percent of cells expressing one cytokine. The middle row represents the cells co-expressing two cytokines, while the top row represents the cells co-expressing three cytokines. The key at the bottom shows how to interpret the phenotypes of the cytokine producing cells.

The CMV pp65 response demonstrates the expression of TNFα and IFNγ in the effector memory (EM) and terminal effector (EF) stages, while the SEB response demonstrate a heterogeneous cytokine response distributed in all the stages.

3. Analysis of plasticity in CD4 T-cells



Peripheral blood from three healthy donors were stained with an 11-color cocktail. A CD4 T-cell GemStone template was used to establish progression of T-cell differentiation. The cocktail also included antibodies to GATA 3 (Th2), FOXP3 (Treg), and T-bet (Th1) to better understand the interaction of the three master regulators in the commitment of cells to specific CD4 T-cell lineages.

In the 2D dotplots (3a), a majority of the cells demonstrate expression of one of the three transcription markers. In the parameter profiles (3b), the stage of T-cell progression is evident for the populations of cells expressing each of the transcription markers.

In the TriCom analysis (3c), the X-axis depicts the four stages of CD4 antigen-experienced progression. The bottom row depicts the percent of cells expressing one transcription marker. The middle row represents the cells co-expressing two transcription marker, while the top row represents the cells co-expressing three transcription markers. The key at the bottom shows how to interpret the phenotypes of the cells. Three donors are depicted as an example of the diversity of expression in the multiple healthy donors.

In general, the largest circles on the bottom rows indicate that the majority of cells expressing a transcription marker is expressing one transcription marker in all the donors. Transcription factors are thought to be master regulators that drive the commitment of cells the a specific T-cell lineage (Th1, Th2, Treg). More recently, several studies have demonstrated the plasticity of these cells[2]. The co-expression of transcription markers as demonstrated in the middle rows and top rows may be an indication of cells in transition between lineages.

Summary

- The four canonical CD4+ T-cell progression stages, Naïve, central memory (CM), effector memory (EM) and Terminal Effector (EF), can be determined using CCR7 (CD197), CD28 and CD45RA.
- Cytokine polyfunctional T-cells can be easily associated with stages of T-cell progression using the TriCom™ feature.
- TriCom™ analysis confirms the exclusive expression of the three transcription markers in the majority of the cells, representing the lineage commitment to Th1, Th2 and Treg cells.
- The co-expression of three transcription markers in a small representative of the cells be an indication of cells in transition between lineages.

References

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23-13467-00
PB# B156
ISAC 2011

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