Program #241

Modeling Peripheral Blood B cells with GemStone™ Analysis Software and 39-Parameter CyTOF Data

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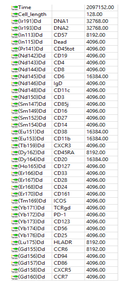
Background

Circulating peripheral blood B-cell subsets have been poorly defined until the advent of greater than six color flow cytometry[1]. Trafficking of these subsets between tissues reflects the immune status of an individual and can signal disorders of B cell development as well as the presence of autoimmune disease or lymphoproliferative diseases. Having the ability to determine micro-populations of existing B-cell subsets could help researchers and clinicians to hone in on a diagnosis in a relatively non-invasive way and also track an individual's response to infection, vaccine administration or therapeutic treatment.

With many groups having access presently to more than 40-parameter CyTOF mass cytometry data, the need for analysis software that is reproducible, interpretable and able to handle high-dimensional data is greater than ever. These many-parameter panels would be extremely helpful in elucidating immune subsets, such as B-cell micro-populations, especially if the analyses were consistent between multiple files, multiple users, and from lab to lab. This study investigates the use of Probability State Modeling (PSM) for determining the maturation and progression of peripheral blood B cells using a 39-marker CyTOF-derived dataset.

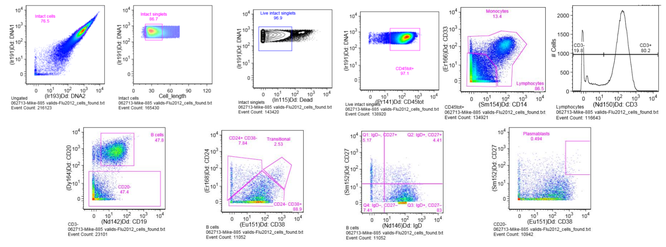
Methods

CyTOF Immunophenotyping: This assay was performed in the Human Immune Monitoring Center at Stanford University (Stanford, CA, USA)[2]. PBMCs were thawed in warm media, washed twice, resuspended in CyFACS buffer (PBS supplemented with 2% BSA, 2 mM EDTA, and 0.1% soium azide), and viable cells were counted by Vicell. Cells were added to a V-bottom microtiter plate at 1.5 million viable cells/well and washed once by pelleting and resuspension in fresh CyFACS buffer. The cells were stained for 60 min on ice with 50 uL of the following antibody-polymer conjugate cocktail: [insert Ab list here]. All antibodies were from purified unconjugated, carrier-protein-free stocks from BD Biosciences, Biolegend, or R&D Systems. The polymer and metal isotopes were from DVS Sciences. The cells were washed twice by pelleting and resuspension with 250 uL FACS buffer. The cells were resuspended in 100 uL PBS buffer containing 2 ug/mL Live-Dead (DOTA-maleimide (Macrocyclics) containing natural-abundance indium). The cells were washed twice by pelleting and resuspension with 250 uL PBS. The cells were resuspended in 100 uL 2% PFA in PBS and placed at 4C overnight. The next day, the cells were pelleted and washed by resuspension in fresh PBS. The cells were resuspended in 100 uL eBiosciences permeabilization buffer (1x in PBS) and placed on ice for 45 min before washing twice with 250 uL PBS. If intracellular staining was performed, the cells were resuspended in 50 uL antibody cocktail in CyFACS for 1 hour on ice before washing twice in CyFACS. The cells were resuspended in 100 uL iridium-containing DNA intercalator (1:2000 dilution in PBS; DVS Sciences) and incubated at room temperature for 20 min. The cells were washed twice in 250 uL MilliQ water. The cells were diluted in a total volume of 700 uL in MilliQ water before injection into the CyTOF (DVS Sciences).



CyTOF Panel

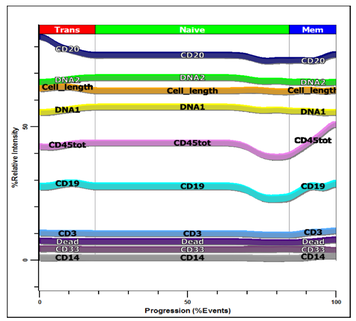
Data Analysis: Analysis of the collected data was performed using GemStone™ Probability State Modeling and Graphics System from Verity Software House (Topsham, ME, USA).



Original gating analysis of peripheral B cells performed by Maecker Lab.

	GemStone			FlowJo		
	Percent	Events	% Total	Percent	Events	% Total
Total B Cells		11,748	5.44		11,052	5.1
Trans	7.64	897	0.42	2.53	279	0.12
Naïve	77.23	9073	4.2	88.9	9825	4.5
Mem	15.13	1778	0.82	7.84	866	0.4
		11748			10970	

Comparison of percentages and number of events between modeling vs. gating analysis. Total B cells selected and analyzed were very similar between the two methods. Subset percentages and events vary due to the ability of GemStone to account for transitional events and branching of markers. For example, CD27 positivity marks the beginning of the memory B cell stage. In GemStone we model the memory stage as starting when B cells are becoming positive for CD27, while in gating, the memory stage is defined when CD27 is fully positive.



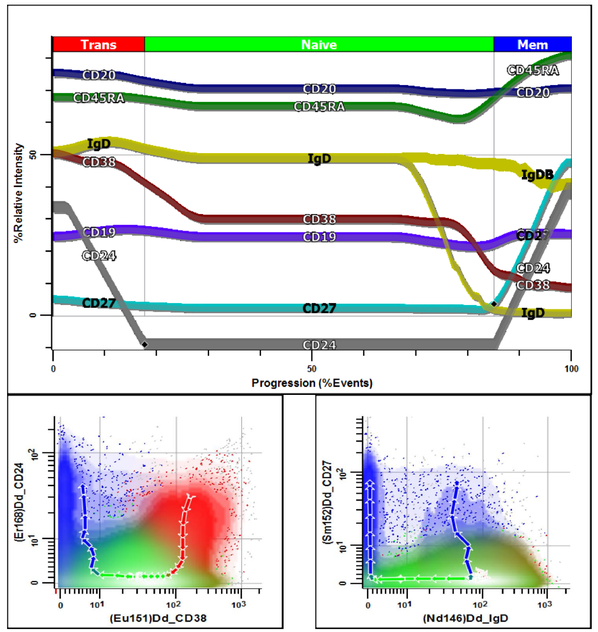
Selection Markers for GemStone Analysis

Results

Peripheral B cells were selected using the markers DNA1, DNA2, Cell Length, Dead, CD45tot, CD33-, CD14-, CD3-, CD19+ and CD20+. The markers CD27, CD24, CD38 and IgD were modeled to stratify the B cells into Transitional, Naïve and Memory subsets as described by Warnatz and Schlesier, 2008[3 a] and as gated by the Maecker Lab shown above. In addition, CD45RA, CXCR3, CXCR5, CCR6, HLADR, CD11c, CD86, and CD86j all exhibited stage-related modulations and allowed us to identify subpopulations that exist within these compartments.

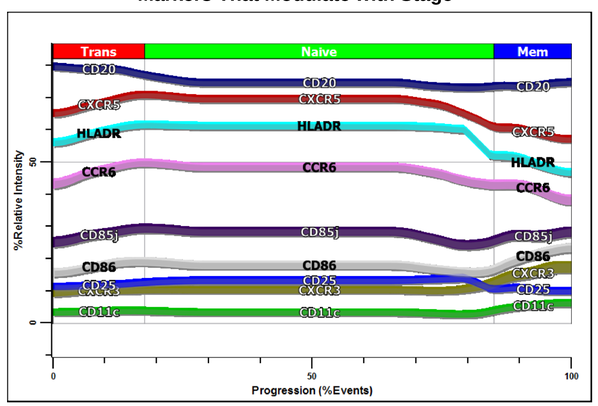
Plasmablasts were modeled as a separate cell type and identified as CD19+, CD20-, CD38++, and CD27+ events. GemStone identified 52 plasmablasts compared to 54 identified by gating.

Stratification Markers



Stratification markers are used to delineate subsets in the cell type of interest. Peripheral B cells are divided into immature or transitional B cells based on positivity for CD24 and CD38, Naïve B cells are designated CD24 negative and CD38 positive, and memory B cells are CD27 positive and CD38 negative. IgD is branched; some events remain positive while some events downregulate as B cells mature. Two-parameter dot plots are shown with arrows showing the direction of maturation, demonstrating the usefulness of the GemStone overlay plot to see clearly the direction of maturation even in branching profiles and how other known markers modulate with the stratification markers.

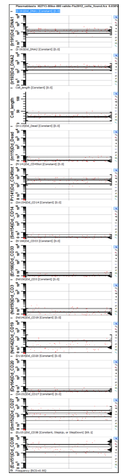
Markers That Modulate with Stage

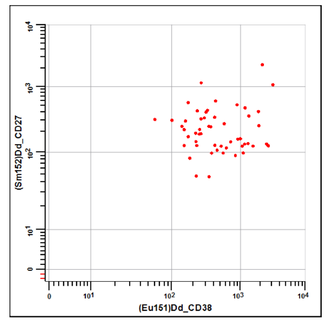


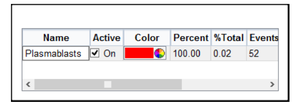
After the stratification markers are modeled and the subsets are defined, any number of markers included in the panel can be added to the analysis. We are only showing markers present in the panel that demonstrated modulation with staging. One can clearly see the transition points as well as the up-regulation or down-regulation of markers that occurs biologically as B cells mature.

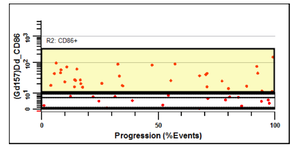
Click here or download the poster from www.vsh.com/publications

Plasmablasts



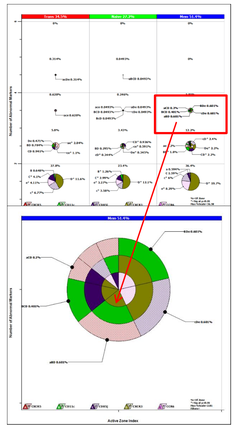


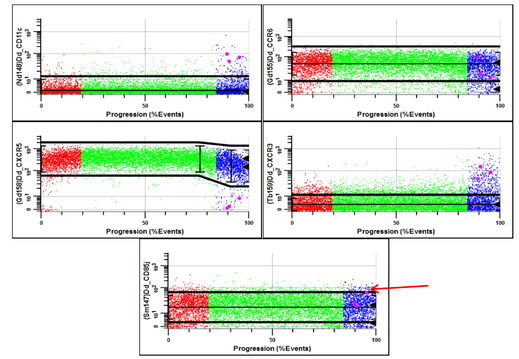




Plasmablasts were modeled as a separate cell type in GemStone using the selection markers described and defined as CD19 positive/dim and CD20 negative. These cells were modeled with constant expression profiles only as shown to the left. There is a known subset of plasmablasts that are CD86 positive which are shown in the expression profile on the right.

B-Cell Micro-subsets





One of the exciting and novel uses of GemStone is for discovery, resulting in the ability to look at events in ways we have not previously been able to see them. We used the TriCOM feature of GemStone to see phenotypes based on the markers CXCR5, CD11c, CXCR3, CD85j and CCR6. The TriCOM is shown in the upper left, the boxed area and arrow point to the enlarged area below. The pie wedge represents events low for CXCR5, positive for CD11c, positive for CXCR3, low for CCR6 and positive for CD85j. When you right click on the wedge of interest in GemStone you can animate the selected events. The expression profiles above right have the selected, animated events shown as pink dots on each individual expression profile.

Conclusions

Probability State Modeling with GemStone was successfully employed to analyze a 39-marker CyTOF data file. A modeling template was designed and employed for the analysis. Although our subset percentages did not match the gating analysis exactly, the total number of B cells and plasmablasts identified were very similar between the two methods. The GemStone method reduces subjectivity, accounts for the transitions between stages and does not exclude or gate out events, therefore, differences in staging are expected.

The TriCOM feature of GemStone can phenotype subpopulations present within the cell type and stages. We demonstrated only one of the subpopulations found in this sample, but this illustrates the sensitivity of GemStone and its usefulness in exploring interactions.

This approach allows a user to analyze any number of files in a consistent and reproducible manner, thus enabling, for example, the potential to follow patients over time and during the course of treatment. The ability to see the changes in these micro-subpopulations would greatly add to our knowledge of normal immune function as well as the response to infection and the dysregulation that occurs in disease.

Acknowledgements

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References

1.Perez-Andres M, et., al. 2010. Human peripheral blood B-cell compartments: A crossroad in B-cell traffic. Cytometry Part B; 78B: S47-S60.
2.Horowitz A, et.al. 2013. Genetic and environmental determinants of human NK cell diversity revealed by mass cytometry. Science Translational Medicine; 5: 208ra145.
3.Warnatz K, Schlesier M. 2008. Flow cytometric phenotyping of common variable immunodeficiency. Cytometry Part B; 74B: 261–271.