Program Number: 236

Probability State Modeling Analysis of CD38, CD10, and CD19 Up-regulation in Early Human B-Cell Development

C. B. Bagwell[1], C. C. Stewart[2], B. Wood[3], B. Greig[4], F. Preffer[5]

[1]Verity Software House, Topsham, Maine 04086 USA
[2]Laboratory of Flow Cytometry, Roswell Park Cancer Institute, Buffalo, NY 14063 USA
[3]University of Washington, Dept. of Laboratory Medicine, Seattle WA 98195 USA
[4]Vanderbilt University Medical Center, Dept. of Pathology, Nashville TN 37232 USA
[5]Harvard Medical School, Massachusetts General Hospital, Dept. of Pathology, Boston 02114 USA

Human B-cell development is a regulated process characterized by the ordered differential expression of numerous cell surface and intra-cytoplasmic antigens, immunoglobulin gene rearrangements, and ultimately class switching. This process originates from self-renewing hematopoietic stem cells in the fetal liver and postnatal bone marrow and ends with the ultimate export of mature naïve B-cells from the marrow into the peripheral blood circulation.

B-cell precursors have been extensively studied in mouse and human systems and there is general agreement that CD34 and CD38 help identify early B-cell progenitors. However, the relative order of CD19 and CD10 up-regulation is not well established, with numerous conflicting published reports.

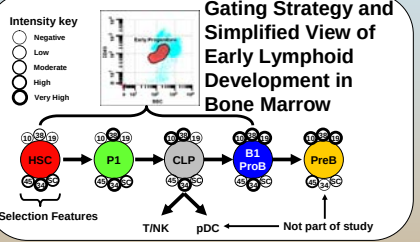
Recently, a new type of modeling program has been developed that allows a detailed objective analysis of the relative order of antigen up- and down- regulation for high-dimensional cytometry data. In this study, Probability State Modeling was used to determine the relative order of CD38, CD19, and CD10 up-regulation for a number of uninvolved bone marrow specimens. It also provided detailed correlated information on other cytometric features such as forward angle light scatter (FSC), side scatter (SC), CD20, CD9, and CD81.

1 Introduction

Early development of human B-cells occurs in distinct steps or stages in the bone marrow (BM). Many of these stages are defined by changes in the rearrangement status of immunoglobulin (Ig) heavy (H) and light chains (L), the expression of transcription factors, as well as specific phenotypic changes on the cell surface. Although much is known about B-cell development from a molecular genetics point-of-view, the functions of many of the surface and cytoplasmic proteins that appear and disappear as progenitor and B-cells develop remain unknown. Also, the literature contains a number of conflicting reports on the progression of many of these phenotypic changes. The exact timing of these cellular changes are important to document since many basic science studies are based on purification methods that use specific marker protein combinations. Also, many hematologic malignancies are defined in terms of having similar phenotypic patterns to these normal stages of differentiation.

This study examines very early lymphoid and B-cell phenotypic changes by the technique of Probability State Modeling (PSM). PSM uses an relatively objective and accurate modeling approach that orders events along a progression axis. Once ordered, the correlated changes of other cell features can also be studied. The cellular features examined are CD34, CD38, CD10, CD19, CD45, CD9, CD81, forward angle scatter (FS), and side scatter (SC).

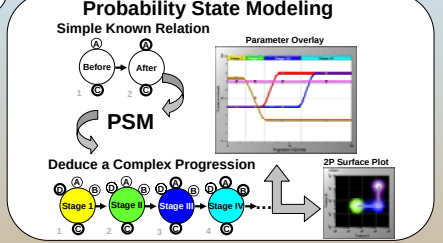
2 Gating Strategy and Simplified View of Early Lymphoid Development in Bone Marrow



A simplified consensus view of early lymphoid development is shown above along with the gating strategy used in this study. The selection features for the studied developmental progression were CD34+ and the above early progenitor CD45/SSC gating region. The progression stratification features were CD38, CD10, and CD19. The stages of the progenitor progression were defined as 1) hematopoietic stem cells (HSC, CD38-blim, CD10-, CD19-); 2) early progenitor cells (P1, CD38+/-,CD10-,CD19-); 3) common lymphoid progenitor cells (CLP, CD38+, CD10+, CD19-); and 4) progenitor B-cells (Pro/B labeled as B1, CD38+, CD10+, CD19+). T-cells, NK-cells, and Pre-B cells were not part of the study.

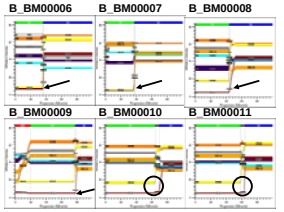
Many of these stages such as HSC have incomplete phenotypes and may not represent their true defined stage. Also, the existence of some of these stages is controversial.

3 Probability State Modeling



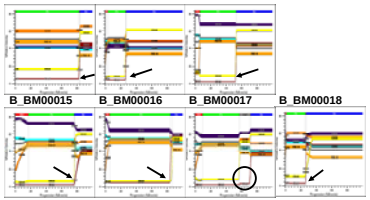
Probability State Modeling is a tool that scientists use to leverage a simple known relation (top) to deduce a much more complex progression (bottom). The simple relation in this example is the knowledge that cells selected by feature C initially are A- but later are A+. With this simple relation it is possible to unravel a very complex progression involving many features (bottom). Not only can the system determine order but it can produce a single graph that shows the order and percentages of all the intermediate stages (Parameter Overlay, middle-right) and two-dimensional contextual surface plots that appropriately blend colors from defined stages (bottom-right). For more details see poster P96, program number 182.

4 Probability State Modeling Results



Probability State Modeling (PSM) analyses were performed on 13 high-dimensional listmode files from clinical samples obtained by University of Washington's Cytometry Laboratory. Only very early progenitors and early B-cells were selected for the analysis (CD34+, gated on CD45/SSC (top-left)) in this study. The only information provided to the model was that CD38, CD10, and CD19 up-regulate at some point in the progenitor progression. The progression was divided into four stages. The first progenitor stage (HSC, red) was defined as events that were CD38- or in transition to CD38+. The second progenitor stage (P1, green) ended when CD10 up-regulated. The third progenitor stage (CLP, grey) ended when CD19 up-regulated, and the fourth progenitor stage directly followed the third (B1, blue).

5 Probability State Modeling Results



In ten of the thirteen samples, CD10 and CD19 apparently up-regulated nearly together (black arrows); whereas, in three samples, CD10 seemed to up-regulate slightly before CD19 (black open circles). Other pertinent observations were that FSC was highest in the P1 stage. CD34, CD45, and SSC stepped down through the HSC, P1, CLP, and B1 stages for many of the files.

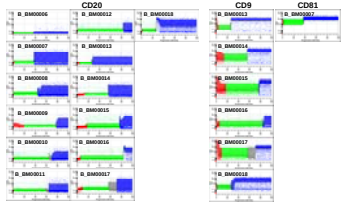
6 CD10 and CD19 Positions

Files	CD19 %	CD10 %	Diff'	Cytometry Features and Models\nTotal Events
B_BM00006	36.83	35.98	0.85	CD10,10,19,20,34,38,45,K-L_FS,SC (4,402288,218)
B_BM00007	38.10	38.21	-0.11	CD10,10,20,34,38,45,81,138,FS,SC (7,163/237,097)
B_BM00008	44.02	44.17	-0.15	CD10,10,20,34,38,45,K-L_FS,SC (11,323/417,911)
B_BM00009	78.68	77.01	1.67	CD10,10,20,34,38,45,K-L_FS,SC (5,972/422,432)
B_BM00010	69.66	64.55	5.11	CD10,10,20,34,38,45,K-L_FS,SC (13,528/632,595)
B_BM00011	64.38	58.69	5.69	CD10,10,20,34,38,45,K-L_FS,SC (14,595/742,220)
B_BM00012	83.60	82.59	1.01	CD10,10,20,34,38,45,K-L_FS,SC (8,787/522,838)
B_BM00013	26.79	26.20	0.59	CD9,10,19,20,34,38,45,FS,SC (2,230/171,218)
B_BM00014	53.04	53.04	0.00	CD9,10,19,20,34,38,45,FS,SC (5,526/358,633)
B_BM00015	76.34	77.01	-0.67	CD9,10,19,20,34,38,45,FS,SC (14,914/321,185)
B_BM00016	83.97	86.53	-2.56	CD9,10,19,20,34,38,45,FS,SC (5,458/290,698)
B_BM00017	71.49	56.67	14.82	CD9,10,19,20,34,38,45,FS,SC (16,347/371,037)
B_BM00018	27.05	25.78	1.28	CD9,10,19,20,34,38,45,FS,SC (7,269/415,403)

$^{*}H_0$: Diff = 0; t=0.48; since $t_{0.45@n=12} = 2.18$, H_0 could not be rejected.
Positive Sign Test for Diff; P(X>=8), n=13 is >0.26, no proclivity for CD10 up-regulating before CD19 can be deduced.

The above table summarizes the relative timing of CD19 and CD10 up-regulation for this study. The units are in % progenitor events. For example, a value of 36.83 for CD19 means that 36.83 percent of the progenitors selected were earlier in the progression. The difference data between the up-regulation of CD19 and CD10 were subjected to a t-test to determine whether CD19 preceded or followed CD10 up-regulation. The null hypothesis was that the mean difference between CD19 and CD10 up-regulation positions was zero. The observed t value for the differences was 0.48 and since the critical t-value for both tails of the t-distribution at p=0.05 with 12 degrees of freedom was 2.18, the null hypothesis could not be rejected. A Sign Test also failed to show that CD10 up-regulation generally precedes CD19. The statistics suggests that the CLP stage defined by the phenotype of CD34+, CD38+, CD10+, CD19- may either be too small to reliably measure or may not exist. The data suggests that CD10 and CD19 tend to up-regulate together.

7 CD20, CD9, and CD81 Hi-Lo Progressions



The above Hi-Lo progression plots show the modulation of CD20, CD9, and CD81 for a number of listmode files. The low-boundary in these plots was set to a 0.5 fraction, which is the median of all the events in each progression state along the axis. The high-boundary was set to 0.95 to show the upper 95% limit of the data.

When both CD10 and CD19 are expressed, CD20 is expressed on many of the events (first 3 rows). Some files showed relatively high CD9 expression early in the HSC stage (4th row). When CD19 is expressed, most of the events express high levels of CD9, but there is a significant fraction that don't express CD9. For the one file that had CD81 (last row), it seems to be up-regulated slightly when CD10 and CD19 are up-regulated.

8 Summary

The results of these analyses show that CD38 is up-regulated well before CD19 and CD10. There does not seem to be statistically significant proclivity of cells to express CD10 before CD19, suggesting that either the CLP phenotype of CD34+, CD38+, CD10+, CD19- may be too small to measure for many bone marrow specimens or that it does not exist as a real intermediate lymphoid progenitor stage. A more consistent conclusion from this data is that CD10 and CD19 are commonly co-expressed.

Other observations from this study are that FSC is very high once CD38 is expressed and then drops after CD10 is expressed. CD34, CD45, and SSC commonly drop in intensity in a step-wise manner until the B1 or ProB stage. After CD10 and CD19 are expressed; CD20, CD9, and CD81 are up-regulated for many events.

After CD19 was up-regulated, many events showed variable degrees of positivity for CD20. Some samples showed high CD9 expression in the HSC stage. In P1 and CLP stages, CD9 expression dropped to intermediate levels. Most events showed positive CD9 expression after CD19 was up-regulated, but a significant number of events remained negative.