BD LSRFortessa™ X-20 System
5-Color Panels Designed for Minimal Compensation

In this experiment, a five-laser BD LSRFortessa™ X-20 flow cytometer was used in combination with BD reagents to design panels that are optimized for minimal compensation and optimal signal by selecting one bright fluorochrome per laser. Three different panels are shown: human T cell, human B cell, and mouse B cell.

Analyzer Configuration

Laser	Filter	Fluorochrome	Human T-Cell Panel	Human B-Cell Panel	Mouse B-Cell Panel
Blue 488 nm	530/30	FITC	CD8	IgD	IgD
Yellow-Green 561 nm	610/20	PE-CF594	CD27	CD38	IgM
Red 640 nm	670/30	APC	CD45RA	IgM	CD21
Violet 405 nm	450/40	BV421	CD3	CD27	CD23
Ultraviolet 355 nm	379/28	BUV395	CD4	CD19	CD19

Compensation*

Fluorochrome	(-)% Fluorochrome	% Compensation
FITC	BV421	0.00%
PE-CF594		0.00%
APC		0.00%
BUV395		0.00%
BV421	FITC	**0.02%**
PE-CF594		0.00%
APC		**0.04%**
BUV395		0.00%
BV421	PE-CF594	0.00%
FITC		**0.29%**
APC		**1.39%**
BUV395		0.00%
BV421	APC	0.00%
FITC		0.00%
PE-CF594		**0.03%**
BUV395		0.00%
BV421	BUV395	**1.57%**
FITC		0.00%
PE-CF594		0.00%
APC		**0.24%**

*Representative compensation values. Compensation varies as a function of PMT voltage.

BD

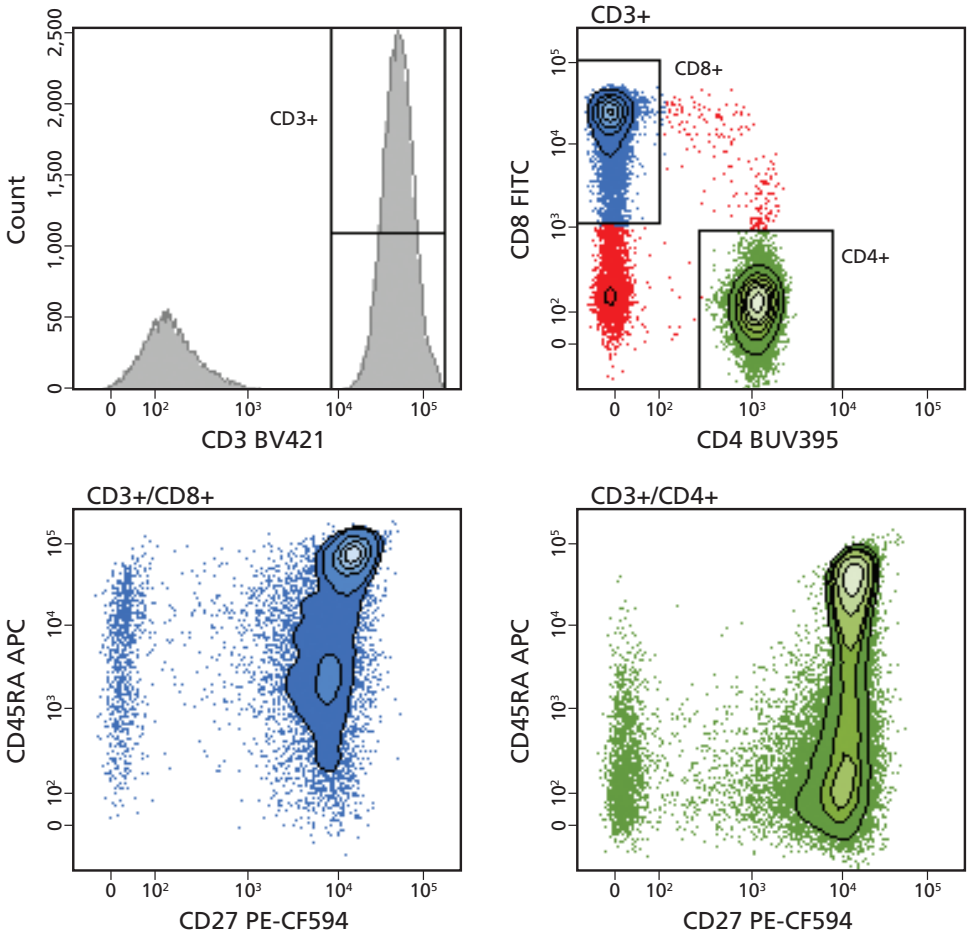
Protocol

PBMCs were prepared by using Ficoll-Paque™ Plus according to the manufacturer's directions and incubated with antibodies at room temperature protected from light for 20 minutes, washed, and acquired on a BD LSRFortessa X-20 flow cytometer. Single cells were identified by gating on FSC-A vs FSC-H. Lymphocytes were then identified based on FSC vs SSC and further analyzed as described in the subsequent figures.

For mouse experiments, C57BL6 spleen was harvested into a single-cell suspension using a cell strainer and syringe plunger. Red blood cells were lysed using BD Pharm Lyse™ lysing buffer (Cat. No. 555899) on ice for 2 minutes. Cells were subsequently washed, stained with antibodies for 30 minutes on ice, washed, and acquired on a BD LSRFortessa X-20 flow cytometer.

Data

Analysis of Human Naïve, Effector, and Memory T Cells

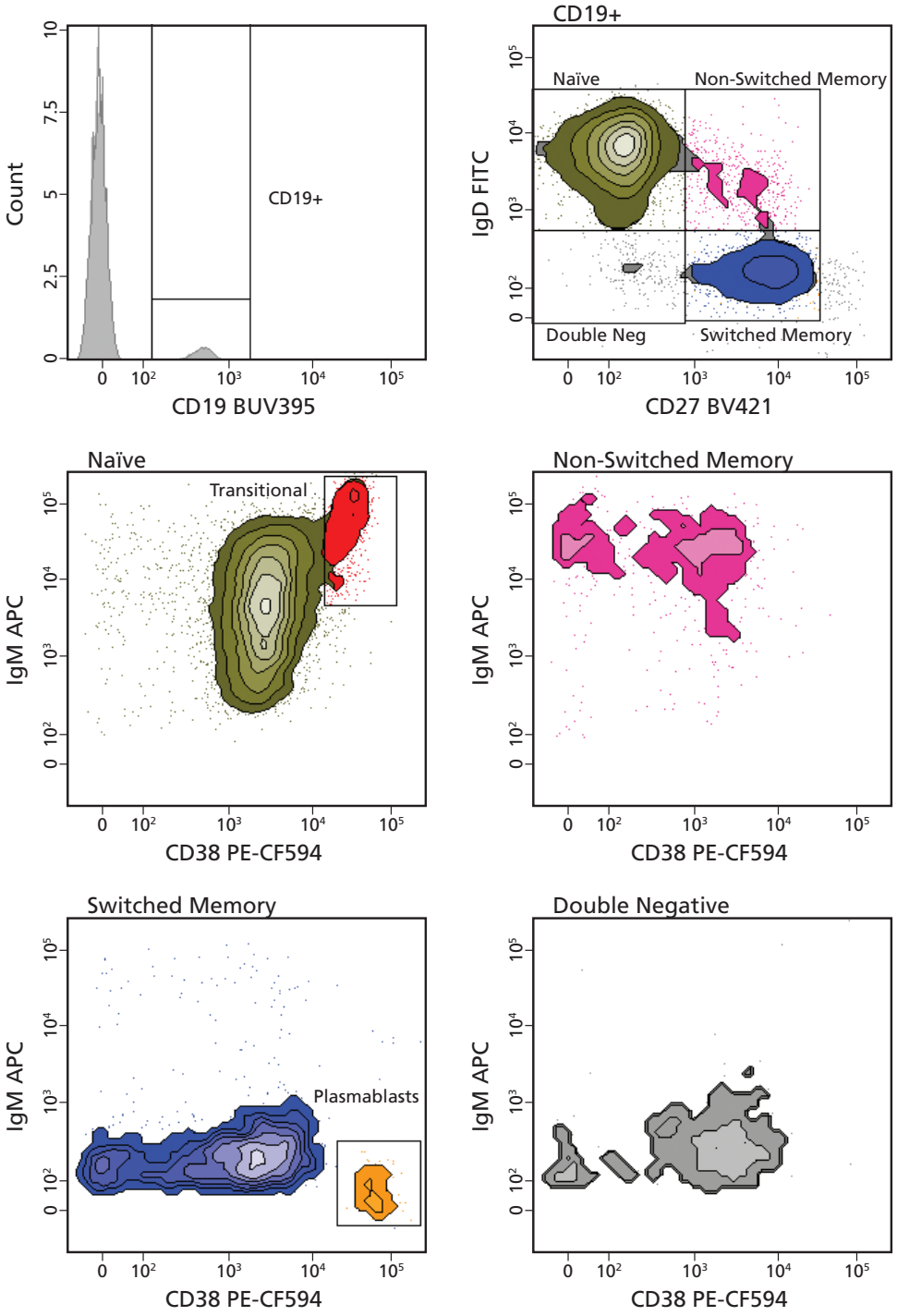


Discussion

Human PBMCs were stained with CD4 BD Horizon™ Brilliant Ultraviolet™ 395 (BUV395), CD8 FITC, CD3 BD Horizon™ Brilliant Violet™ 421 (BV421), CD27 PE-CF594, and CD45RA APC. Singlet lymphocytes were discriminated based on light scatter properties, and the $CD3^+$ population was identified (top left). Traditional CD4 and CD8 T-cell subsets can be gated on from the $CD3^+$ lymphocyte population (top right). Within the $CD8^+$ (bottom left) and $CD4^+$ (bottom right) T-cell subsets, naïve ($CD27^+CD45RA^+$), memory ($CD27^+CD45RA^-$), and effector ($CD45^{+/-}CD27^-$) T cells can be identified.

Data

Analysis of Human B-Cell Subsets

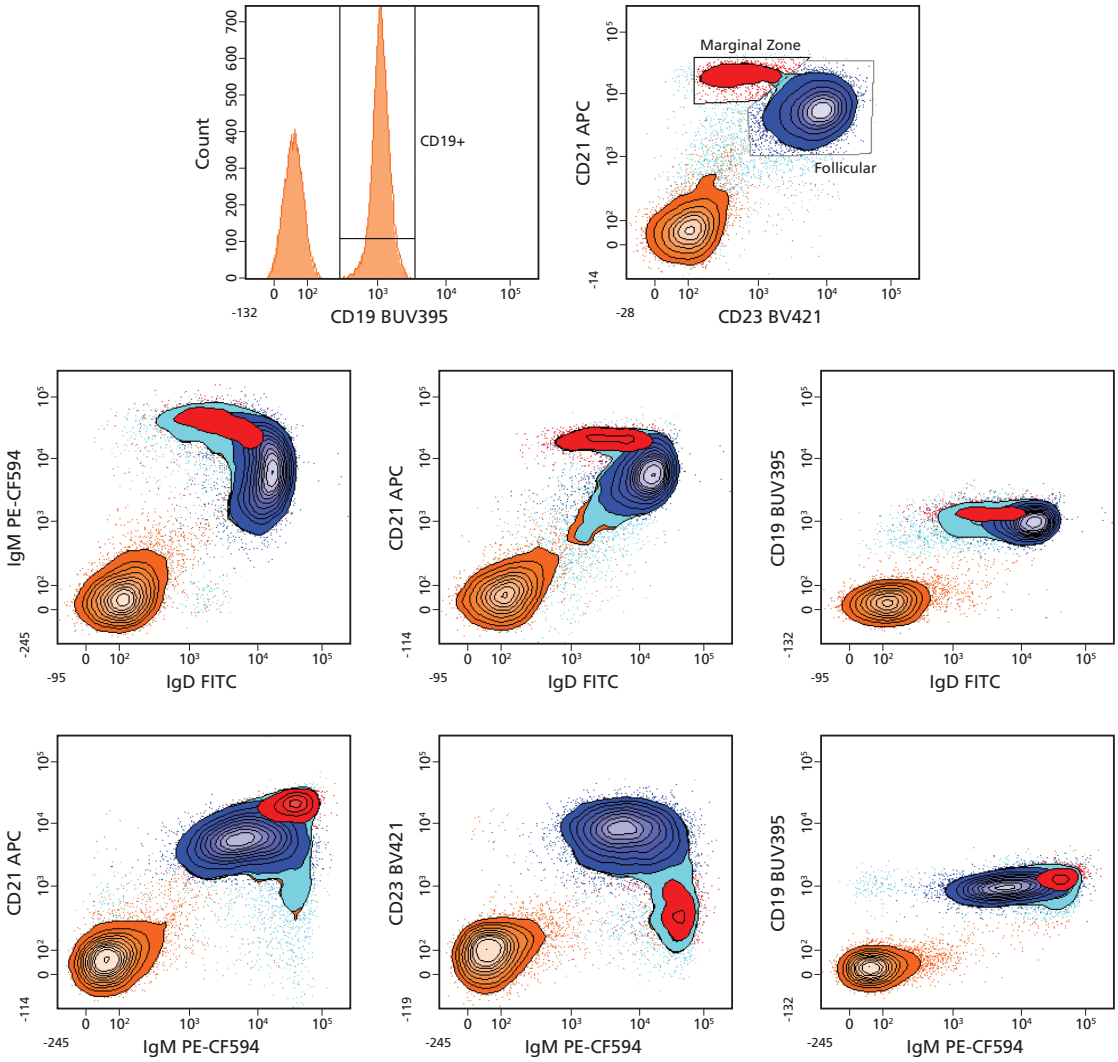


Discussion

Human PBMCs were stained with CD19 BUV395, CD27 BV421, IgD FITC, CD38 PE-CF594, and IgM APC. Singlet lymphocytes were discriminated based on light scatter properties, and the $CD19^{+}$ population was identified (top left). Naïve, non-switched, switched memory, and double negative B-cell subsets can be identified from the $CD19^{+}$ lymphocyte population based on expression of CD27 and IgD (top right). Within all subsets, IgM vs CD38 expression was analyzed (middle and bottom). Within the naïve B-cell subset (middle left), transitional B cells can be identified as $IgM^{++}CD38^{++}$. Within the switched memory subset (bottom left), plasmablasts can be identified as $IgM^{-}CD38^{++}$.

Data

Analysis of Mouse Marginal Zone and Follicular B Cells



Discussion

C57BL6 splenocytes were stained with CD19 BUV395, CD23 BV421, IgD FITC, IgM PE-CF594, and CD21 APC. CD19^{+} B cells (top left) can be subdivided into follicular and marginal zone cells based on expression of CD23 and CD21 (top right). The variable expression of CD21, CD23, IgM, and IgD is shown for the two subsets in the middle and bottom rows. The marginal zone cells show higher expression of IgM and CD21 compared to the follicular cells. In comparison, the expression of CD23 and IgD is higher on the follicular cells.

Conclusion

The five-laser configuration of the BD LSRFortessa X-20 system, combined with novel BD Horizon™ Brilliant Violet™ and BD Horizon™ Brilliant Ultraviolet™ reagents, enables optimal panel design based on antigen density and fluorochrome brightness while optimizing signal by using all five lasers simultaneously.

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