BD LSRFortessa™ X-20
15-Color Immunophenotyping

In this experiment, the BD LSRFortessa™ X-20 system was used in combination with BD reagents to stain human peripheral blood mononuclear cells (PBMCs) for analysis of common T-cell, B-cell, NK-cell, dendritic-cell (DC), and monocyte subsets. Three different gating strategies enabled identification of T cells, NK-T cells, and regulatory T cells (Tregs); B cells, NK cells, and DCs; and monocytes. The five-laser configuration of the BD LSRFortessa X-20 flow cytometer combined with novel BD Horizon™ Brilliant Violet™ and BD Horizon™ Brilliant Ultraviolet™ reagents enables optimal panel design based on antigen density and fluorochrome brightness.

Instrument Configuration

Laser	Filter	Fluorochrome	Specificity	Clone	Cat. No.
Blue 488 nm	530/30	FITC	CD57	HNK-1	347393
	695/40	PerCP-Cy™5.5	CD3	SK7	340949
Yellow-Green 561 nm	586/15	PE	CD11c	S-HCL-3	347637
	610/20	BD Horizon™ PE-CF594	CD16	3G8	562293
	780/60	PE-Cy™7	CD33	P67.6	333946
Red 640 nm	670/30	APC	CD56	NCAM16.2	341025
	730/45	Alexa Fluor® 700	CD20	2H7	560631
	780/60	APC-H7	HLA-DR	L243	641393
Violet 405 nm	450/40	BD Horizon™ BV421	CD123	9F5	562517
	525/50	BD Horizon™ V500	CD14	MφP9	562693
	610/20	BD Horizon™ BV605	CD25	2A3	562660
	660/20	BD Horizon™ BV650	CD335 (NKp46)	9E2/Nkp46	563230
	710/50	BD Horizon™ BV711	CD8	RPA-T8	563676
	780/60	BD Horizon™ BV786	CD19	SJ25C1	563325
Ultraviolet 355 nm	379/28	BD Horizon™ BUV395	CD4	SK3	563550

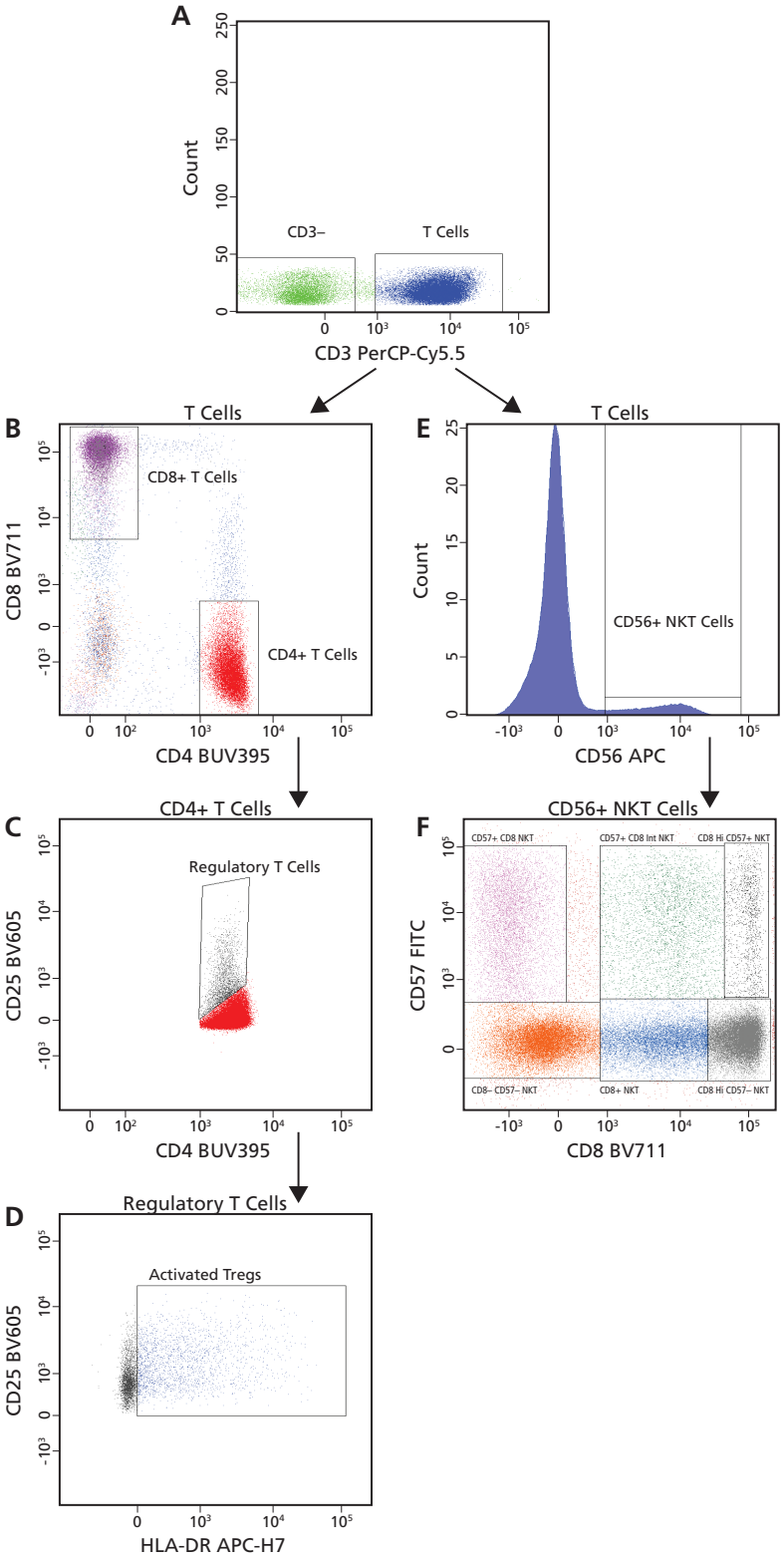
Protocol

PBMCs were isolated by preparing a mononuclear cell fraction using Ficoll-Paque™ Plus. Briefly, 15 mL of whole blood was diluted with 15 mL of phosphate buffered saline (PBS) + 2% fetal bovine serum (FBS). The entire 30 mL of diluted blood was layered over 15 mL of Ficoll-Paque Plus in a 50-mL Falcon® tube. Blood was centrifuged at 400*g* for 30 minutes at room temperature with the brake off. PBMCs were removed from the plasma-Ficoll interface and washed twice with BD Pharmingen™ stain buffer. Cells were counted and aliquoted at 1 x 10^6 cells per tube and incubated with antibodies on ice 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 or monocytes were then identified based on FSC vs SSC and further analyzed as described in the subsequent figures.

BD

Data

Distinguishing helper and cytotoxic T cells, Tregs, and NK-T cells

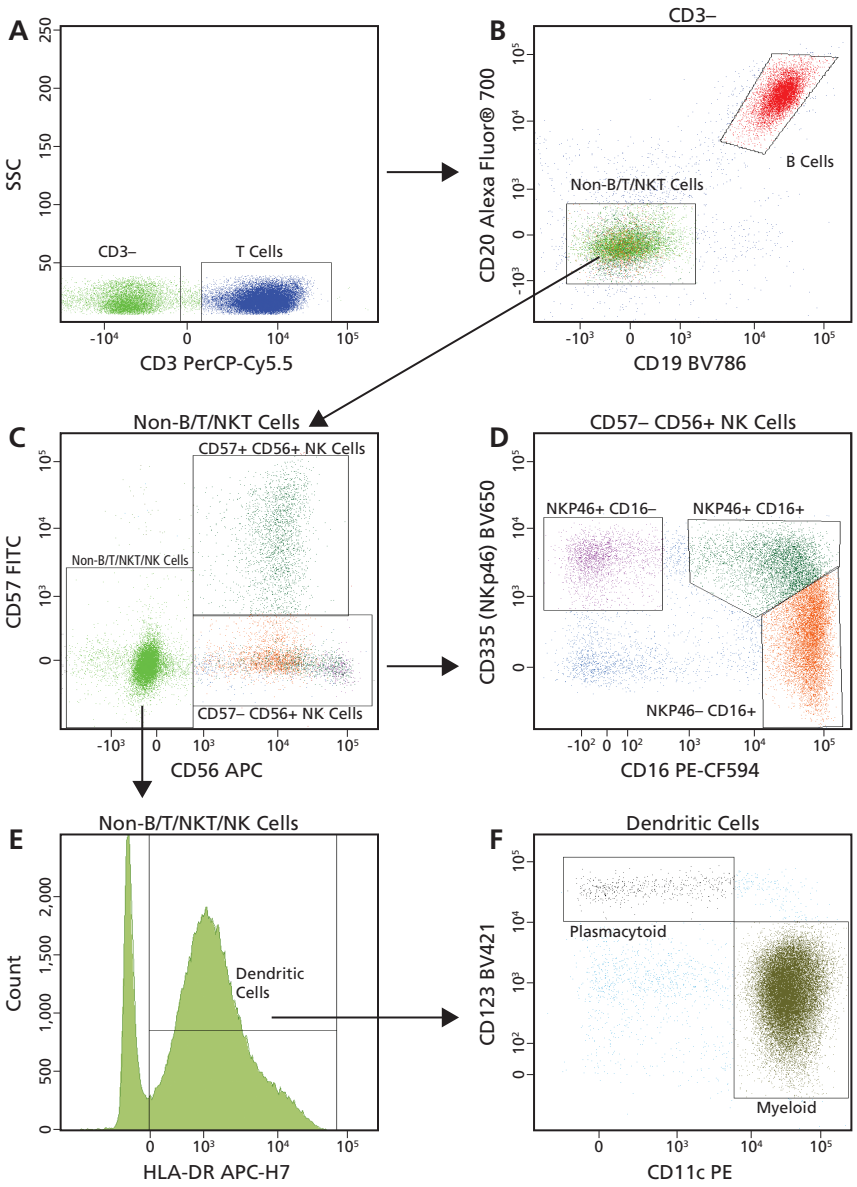


Discussion

The CD3⁺ population (A) contains traditional CD4 and CD8 T cells in addition to Tregs and natural killer T cells. Gating on CD4⁺ T cells (B) allows the identification of CD25⁺ Tregs (C) that can be further analyzed for activation based on HLA-DR expression (D). Natural killer T cells are characterized by CD56 expression within the CD3⁺ subset (E). With the use of multicolor flow cytometry, we can identify NK-T–cell subsets based on CD57 and CD8 expression (F).

Data

Distinguishing B cells, NK cells, and DC subsets

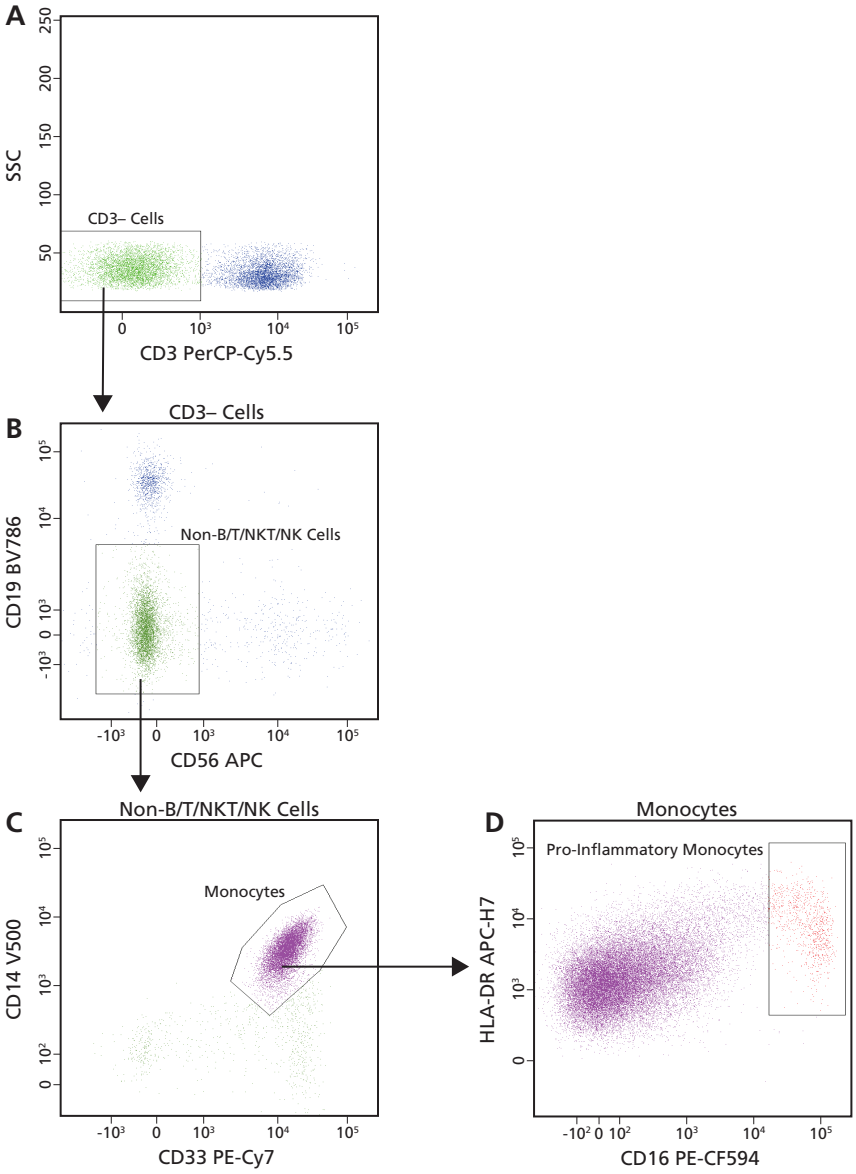


Discussion

Gating CD3⁻ negative cells (A) allows for identification of B cells, NK cells, and DCs. CD19 and CD20 identify the B-cell population, while cells negative for CD19/CD20/CD3 (B) include NK cells and DCs (C). CD56 and CD57 identify functionally distinct mature NK cell populations from other subsets of NK cells based on CD16 and CD335 expression (D). Remaining PBMCs contain dendritic cells that can be identified as $CD123^{hi}CD11c^{-}$ plasmacytoid and $CD123^{-}CD11c^{hi}$ myeloid subsets (E, F).

Data

Identification of pro-inflammatory monocytes



Discussion

Monocyte gates based on light scatter can be further refined by gating out T, B, and NK cells based on CD3, CD19, and CD56 respectively (A, B). Monocytes are clearly identified based on CD14 and CD33 expression (C). Pro-inflammatory monocytes are identified as $CD16^{hi}HLA\text{-}DR^{hi}$ cells (D).

Conclusion

A five-laser BD LSRFortessa X-20 system and BD flow cytometry reagents enable high-order multiplexing of results. In this 15-color example, we were able to subset PBMCs into 7 unique populations.

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