Improved resolution of plasma cell subpopulations by flow cytometry

Joel R. Wilmore, Derek D. Jones, and David Allman

Supporting Information

Materials and Methods

Mice

C57/BL6 and B6.Blimp$^{GFP/+}$ mice were bred and housed in our colony at the University of Pennsylvania Perelman School of Medicine. Animals were housed and maintained according to procedures approved by the University of Pennsylvania IACUC.

Flow Cytometry

Mice were euthanized by approved methods and then tissues were immediately removed from the mice and placed in FACS buffer on ice. FACS buffer consists of DPBS with 0.5% Bovine Serum Albumin and 2mM EDTA. Experiments comparing FACS buffers with several different concentrations of sodium azide were performed with the indicated percentage of azide added, but typically azide is absent from our FACS buffer. Bones were then flushed using FACS buffer and a 23G needle and marrow was broken up by gently pipetting before being passed through a 70μm strainer. Spleens and Peyer's patches were broken up by gently disassociating the cells with frosted glass slides before pipetting through a 70μm strainer. Cells were then washed and red blood cells were lysed using ACK lysis buffer. Following lysis the cells were washed with PBS and roughly 1/10th of the bone marrow prep (obtained from 2 femurs and 2 tibia) was stained with the fixable viability dye Zombie Aqua

(Biolegend, San Diego, CA, USA) according to manufacturers protocol. Next, a cocktail of antibodies including anti-CD138 PE (clone 281-2 BD Biosciences San Jose, CA, USA), anti-B220 APC (RA3-6B2, Tonbo Biosciences, San Diego, CA, USA), anti-IgD APC-Cy7 (clone 11-26c.2a, Biolegend), anti-Sca-1 Brilliant Violet 605 (clone D7, Biolegend), and anti-CD4 (GK1.5), anti-CD8α (53-6.7), anti-F4/80 (BM8), and anti-TER119 all in PE-Cy7 (Biolegend). Cells were incubated on ice in 100 µl of antibody cocktail for 20 minutes followed by two washes with FACS buffer. The cell suspension was brought up in 400 µl of FACS buffer then run on a LSR II flow cytometer (BD Biosciences, configuration in Supplementary Table 1). At least 2 million events were acquired for each sample, which were then analyzed using FlowJo 8.8.7 (Tree Star, Ashland, OR, USA).

Supplementary Table 1

Target	**Clone**	**Fluorophores**	**Dilution**	**Laser (nm)**	**Filter**
Blimp1	Transgene	GFP		488	515/20
CD138	281-2	PE	1-400	532	575/26
CD4	GK1.5	PE-Cy7	1-400	532	780/60
CD8	53-6.7	PE-Cy7	1-400	532	780/60
F4/80	BM8	PE-Cy7	1-400	532	780/60
TER119	TER119	PE-Cy7	1-400	532	780/60
B220	RA3-6B2	APC	1-200	640	660/20
IgD	11-26c.2a	APC-Cy7	1-200	640	780/60
Sca-1	D7	BV605	1-200	405	605/40
Zombie (Viability)		Aqua		405	515/20