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Elimination of Non-Specific Staining in Murine Cell Samples

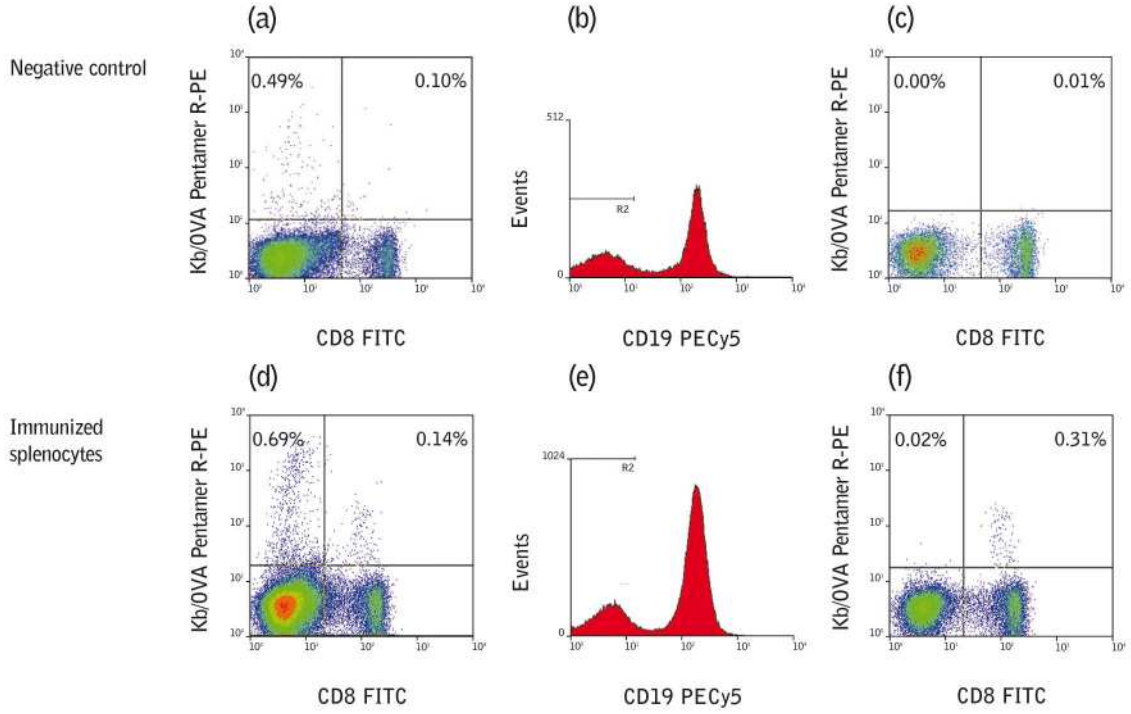
When performing flow cytometric analysis of murine samples stained with Pro5® MHC Pentamers, high background (non-specific) staining is sometimes observed, even when a tight gate is set around the live lymphocyte population. This can interfere with the interpretation of results and make it difficult to visualize true Pentamer staining. This non-specific staining is caused by B cells and can be eliminated by gating out CD19, as illustrated in the results below.

Experiment

To produce antigen-specific cells, C57BL/6 mice were immunized with the H-2Kb/SIINFEKL epitope from the model antigen ovalbumin (OVA). 1 x 10^6 naïve or OVA-immunized C57BL/6 splenocytes were depleted of red blood cells by lysis using Pharm Lyse™ (BD Biosciences) according to the manufacturer's instructions. Cells were then incubated with 1 test unlabeled H-2Kb/SIINFEKL (OVA) Pentamer for 10 minutes at room temperature (22°C) in 50 µl volume. Samples were washed once with 10 volumes of buffer (0.1% BSA, 0.1% sodium azide in PBS) and spun down (5 minutes at 500 x *g*), then incubated with 0.5 tests R-PE-Fluorotag, 1 test anti-mouse CD8-FITC (clone KT15) and 1 test anti-mouse CD19-PECy5 (clone 6D5) for 30 minutes at 4°C. Cells were again washed and spun down then fixed (1% heat-inactivated fetal calf serum, 2.5% formaldehyde in PBS) before analysis by flow cytometry.

Results

The figure below shows the effect of gating out B cells from a negative control sample (a) - (c), and immunized splenocytes (d) – (f).



In (a) & (d) the live splenocyte population shows considerable non-specific staining in the CD8-negative, Pentamer-positive quadrant. In (b) & (e) the region (R2) is set upon the CD19-negative cells. (c) & (f) The live splenocyte population is re-gated to show only events within R2 (which excludes B cells), and this results in elimination of the non-specific staining. The antigen-specific population in the upper right quadrant of (f) can now be clearly distinguished.

Conclusions

B cells can contribute to the non-specific staining observed with mouse splenocytes when staining samples with Pro5® MHC Pentamers. This non-specific staining can be reduced or eliminated by co-staining with anti-CD19 antibody and gating on the CD19-negative cells when performing analysis. Alternatively, anti-CD19 magnetic beads could be used to remove B cells prior to staining.