Staining Murine Splenocytes for Antigen-Specific Responses Following Peptide Immunization

Introduction

Peptide immunization is a common method used to elicit antigen-specific T cells in vivo. In this example, the aim was to generate murine CD8+ T cell responses targeting the H2-Kb restricted response to the SIINFEKL epitope from the model antigen ovalbumin (OVA). In the example below the immunogen (AVHAHAINEAGSIIIFEKL peptide) contains an OVA-specific T helper epitope followed by the OVA-specific SIINFEKL CTL epitope.

Experiment

Four C57BL/6 female mice were immunized with 100µg AVHAHAINEAGSIIIFEKL peptide and incomplete Freund's adjuvant in a 1:1 ratio (day 0). A control mouse was immunized with PBS only. On day 12 and day 26, all mice received boosters as for day 0. On day 36, tail vein bleeds were taken from each mouse and analyzed for an antigen-specific response. Three of the immunized mice had a positive response. Splenocytes were obtained on day 44 and lysed to remove red cells. The splenocytes from two peptide-immunized mice were pooled together for staining.

Cells were incubated with either 1 test R-PE-labeled Kb/OVA (SIINFEKL - specific peptide) or 1 test R-PE labeled Kb/HSV (SSIEFARL - irrelevant peptide) Pentamer for 10 minutes at room temperature (22°C) in 50 µl volume. Samples were washed 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 1 test anti-CD8 FITC (clone KT15) and 1 test anti-CD19 PECy5 (clone 6D5) for 20 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 figures shown are gated on live, CD19-negative cells to eliminate non-specific staining of B cells from the results.

The Kb/OVA Pentamer does not stain cells from a control mouse injected with PBS (a). Peptide immunized mice also give a negative response when stained with a Pentamer containing an irrelevant peptide (Kb/HSV) in (b). However, a specific response (0.39%) is clearly detected in immunized splenocytes stained with Kb/OVA Pentamer (c).

Conclusions

Immunization of C57BL/6 mice with OVA-specific peptide yields a response that is clearly detected by staining splenocytes with H-2Kb/SSINFEKL Pro5® MHC Pentamer. This immunization model illustrates a useful approach for generating antigen-specific T cell responses with other peptide antigens.

1 HSV = Herpes Simplex virus