Evaluation of antigen binding by CD8+ T cells after *in vivo* treatment with myeloid derived suppressor cells

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Introduction

This protocol is adapted from previous work of Dr. Schneck and used to evaluate binding of OT-1 and 2C specific pMHC by CD8+ T cells.

Reagents

ACK lysing buffer 8.29g NH_4Cl (0.15M), 1g $KHCO_3$(10.0mM), 37.2 mg Na_2EDTA(0.1mM) Add 800ml H_20 and adjust pH to 7.2-7.4, Add H_20 to 1L. Filter sterilize and store at RT.

Wash buffer PBS+2%FBS+.05% azide

Dimers SIINKb-Ig and SIYKb-Ig dimmers

Biotin-Gr-1 antibody (Pharmingen, CA)

Strepatavidin beads (Miltenyi biotech, CA)

Specific peptide SIINFEKL and incomplete Freund's adjuvant

T cell column (R & D systems, MN)

Magnetic Columns (Miltenyi biotech, CA)

Equipment

Centrifuge with temperature control

Flow cytometer (BD FacsCalibur)

Flow analysis software Flowjo vers 8.2

Procedure

Day 1: Isolate T cells from OT-1 mice using T cell enrichment column and transfer 5-6 X 10^6 cells intravenously into CD45.1 mice.

Day 3. Isolate MDSC from 3 week tumor-bearing mice (EL4 or MC38) using biotin-Gr-1 and streptavidin beads (Miltenyi biotech, CA) antibody and transfer 4-5 X 10^6 cells intravenously into mice which have received OT-1 T cells. Immunize mice with SIINFEKL peptide 100 ug in 100 ul PBS plus 100 ul of incomplete Freund's adjuvant.

Day 10:

1. Harvest spleens from mice and prepare a single cell suspension.
2. Pellet the cells by centrifugation (1700 RPM 5 min) at room temperature (RT) and

aspirate the supernatant.

3. Resuspend the pellet in 5 ml/spleen of ACK Lysis Buffer.
4. Incubate at RT for 4-5 minutes with occasional shaking.
5. Stop the reaction by adding 40-50 µl of PBS.
6. Wash the cells with PBS twice (1700 RPM 5 min) at RT.
7. Use Cy5 (Molecular probes, OR) labeled SIINKb-Ig and SIYKb-Ig dimers as specific and non-specific MHC-Ig ligands, respectively.
8. Prepare a serial dilution (2000 nM – 12.5 nM) of 20 µl of Cy5 labeled MHC-Ig dimer in a FACS tube.
9. Adjust cells to a concentration of 10^7 cells/ml in FACS wash buffer
10. Add 10 ul aliquot of cells to the FACS tube with the MHC-Ig dimer.
11. Incubate cells with the MHC-Ig dimers for 60-90 min on ice.
12. Without any washing add FITC labeled anti-CD8 and PE labeled anti-CD45.1 antibodies to each aliquot.
13. Incubate cells for an additional 20 min on ice and wash two times before flow cytometric analysis.
14. Specific MHC-Ig staining is calculated by subtracting the non-specific MHC-Ig staining from the total MHC-Ig staining within the population of CD45.1 negative CD8 positive cells.

Timing

Approximately 11 days

Anticipated Results

See accompanied paper

Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer

by Srinivas Nagaraj, Kapil Gupta, Vladimir Pisarev, +5