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

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Method Article

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Abstract

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