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Culture and expansion of OT-1 TCR transgenic T cells

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Method Article

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Abstract

Introduction

This protocol was used in the above _Nature Immunology_ paper.

Reagents

Complete media for SAMBOK cell line: DMEM \(Gibco11960) high glucose supplemented with 10% FBS \(v/v), 2% pen/strep \(v/v), 1mM sodium Pyruvate \(Gibco 11360) 150µg/ml of G418 \(clontech 631308), 150µg/ml of Hygromycin B \(Mediatech 30240CR) **Complete media for primary splenic cultures:** RPMI 1640 \(Gibco 11875) supplemented with 10% FBS \(v/v), 2% pen/strep \(v/v), 1mM sodium Pyruvate and 500µl of B-ME solution \(39µl of B-ME in 10 ml of media, filter sterilized)

Procedure

Incubate all cells at 37 degrees C in 5% CO2 1) Two days prior to your experiment plate out 100,000 SAMBOK APCs \(ref. 1) in complete media supplemented with 4ng/ml of IFN-g \(Peprotech 315-05) per well in a 12 well plate. Use one 12 well plate per spleen. 2) On the day of experiment collect spleen and lymph nodes from CD8+OT-1 TCR transgenic mice and make single cell suspensions. Pool cells and count 3) Keep cells at 2 X 10^7 /ml in complete media for primary splenic cultures. 4) Wash SAMBOK APCs in PBS once to get rid of any residual media and IFN-g. 5) Place 1 mL of cells onto SAMBOK APCs and culture for 48 hours. Ensure that the media does not become too yellow. It is best to place an additional 1ml of media in each well after 24 hours. 6) Pool all cells in and spin down in a 50 mL conical and resuspend in complete media for primary splenic cultures supplemented with 200 U/ml of IL-2 at 2 X 10^7/ml. Allow these cells to sit for an additional 48 hours in a 24 well plate \(1ml/well). 7) After 48 hours take some cells for FACs analysis to check purity of the population should be > 95% CD8 T cells.

References

1. van Stipdonk, M.J., E.E. Lemmens, and S.P. Schoenberger. 2001. _Nat. Immunol._ **2**:423–429