ENCODE – protocol

Originating Product

Leukopaks from healthy adults were obtained from ALLCells and processed to isolate Peripheral Blood Mononuclear Cells (PBMCs) using Ficoll-paque plus (GE Health care, Catalog Number 17-1440-03).

Human T and B cell purification

$CD4^+$ T, $CD8^+$ T and CD19+ B cells were purified from PBMCs using Dynal CD4 Positive (Invitrogen cat# 11331D), CD8 Positive (Invitrogen cat# 11333D) and CD19 Positive (Invitrogen cat# 11143D) Isolation Kits respectively.
CD4+ and CD8+ T cells were surface stained with CCR7 (R&D Systems Cat# FAB197F) and CD45RO (Biolegend cat# 304228) then sorted by FACS (BD FACS ARIA cell sorter) into Naïve (CD45RO- CCR7+) and Memory (CD45RO+ and CD45RO- CCR7-) subsets.

Monocytes purification and activation

CD14+ monocytes were purified from PBMCs using CD14+ microbeads (Miltenyi cat# 130-118-906) and AutoMACS pro separator. For activation, purified monocytes were cultured at 10M/6ml/well in low binding 6-well plates and treated with 100 ng/ml Lipopolysaccharide (LPS, Sigma-Aldrich cat# L4391) or 10 μg/ml Polyinosinic-polycytidylic acid (Poly I:C, Sigma-Aldrich cat# P9582) and cultured at 37°C in a 5% CO_2 incubator for 24 hours.

Generation of Monocyte derived Dendritic cells

Monocyte derived dendritic cells were generated by culturing purified monocytes in complete RPMI 1640 medium (RPMI 1640 supplemented with 10% FBS; Atlanta Biologicals, Lawrenceville, GA), 8% GlutaMAX (Life Technologies), 8% sodium pyruvate, 8% MEM vitamins, 8% MEM nonessential amino acid, and 1% penicillin/streptomycin (all from Corning Cellgro) complemented with 50 ng/ml GMCSF (R&D Systems cat# 7954-GM-050/CF) and 40 ng/ml rhIL-4 (R&D Systems cat# 204-IL-020) for 4 days.

Human T cell activation and Differentiation into T Helper subsets

CD4+ and CD8+ T cells and their subsets were left resting in culture or activated using anti-CD3 and anti-CD28 (aCD3/aCD28) coated beads (Invitrogen cat# 11132D) (1 bead:2 cells ratio) and cultured in complete RPMI 1640 at 10M/6ml/well in 6 well-plate. Resting cells and 90% of activated T cells were collected on day 2 (36 hours) and beads were removed using Dynal magnet. The rest of activated cells was expanded in rhIL-2 (10 ng/ml, R&D Systems cat# 10453-IL-100) containing media for 5 to 6 more

days and collected on day 7 or day 8 post activation. Activation of T cells was confirmed by the upregulation of CD25 and CD69 surface expression analyzed by flow cytometry. For differentiation into different T helper (Th) subsets, Naïve CD4+ (sorted CD45RO-CCR7+) T cells were activated with anti-CD3 and anti-CD28 beads in the presence of the following polarizing cytokine and blocking antibody combinations: Th0, medium only, Th1, human IL-12 (R&D Systems cat# 219-IL-025; 30 ng/ml) and neutralizing anti-IL-4 Antibody (R&D Systems cat# AF-204-NA; 1 µg/ml), and Th2, human IL-4 (R&D Systems cat# 204-IL-020; 100 ng/ml) and neutralizing anti-IFN-γ Antibody (R&D Systems cat# MAB285-100; 5 µg/ml). Cells were cultured and expanded in rhIL-2 (10 ng/ml, R&D Systems cat# 10453-IL-100) containing media for 2 weeks. On day 13 after activation, some of the cells were reactivated with aCD3/aCD28 beads (at 1 bead:3 cells ratio) for another 24 hours.

Human B cell activation

CD19+ B cells were left resting in culture or activated using TLR9 agonist CPG ODN 2006 (Invivogen cat# tlrl-2006) at 0.5μM. Both resting and activated cells were collected on day 1 (24 hours post activation). Activation of B cells was confirmed by the upregulation of CD25, CD69 and CD86 surface expression analyzed by flow cytometry.

Human Natural Killer (NK) purification

NK were purified from PBMCs by negative selection using NK Cell Isolation Kit (Miltenyi cat# 130-092-657) and AutoMACS pro separator.

Freezing cells

After collection, cells were either flash frozen as cell pellets for RNA seq analysis or lively frozen in 50% RPMI+40% FBS+10% DMSO (Dimethyl Sulfoxide, Sigma-Aldrich cat# D2650)