

Supplementary Methods

cDNA Preparation

Fresh tissue samples were transported in RPMI media, and processed within 24 hours. Skin samples were incubated in dispase at 4° for 8 hours prior to mechanical disruption of the epidermal layer. All samples were processed into single cell suspension over a 70 µm mesh filter. Cells from the biopsy samples were then separated by flow cytometry by gating on the propidium iodide-negative population using CD207-PE (Beckman Coulter, Fullerton, CA) and CD3-FITC (BD Bioscience, San Jose, CA) conjugated antibodies. Control tonsil CD3 cells were prepared in the same manner, and then isolated with CD3-FITC dye alone. Control Langerhans cells were isolated from normal skin samples that were digested with dispase, the epidermal layer was processed as described above, and Langerhans were isolated with CD1a-PE (BD Bioscience, San Jose, CA). Cells from all samples were sorted with a MoFlo Sorter (Fullerton, CA) directly into PicoPure RNA Extraction Buffer (Molecular Devices, Sunnyvale, CA). RNA was then processed according to manufacturer's protocol. RNA concentration and quality was verified using the 6000 Pico Chip (Agilent, Santa Clara, CA) at the Baylor Genomics Core. Any samples with detectable RIN<5 were excluded from the study. cDNA amplification was performed with the WT-Ovation Pico System (NuGen, San Carlos, CA) according to manufacturer's protocol.

Polymerase Chain Reaction

PCR were prepared using 20 ng of template cDNA according to standard conditions with annealing temperature 58° and 37 cycles with the following primers:

GAPDH F: 5'-GGCCTCCAAGGAGTAAGACC-3'

GAPDH R: 5'-AGGGGTCTACATGGCAACTG-3'

CD207 F: 5'-CAACAATGCTGGGAACAATG-3'

CD207 R: 5'-GGGGAAGAAAGAGGCATTTTC-3'

OPN F: 5'-GCCGAGGTGATAGTGTGGTT-3'

OPN R: 5'-TGAGGTGATGTCCTCGTCTG-3'

IL-17A F: 5'-CCCCTAGACTCAGGCTTCCT-3'

IL-17A R: 5'-TCAGCTCCTTTCTGGGTTGT-3'

CD3e F: 5'-CCTGTTCCCAACCCAGACTA-3'

CD3e R: 5'-GAGGCAGTGTCTCCAGAGG-3'

Control IL-17A Samples

Control samples were prepared by collecting peripheral blood from 2 healthy volunteers, isolating peripheral blood mononuclear cells over a Histopaque-1077 (Sigma, St. Louis, MO) gradient. 5×10^6 cells were plated into 2 ml RPMI media (Invitrogen, Carlsbad, CA), and after 24 hours, 20 ng/ml PMA and 1 µg/ml of ionomycin (Sigma-Aldrich, St. Louis, MO) were added. Media was collected prior to and 48 hours after addition of ionomycin/PMA then stored at -80°C.

Collection of Plasma Samples

Peripheral blood (10 ml) was collected from consenting donors. Plasma was collected after centrifuging blood over a Histopaque-1077 gradient at 450G for 30 minutes, and then was frozen at -80°C. All plasma samples were frozen only once prior to testing.

ELISA

Enzyme-Linked Immunosorbent Assays were performed using both the R&D Systems (Minneapolis, MN) Human IL-17 Quantikine ELISA system and the eBioscience (San Diego, CA) Human IL-17E ELISA system according to manufacturers' protocols.