Appendix S1

SUPPLEMENTAL MATERIAL AND METHODS

Cell culture

Primary human epidermal keratinocytes (HEK) were cultured in serum-free EpiLife medium (MEPI500CA, ThermoFisher, Waltham, MA, USA) with supplements (S-001-K, ThermoFisher). Primary human dermal fibroblasts (HDF) were cultured in Medium-106 (M-106, ThermoFisher) with supplements (S003K, ThermoFisher). Three independent experiments were performed using cells from different donors. Peripheral blood mononuclear cells (PBMC) were isolated from fresh buffy coats and cultured in RPMI medium (72400-021, ThermoFisher) supplemented with 10% Fetal Bovine Serum. HEK and HDF (5.0×10^4 cells/ml) or PBMCs (5.0×10^6 cells/ml) were seeded into 12-well tissue culture plates. At 60–75% confluence of HEK and HDF cultures, medium containing rhIL-13 (213-ILB, R&D Systems, Bio-techne, Abingdon, UK) was added in presence or absence of a concentration range of tralokinumab or IgG4 isotype control antibody (anti-RSV IgG4, Proteros, Planegg, Germany) pre-incubated for 30 min before addition to the cells. Cells were cultured for 24 h at 37°C in a humidified incubator with 5% CO_2. For assessment of skin barrier markers, keratinocytes were supplemented with 1.2 mM $CaCl_2$ (21115, Merck, Munich, Germany) to induce differentiation. After 72 h in $CaCl_2$-supplemented media, cells were exposed to rhIL-13 in presence or absence of a concentration range of tralokinumab or IgG4 isotype control antibody for 24 h. Cell supernatants were collected and stored at –80°C and cells were harvested for RNA extraction. Biological duplicates were included for all samples within the same experiment.

Gene expression analysis

Total RNA was isolated using QIAsymphony® SP (QIAGEN, Hilden, Germany) and converted to cDNA using the High-Capacity cDNA Reverse Transcription kit (4368813, ThermoFisher). qPCR analysis was performed on Applied Biosystems ViiA7 Real-Time PCR system using TaqMan gene expression assays (ThermoFisher): CCL2-Hs00234140_m1, CCL11-Hs00237013_m1, CCL17-Hs00171074_m1, CCL22-Hs01574247_m1, CCL26-Hs00171146_m1, ELOVL3-Hs00537016_m1, ELOVL6-Hs00225412_m1, FLG-Hs00856927_g1, FLG2-Hs00418578_m1, LOR-Hs01894962_s1, NTRK1-Hs01021011_m1, IL13RA2-Hs00152924_m1, POSTN-Hs01566750_m1, ACTB-Hs01060665_g1, GAPDH- Hs99999905_m1, RPLP0-Hs99999902_m1, B2M- Hs99999907_m1, PPIA- Hs99999904_m1, TBP- Hs99999910_m1. Normalization constants were generated from 3 reference genes. Relative quantification of fold-changes in mRNA expression were calculated as 2^{ddCt}.

Protein analysis

A human CCL-2/MCP-1 Quantikine® ELISA kit (DCP00, R&D Systems) was used for determination of CCL-2 in cell culture supernatants. A U-PLEX MSD assay (K15067L-1, Meso Scale Discovery, Rockville, MD, USA) was used for detection of CCL-17/TARC and CCL- 22/MDC in cell culture supernatants.

Data analysis for IC_{50} determination

Dose-response curves were generated in GraphPad Prism 8 using a non-linear regression fit of antibody concentration vs response to a 4-parameter logistic equation. Where applicable, geometric means +95% confidence intervals (95% CI) were calculated from 3 individual experiments.