Appendix S1

MATERIALS AND METHODS

Evaluation of skin condition and dermatitis

Before IMQ application, transepidermal water loss (TEWL) and stratum corneum (SC) hydration were evaluated daily in each mouse using a Tewameter TM300 and a Corneometer CM825 (Courage and Khazawa, Cologne, Germany), respectively (room temperature; 25.1 ± 0.6°C, relative humidity; 43.4 ± 1.3 %). The severity of psoriasis-like dorsal skin lesions was assessed according to 4 symptoms: erythema/haemorrhage, scarring/dryness, oedema, and excoriation/erosion. Each symptom was graded from 0 to 3 (none, 0; mild, 1; moderate, 2; severe, 3). Dermatitis score was defined as the sum of the individual scores, with total scores ranging from 0 to 12.

Histological analyses

Twenty-four hours after the fifth application of IMQ cream, the mice were anaesthetized with somnopentyl, and skin samples were collected. The samples were fixed in 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (pH 7.4) for 4 h at 4°C. After washing with phosphate-buffered saline (PBS, pH 7.4), small pieces of skin were immersed in PBS containing 20% sucrose overnight at 4°C. These samples were embedded in O.C.T. compound and frozen on dry ice. Cryosections (20 μm thick for PGP9.5 staining and 6 μm thick for haematoxylin-eosin (HE) staining) were cut using a CM1850 cryostat (Leica Biosystems, Wetzlar, Germany) and mounted onto MAS-GP type A coated glass slides (Matsunami Glass, Osaka, Japan). After blocking in PBS containing 5% normal donkey serum (NDS), 2% BSA, and 0.2% Triton X-100, the cryosections on the glass slides were incubated with rabbit anti-PGP9.5 antibody (1:500 dilution; Enzo Life Sciences, Farmingdale, NY, USA) overnight at 4°C. The sections were washed with PBS containing 0.05% Tween 20 (PBS-T) and incubated with AlexaFluor 488-conjugated donkey anti-rabbit IgG (1:300 dilution; Thermo Fisher Scientific, Franklin, MA, USA) for 1 h at room temperature. The sections were washed again with PBS-T and mounted in Vectashield® mounting medium with DAPI (Vector Laboratories, Burlingame, CA, USA). In histological analyses, cryosections 6 μm thick were stained with haematoxylin and eosin solution.

Semi-quantification of epidermal nerve fibres

Three skin specimens from each mouse were incubated with anti-PGP9.5 antibody. Using a confocal microscope, optical sections 0.9 μm thick were scanned through the z-plane of the stained specimens (thickness 20 μm), and the images were reconstructed in 3 dimensions using Leica Confocal Software (Leica Microsystems). The numbers of epidermal nerves were determined by analysing at least 6 confocal images from each mouse. The numbers of epidermal nerve fibres in areas measuring 1.6×10^5 μm^2 were hand-counted in the images. Number of intraepidermal PGP9.5$^+$ fibres (Fig. S3b[1]) indicates the number present within the epidermis. Number of PGP9.5$^+$ fibres penetrating into epidermis (Fig. S3c) indicates the number crossing the epidermis basement membrane. All values are reported as mean ± standard deviation (SD).

Semiquantitative measurements of epidermal thickness

Epidermal thickness was measured in 9 random fields (1.0×10^5 μm^2) per mouse using BZ-H2A software (Keyence, Osaka, Japan). All values represent the mean ± SD.

Quantitative RT-PCR (qRT-PCR) analysis

Dorsal skin samples collected from each mouse under somnopentyl anaesthesia were collected 24 h after the fifth daily application of IMQ cream. For quantitative RT-PCR (qRT-PCR) analysis, total RNA was isolated from skin samples using RNeasy Fibrous Tissue Mini kits (Qiagen KK, Tokyo, Japan), according to the manufacturer's instructions. The expression of mRNAs encoding IL-17A, IL-17F and IL-23p19 was assessed by qRT-PCR using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocols. PCR primer sequences were: IL-17A, 5'-GAA GGC CCT CAG ACT ACC TCA A-3' (forward) and 5'-TCA TGT GGT GGT CCA GCT TTC-3' (reverse); IL-17F, 5'-TGT CCC ACG TGA ATT CCA GA-3' (forward) and, 5'-CAT TGA TGC AGC CTG AGT GTC-3' (reverse); IL-23p19, 5'-ACA TGC ACC AGC GGG ACA TA-3', (forward) and 5'-CTT TGA AGA TGT CAG AGT CAA GCA G-3' (reverse); and RPS18, 5'-TTT GCG AGT ACT CAA CAC CAA CAT C-3' (forward) and 5'-GAG CAT ATC TTC GGC CCA CAC-3' (reverse). The levels of gene expression were calculated relative to expression of mRNA encoding ribosomal protein S18 (RPS18).

Evaluation of H_1 receptor antagonist in histamine-evoked scratching behaviour

Mice were anaesthetized with sevoflurane, and the rostral part of the back was clipped with an electric shaver. After 3 days, mice were orally administered 3 mg/kg bepotastine besilate (Wako Pure Chemical Industries), followed 1 h later by an intradermal injection of 10 μl of 27.1 mM histamine dihydrochloride (Sigma-Aldrich) or vehicle (saline; Otsuka Pharmaceutical Factory, Tokushima, Japan)). Scratching behaviour was monitored for 1 h using a SCLABA®-Real system (Noveltec).