SUPPLEMENTARY MATERIALS

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Immunohistochemical staining and analysis

Skin samples were formalin-fixed and paraffin-embedded. Samples were haematoxylin and eosin-stained and immunostained for Foxp3 with rabbit polyclonal antibodies against Foxp3 (NB100-39002, Novus biologicals, CO, USA) at 1:1000 dilution after antigen retrieving treatment by autoclave. Samples were also immunostained for Ki-67 and keratin 6 with Rabbit monoclonal [SP6]to Ki67 (ab 16667, Abcam, Cambridge, USA) and Rabbit monoclonal [EPR1603Y] to Cytokeratin 6 (ab 52620, Abcam), respectively after antigen retrieving treatment by microwave. Samples were immunostained with rabbit polyclonal antibodies against loricrin and involucrin (Covance Inc. USA) at 1:3000 dilution and Rabbit polyclonal antibody Simple stain mouse MAX-PO(R) (Nichirei, Tokyo, Japan) was used as the secondary antibody, which were visualized using the HPR (DAB) kit (Dako, Denmark). Samples were also immunostained with IL-10 rabbit polyclonal antibody (Proteintech Group Inc. Rosemont, USA) at 1:1000 dilution, which were visualized by Goat Anti-Rabbit IgG H&L(Alexa Flor488) preadsorbed (ab15008, Abcam) using Olympus BX51 microscope with cellSens Standard 1.6 image software (Olympus, Tokyo, Japan) and a fluorescence microscope (BZ-X810; KEYENCE, Japan). Thickness of the epidermis was evaluated in haematoxylin and eosin-stained sections at ten points of whole skin harvested using an Olympus BX51 microscope with cellSens Standard 1.6 image software (Olympus). The number of infiltrating cells in a high-power field (200×) view of the skin taken as a picture was manually counted using iPad. Ten pictures were taken from each skin harvested.

Reverse transcriptase-PCR analysis

Dorsal skin was collected from mice 24 hours after last application of imiquimod using a sterile 5 mm biopsy punch. The dorsal skin was homogenized, and total RNA was isolated using the NucleoSpin® RNA Midi kit (MACHEREY NAGEL, Duren, Germany). Total RNA (2000 ng) was reverse-transcribed using Prime Script RT Master Mix (Perfect Real Time, Takara Bio Inc., Kusatsu, Japan). SYBR® Premix Ex Taq II (Til RNaseH Plus) (Takara Bio Inc.) was used for reverse transcriptase-PCR. The PCR conditions used to amplify all genes were 30 s at 95ºC and 55 cycles for 5 s at 95ºC and 34 s at 60ºC. The forward and reverse primer sequences for IL-10, CCL20, IL-17a, IL-22, IL-23a, Foxp3, IFNγ, and β-actin (control) are mentioned in Supplementary Table S1. Expression analysis was performed with the 7500 SDS v1.4.1 software (Thermo Scientific, Waltham, MA, USA) using the relative standard curve method.

Supplementary Table S1. Sequences of the primers used for quantitative reverse transcriptase-PCR

Gene	Forward	Reverse
IL-10	5′- GCCAGAGCCACATGCTCCTA -3′	5′- GATAAGGCTTGGCAACCCAAGTAA-3′
CCL20	5’ CAGGTGTCCACTCCCAGGTCCAAG 3’	5’ GGCAACTAGAAGGCACAGTCGAGG 3’
IL-17a	5′- GAAGGCCCTCAGACTACCTCAA-3′	5′- TCATGTGGTGGTCCAGCTTTC-3′
IL-22	5′-TCCAGCAGCCATACATCGTC-3′	5′- CTTCCAGGGTGAAGTTGAGCA-3′
IL-23a	5′- ACATGCACCAGCGGGACATA-3′	5′- CTTTGAAGATGTCAGAGTCAAGCAG-3′
Foxp3	5′-AGTGCCTGTGTCCTCAATGGTC-3′	5′-AGGGCCAGCATAGGTGCAAG-3′
IFNγ	5′-CGGCACAGTCATTGAAAGCCTA-3′	5′-GTTGCTGATGGCCTGATTGTC-3′
β-actin	5′-CATCCGTAAAGACCTCTATGCCAA-3′	5′-ATGGAGCCACCGATCCACA-3′

IL: Mus musculus interleukin; IL-17a: Mus musculus interleukin 17A; IL-23a: Mus musculus interleukin 23, alpha subunit p19; Foxp3: Mus musculus forkhead box P3; IFNγ : Mus musculus interferon gamma;

Figure legend for Figure S1

(a) Representative pictures of the psoriasis-like dermatitis in the three groups of BALB/c mice.

(b) Cytokine mRNA expression in the psoriasis-like dermatitis in BALB/c mice.

Skin was harvested from Sham-, OVX, and HRT-mice 24h after the last application of imiquimod. Cytokine mRNA expression in the skin was evaluated by quantitative reverse transcriptase-PCR. Results are expressed as mean ± SD. n=7 for each group. **p<0.01

(c) Number and percentage of Foxp3-positive cells in the psoriasis-like dermatitis of BALB/c mice (×200). Results are expressed as the mean ± standard deviation. N = 6 or 7 for each group. *p < 0.05, ****p < 0.001