Supplementary material has been published as submitted. It has not been copyedited, typeset or checked for scientific content by Acta Dermato-Venereologica

Appendix S1

MATERIALS AND METHODS

Skin biopsies

Skin biopsies were collected from the lesional and non-lesional skin of 10 patients with AD, from the lesional skin of patients with PsV, and from the corresponding skin regions of 10 healthy individuals (**Table I**). Using adequate region-matched healthy controls is essential, as distinct skin regions can be characterized by significantly different immune activity including AMPs (2, 6, 7). Written, informed consent was obtained, according to the Declaration of Helsinki principles and the study was also approved by the local ethics committee of the University of Debrecen, Hungary. One part of the biopsies was stored in RNAlater (Qiagen, Hilden, Germany) at -70°C until RNA isolation for RT-PCR, the other part of the biopsies was formalin-fixed and paraffin-embedded and used for IHC.

RNA Isolation, Reverse Transcription, and Real-Time Quantitative PCR

All samples were homogenized in TriReagent solution (Sigma-Aldrich, Dorset, UK) with Tissue Lyser (QIAGEN) using previously autoclaved metal beads (QIAGEN). Total RNA was isolated from the human skin. The concentrations and purities of the RNA samples were measured using a NanoDrop spectrophotometer (Thermo Scientific, Bioscience, Budapest, Hungary). RNA quality was checked using an Agilent 2100 Bioanalyser (Agilent). RNA was reverse transcribed into complementary DNA using the High Capacity cDNA Archive Kit (Invitrogen, Life Technologies, San Francisco, CA) according to the manufacturer's instructions. Samples were pretreated with DNase I (Applied Biosystems, Foster City, CA, USA). The qRT-PCR measurements were carried out in triplicate using pre-designed FAM-MGB assays as well as TaqMan® Gene Expression Master Mix (Applied Biosystems, Life Technologies). The following oligo sets were used: PPIA (Hs99999904_m1), ADM (Hs00969450_g1), ANG (Hs04195574_sH), CCL20 (Hs00355476_m1), DEFB1 (Hs00608345_m1), DEFB4B (Hs0017547_m1), DEFB103 (Hs04195435_g1), DEFB104 (Hs00175474_m1), LCN2 (Hs01008571_m1), LL-37 (Hs00189038_m1), LYZ (Hs00426232_m1), PI3 (Hs00160066_m1), RNASE7 (Hs00922963_s1), S100A7 (Hs00161488_m1), S100A8 (Hs374264_g1), S100A9 (Hs00610058_m1), SLPI (Hs00268204_m1), and TSLP (Hs00263639_m1. All reactions were performed using a LightCycler® 480 System (Roche). Relative mRNA levels were calculated using either the comparative Ct method or based on standard curves, and normalized to the expression of PPIA mRNA.

Immunohistochemistry

For IHC analyses, freshly prepared paraffin-embedded sections from AD and PsV patients and healthy control skins were used. After deparaffinizing and rehydrating the samples, endogenous peroxidase activity was eliminated by treating samples with 3% H_2O_2 for 15 minutes. Subsequently, heat-induced antigen retrieval was performed. After blocking in 1% bovine serum albumin (BSA) solution, sections were incubated with primary antibodies overnight at 4°C. The following primary antibodies were used: S100A8 (rabbit polyclonal IgG [HPA024372]: Sigma-Aldrich), human lipocalin/NGAL (rabbit polyclonal IgG [PA5-32476]: Invitrogen), Anti-beta 2 Defensin (rabbit, polyclonal IgG [ab63982]: Abcam), human RNase7 (mouse, monoclonal IgG [MA5-24591]: Invitrogen), Adrenomedullin (mouse monoclonal, [MA524772]: Fisher scientific), human CCL20/MIP-3-a (rabbit polyclonal IgG [ab9829] Abcam), Anti-beta Defensin 4 (rabbit polyclonal IgG [PA567558]: Invitrogen), Anti-beta Defensin 1 (rabbit, polyclonal IgG [ab203307]: Abcam), and Cathelicidin (rabbit, polyclonal IgG [PA5110987]: Fisher scientific).

Subsequently, anti-mouse/rabbit (Dako) HRP-conjugated secondary antibodies were employed. Before and after incubating with antibodies, samples were washed 3 times with TBST for 5 minutes each. Signals were detected with the Vector® VIP and ImmPACT™ NovaRED™ Kit (VECTOR Laboratories, Burlingame, CA, USA). Background staining was performed with methylene green. The detection of each protein was carried out on all sections in parallel at the same time to evaluate comparable protein levels. Positive, Ig, and isotype controls were also used to normalize staining against all proteins. The sections were digitized using Whole Slide Imaging technology and the Pannoramic Viewer (3DHistech, Budapest, Hungary) software was used for the evaluation of the degree of staining.

Statistical analysis

Statistical significance was determined by one-way analysis of variance and Newman-Keuls post hoc tests. Graphs show the means and the corresponding 95% confidence intervals (boxes) and max/min values of protein levels measured by Panorammic Viewer. Statistical analyses were performed using GraphPad Prism v6 (GraphPad Software Inc., San Diego, CA, USA).