Appendix S1

MATERIALS AND METHODS

Host E. coli *strain and plasmid*

The host *E. coli* used was *E. coli* XL1-blue (Stratagene, La Jolla, CA, USA) and BL21 (DE3) (Novagen, Darmstadt, Germany). For DCD protein expression, decahistidine-tagged ubiquitin fusion system was used (26). Plasmid pTKK19-ubinew (2,557 bp) for protein expression and plasmid pYUH20b for yeast ubiquitin hydrolase (YUH) expression were provided by Dr T. Kohno at Mitsubishi Kasei Institute of Life Sciences, Tokyo, Japan. For cloning the DCD gene, plasmids pGEM® -T Easy (Promega, Madison, WI, USA) and pET32a (+) (Novagen) were used.

Strain for antimicrobial activity evaluation and culture method

E. coli ATCC25922 strain and *S. aureus* ATCC25923 strain were provided by Dr R. Okamoto of the Department of Microbiology, Kitasato University School of Medicine, Kitasato, Japan. *P. acnes* was provided by Dr M. Mitsuyama of the Department of Microbiology, Graduate School of Medicine, Kyoto University, Kyoto, Japan. *E. coli* (ATCC25922) was incubated while shaking at 37°C for 16 h in 10 ml Columbia broth (Becton Dickinson, Franklin Lakes, NJ, USA), and then harvested by centrifugation. *S. aureus* (ATCC25923) was incubated overnight at 37°C in 10 ml Columbia broth, and then 1/10 of the broth was inoculated into 10 ml fresh Columbia broth for further incubation while shaking at 37°C for 3 h. It was then harvested by centrifugation. *P. acnes* was cultured anaerobically in modified Gifu anaerobic medium (GAM) agar plate (Nissui Pharmaceutical, Tokyo, Japan) at 37°C for 4 days using Anaero Pack® Kenki (Mitsubishi Gas Chemical, Tokyo, Japan).

Antibodies

Anti-DCD 10C3 mAb, prepared in our laboratory using 6×histidine fusion DCD as the immunogen, is an IgG antibody that recognizes the C-terminus of DCD.

Cloning of human DCD gene

Gene region (273 bp), corresponding to 91 amino acids of human DCD gene, excluding the signal sequence, was amplified by PCR. rDCD, having the same N-terminus sequence as the amino acid sequence of human DCD, was obtained using the method reported by Kohno et al. (26). The base sequence of the plasmid was verified using a sequencer (Genetic Analyzer Model 3130, Applied Biosystems, Foster City, CA, USA).

Yeast ubiquitin hydrolase purification

Recombinant yeast ubiquitin hydrolase (rYUH) was expressed and purified using the method reported by Kohno et al. (26). The protein concentration was determined using Lowry method (DC Protein Assay, Bio-Rad, Richmond, CA, USA).

Expression and purification of rDCD

The *E. coli* BL21 (DE3) strain harbouring the DCD expression plasmid was incubated overnight in 60 ml superbroth (1.5% tryptone, 2.5% yeast extract, 0.5% NaCl, 1% D-glucose) containing 50 µg/ml kanamycin sulphate (Km), then inoculated into a 6 l Km-superbroth. When the OD600 reached 0.6, 1 mM isopropyl-beta-D-thiogalactopyranoside (IPTG) was appended, and incubated for 3 h. The cells were harvested and washed with buffer A (50 mM Tris-HCl pH 8.0, 100 mM NaCl, 1 mM β-mercaptoethanol). BugBuster protein extraction reagent, with Benzonase nuclease (Sigma-Aldrich, St Louis, MO, USA) and protease inhibitor cocktail set III (Merck KGaA, Darmstadt, Germany), was used for cell lysis. TALON® sepharose (Takara Bio, Shiga, Japan) was used to bind to rHis-ubiquitin-DCD in the cell supernatant. Following wash steps with buffer A containing 500 mM NaCl and buffer A, rHis-ubiquitin-DCD was eluted with 100 mM imidazole-appended buffer A. The eluent was dialysed (MWCO: 8000) against buffer A. rYUH was reacted with the fusion protein to cleave the 10×His tag using the method reported by Kohno et al. (26). The 10×His and rYUH in the mixture were removed with Ni sepharose to obtain purified rDCD. This rDCD was dialysed (MWCO: 5,000) against 10 mM sodium phosphate buffer (pH 7.4) for use in the antimicrobial activity test. The protein concentration was determined by Lowry method, and rDCD molecular weight was analysed using Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS, Applied Biosystems).

Western blot analysis

Purified rDCD was electrophoresed on a 15% sodium dodecyl sulphate (SDS) acrylamide gel using the Laemmli method under reducing conditions, and then transferred to a nitrocellulose membrane by electroblotting. The nitrocellulose membrane was blocked for 2 h at room temperature with 0.1% bovine serum albumin (BSA) (fraction V, Boehringer, Ingelheim, Germany) appended with Tris Buffered Saline with Tween 20 (TBST) (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20) solution. The nitrocellulose membrane was incubated for 1 h with 10C3 mAb diluted to 0.5 mg/ml (final concentration) with 0.1% BSA appended TBST solution as the primary antibody. After washing with 0.1% BSA appended TBST solution, the blot was incubated with alkaline phosphatase-labelled anti-mouse IgG antibody (Cappel Laboratories, Cochranville, PA, USA), as the secondary antibody, diluted 2,000 times with a 0.1% BSA-appended TBST solution for 30 min. Then, following a similar protocol to that used with the primary antibody, the blot was stained with 5-bromo-4-chloro-3-indolyl-phosphate (Moss, Pasadena, MD, USA).

Antimicrobial assay of rDCD against E. coli*,* S. aureus *and* P. acnes

The antimicrobial activity of rDCD was determined via a colony-forming unit (CFU) assay. Harvested *E. coli*, *S. aureus* and *P. acnes* were washed twice by centrifugation using saline solution, adjusted to 5×10^4 CFU/ml for *E. coli* and *S. aureus* or 2×10^6 CFU/ml for P. acnes, with 10 mM sodium phosphate buffer. Accurate adjusted bacterial counts were determined by applying a 10-fold diluted solution to the medium each time. Antimicrobial activity against *E.coli* and *S. aureus* were determined by reacting 50 µg/ml rDCD (final concentration) with *E. coli* and *S. aureus* adjusted to 2.5×10^4 CFU/ml (final concentration) at 37°C for 3 h in 10 mM sodium phosphate buffer, followed by diluting the mixture 10-fold with saline solution (0.85% NaC1). Diluted solutions of different concentrations were applied to Columbia agar medium, and incubated at 37°C for 16 h. After incubation, colonies were counted. The antimicrobial activity of 2 different rDCD concentrations against *P. acnes* were studied, as follows: 50 µg/ml and 270 µg/ml rDCD were incubated with 1×10^6 CFU/ml *P. acnes* in 10 mM sodium phosphate buffer, at 37°C for 4 h, and then diluted 10-fold with saline solution. Diluted solutions were applied to

modified Gifu anaerobic medium agar (GAM). After being incubated anaerobically at 37°C for 3 days, the colonies were counted. Antimicrobial activity is expressed as killing rate, thus: [1 – (number of colonies in the presence of DCD)/(number of colonies in the absence of DCD)]×100].

Collection of sweat samples

In accordance with the principles of the Declaration of Helsinki, the study was approval by the ethics committee of Kitasato University. Signed informed consent was obtained from all research participants. Fifteen patients with acne vulgaris, with a history of inflammatory acne before high-school age, and mild-to-medium levels of inflammatory acne at the time of sweat sampling (Table SI) were evaluated. Acne severity was evaluated based on the classification of acne vulgaris severity set out by the Japanese Dermatological Association (27). The history of inflammatory lesions was determined based on self-reporting and the presence/absence of scarring lesions. Samples were collected from 14 healthy Japanese volunteers with no history of inflammatory acne before high-school age and no current acne. None of the participants had underlying disease. A 100 ml sample of freshly secreted sweat was collected from the back of each of the subjects, during bicycle ergometer exercise, within 5 min of the subjects starting to sweat.

DCD concentrations in sweat samples

The concentration of DCD peptides in sweat samples was determined by solid-phase enzyme-linked immunosorbent assay (ELISA). The wells of microtitre plates were coated with native sweat diluted to 1/100 with phosphate-buffered saline (PBS). 10C3 mAb was used as the primary antibody. Horse-radish peroxidase-conjugated goat anti-mouse IgG antibody was used as the secondary antibody. After staining with O-phenylene diamine (WAKO, Osaka, Japan), the colours were analysed with the Spectra Fluor Plus (TECAN, Männedorf, Switzerland) at 492 nm. High-performance liquid chromatography purified synthetic DCD-1L (N-terminally truncated DCD) peptide served in the assays as a standard for quantification of the concentration of DCD peptide in each of the sweat samples. The total protein concentration in human sweat was determined by bicinchoninic acid test with Protein Assay Reagent (Thermo Scientific, Waltham, MA, USA).

Statistical analysis

Statistically significant differences between patient groups and controls were found in the concentration of DCD peptides in sweat samples calculated using the Mann-Whitney *U* test. Level of significance was set at $p < 0.05$. JMP® 10 software (SAS Institute Inc., Cary, NC, USA) was used for all analyses.