SUPPLEMENTARY INFORMATION

MATERIALS AND METHODS

P. acnes culture

A single colony of P. acnes bacteria was inoculated in Reinforced Clostridium Medium (Oxford, Hampshire, England) and cultured at 37°C under anaerobic conditions until logarithmic growth phase. Bacterial pellets were harvested by centrifugation at 5,000 g for 10 min washed with PBS, and suspended in PBS.

P. acnes (266; 1-1a, ST18) and Δcamp2, (PPA0687) were obtained from Professor Holger Brüggemann at Max Planck Institute for Infection Biology, Department of Molecular Biology, D-10117 Berlin, Germany. All P. acnes strains were cultured on Brucella broth agar plates supplemented with 5% (v/v) vitamin K (Remel, KS, USA) and hemin (Remel) under anaerobic conditions using a Gas-Pak (BD Biosciences, CA, USA) at 37°C.

Mass spectrometric analysis

An ICPL kit (SERVA Electrophoresis, Heidelberg, Germany) was used for isotopic labeling of proteins. Lysates (50 µg) of P. acnes (ATCC 6919) from aerobic and anaerobic growth were labeled with light (12C6) and heavy (13C6) forms of Nic-NHS, respectively at room temperature for 2 h. The protein
concentration was measured by using a BCA Protein Assay Kit (Pierce, IL, USA). The disulfide bonds were reduced by tris-2-carboxyethyl phosphine and alkylated by iodoacetamide. All lysine side chains of proteins in lysates were modified by nicotinoylation selectively. After mixing $^{12}$C$_6$-Nic-NHS- with $^{13}$C$_6$-Nic-NHS-labeled samples, the mixture was enzymatically digested with trypsin (0.2 µg, 25 mM Tris, 4 M urea, pH 7.8) at 37°C overnight. Tryptic peptide digests were subjected to a LC-LTQ mass spectrometer (Thermo Scientific, MA, USA) for protein identification and quantification. Samples from three separate experiments were used for ICPL labeling and quantitation. One representative of LC-LTQ mass spectra was illustrated.

**Database searching and spectrum analysis**

Large-scale MS/MS spectra were extracted using default value by BioTools 3.1 (Bruker Daltoniks, MA, USA) and analyzed by an in-house MASCOT 2.1 server (Matrix Science, London, UK) for protein identification. WarpLC 1.1 software (Bruker Daltoniks) was applied for quantifying the peak area of individual peptide peak and the ratios of related peaks. MASCOT was used to search the *P. acnes* databases downloaded from National Center for Biotechnology Information (NCBI) website http://www.ncbi.nlm.nih.gov/ which contains protein
sequences in both forward and reverse orientations. The relative abundance of each identified protein in lysates of *P. acnes* from aerobic and anaerobic growth was calculated by quantifying the areas under the curves in the elution profiles for each of the two peptides. The proteins with ratios > 1.5-fold or < 0.6-fold were preferred as a set of proteins that expressed differentially in two groups (aerobic vs anaerobic growth) of samples. The labels of $^{12}$C$_6$-Nic-NHS with $^{13}$C$_6$-Nic-NHS introduced a mass difference of 6.0 Da per labeled site in mass spectra. CAMP factor with double charges and 3.0 Da mass differences was detected.

**Injection of *P. acnes* into mouse ear**

A 6 to 8-week-old female ICR mouse strain (River Lab, CA, USA) was used in all animal experiments. The ears of ICR mice were injected intradermally with wild-type *P. acnes* (266; 1-1a, ST18) bacteria or a CAMP factor 2 mutant ($\Delta$camp2) *P. acnes* strain ($10^7$ CFU in 20 μl PBS) using a 28-gauge needle. Injection of PBS (20 μl) served as a control. Three days after injection, ears were excised and homogenized for cytokine detection and bacterial counts. All experiments using mice were conducted in a biosafety level 2 (BSL-2) facility and in accordance with institutional guidelines for animal experiments.
ELISA

The levels of MIP-2 in the supernatants of ear homogenates and IL-8 or IL-1β in the supernatants of homogenates of *ex vivo* explants were measured by ELISA kits according to the manufacturer’s protocol (R&D System, MN, USA).

Bacterial counts

The excised mouse ears were homogenized in 200 μl of sterile PBS by a tissue grinder. Bacterial CFUs in the mouse ears were enumerated by plating serial dilutions (1:10<sup>1</sup>-1:10<sup>5</sup>) of the homogenates on Brucella broth agar plates (BD Biosciences) supplemented with 5 % (v/v) defibrinated sheep blood (LAMPIRE Biological Laboratories, Pipersville, PA, USA), 5 mg/ml vitamin K (Remel) and 50 mg/ml hemin (Remel). The plates were incubated for 3 days at 37°C under anaerobic conditions using a Gas-Pak (BD Biosciences) to count colonies. The CFUs per gram of excised ears were calculated.

Expression of recombinant CAMP factor and vaccination

The *P. acnes* CAMP factor (accession number: WP_002518322) or GFP was expressed in a [(*E. coli*), BL21 (DE3)] competent cells (Invitrogen, CA, USA). After induction of competent cells with 1 mM isopropyl-β-D-thiogalactoside
(IPTG) (Sigma-Aldrich, MO, USA) for 4 h. The recombinant CAMP factor or GFP was purified by using a column with 2 ml Ni-NTA agarose (QIAGEN, CA, USA).

The ICR mice were intranasally vaccinated with UV-inactivated $10^7$ CFU *E. coli* over-expressing CAMP factor or GFP as previously described in our publication (Nakatsuji *et al*., 2011). For intradermal vaccination, mice were intradermally injected recombinant CAMP factor or GFP. Recombinant CAMP factor or GFP was dissolved in PBS and mixed with an equal volume of 2% aluminum hydrogel gel (alhydrogel; InvivoGen Inc., CA, USA) adjuvant. For the first vaccination, 10, 20 or 50 µg of recombinant protein with or without alhydrogel in a total of 200 µl was injected subcutaneously into the dorsal skin of mouse neck. Two weeks later, the same amount of recombinant protein with or without alhydrogel was subcutaneously injected for second boost. One week after the second boost, serum containing immunoglobulin G (IgG) antibody was harvested for measurement of antibody titers. Three weeks after the second boost, live *P. acnes* ($10^7$ CFU/20 µl in PBS) bacteria were injected intradermally into the right ears of vaccinated mice. The same volume of PBS was injected into left ears of the same mice. Ear thickness was measured using a micro caliper (Mitutoyo, Aurora, IL). Three days after bacterial injection, ears were excised and homogenized for bacterial counts and MIP-2 quantification by
ELISA as described above. Three independent experiments were conducted using five mice per group.

Quantification of titers of antibodies to *P. acnes* CAMP factor

Recombinant CAMP factor or GFP (0.1 μg/well) diluted in 50 μl PBS was coated onto a 96-well microplate for 4°C overnight. After blocking in PBS with 0.05% Tween 20 and 1% bovine serum albumin (BSA) at room temperature for 1 h, diluted sera from humans or mice were added to the wells and incubated for 2 h. A goat anti-human or -mouse IgG (H+L) IgG-horseradish peroxidase (HRP) conjugate (Promega, WI, USA) (1:10,000 dilution) was added and incubated for 1 h. HRP activity was determined with an OptEIA™ Reagent Set (BD Biosciences). The OD of each well was measured at 450 nm subtracted from 570 nm (OD\_570-450).

In vitro neutralization

A murine macrophage cell line, RAW264.7 (ATCC, VA, USA), was cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS). Complements in the sera were inactivated by heating at 56°C for 30 min. *P. acnes* (ATCC 6919) was pre-
treated with 2.5 % (v/v) different titers of inactivated sera obtained from healthy
subjects, acne patients, and mice vaccinated with UV-inactivated *E. coli* over-
expressing CAMP factor or GFP in the PBS at 37°C for 2 h. For neutralization
of *P. acnes* cytotoxicity, RAW264.7 cells (2×10^5 /well) were incubated with *P.
acnes* (2×10^6 CFU/well) along with different titers of inactivated sera in RPMI
media containing 1% FBS for 18 h. After incubation, cell viability was
determined by an ACP assay (Martin and Clynes, 1991). The cytotoxicity of *P.
acnes* was calculated as the percentage of cell death caused by Triton X-100
(0.1%, v/v) (Nakatsuji *et al*., 2008a).

Production of mAb to CAMP factor

The mAb to *P. acnes* CAMP factor was produced in the GenScript Biotech
Corporation (NJ, USA). In brief, the spleen was removed from mice immunized
with recombinant *P. acnes* CAMP factor. Splenocytes were collected and fused
with a mouse myeloma cell line SP2/0-Ag14 to form hybridomas. The
hybridomas was injected into mice to produce the ascites. The mAb to CAMP
factor the ascites was purified using protein A/G as binding ligands (Thermo
Scientific) as previously described. The specificity of mAb to *P. acnes* CAMP
factor was examined by western blotting using recombinant CAMP factor.
**Immunohistochemical staining**

Non-lesional skin and lesions of patients with acne vulgaris were embedded in Tissue-Tek O.C.T. (Sakura Finetek, Netherlands), and subjected to Haemotoxylin and Eosin (H&E) or immunohistochemical staining using mAb to *P. acnes* CAMP factor. Acne biopsies or tissue sections were visualized with the Bx51 microscope (Olympus, NY, USA) in conjunction with X-Cite 120 fluorescence illumination systems (EXFO, Quebec, Canada).

**RT-qPCR analyses**

To determine the mRNA expression levels of *P. acnes* CAMP factor and Th1/Th2/Th17-related pro-inflammatory cytokines (IFNγ, TNFβ, IL-8, IL-4, IL-9, IL-10, IL-13, IL-1α, IL-1β, IL-17A and TGF-β1), total cellular RNA was extracted from samples using the RNeasy Mini Kit (QIAGEN). The RNA was reverse transcribed to cDNA using an iScript cDNA synthesis kit (Bio-Rad, CA, USA) and amplified by RT-qPCR on the CFX96 real time system (Bio-Rad). The method of comparative delta-delta cycle threshold (ΔΔCT) was used to quantify the gene expression which was normalized to the expression level of GAPDH or 16S rRNA of *P. acnes*. 
**Ex vivo acne model**

The biopsies (4 x 4 x 8 mm) of non-lesional skins were collected from healthy subjects and acne patients aged 18-42 years. The lesions of patients with acne vulgaris within two to fourteen days of onset of inflammatory papules were obtained by punch biopsies from back skins. Non-lesional skins were collected at least 5 cm away from lesional skins in acne patients. To establish the ex vivo acne model, acne lesions and non-lesional explants were cut in half, and half was incubated with 5 µg mouse mAb (IgG1) to *P. acnes* CAMP factor for 24 h in antibiotic-free the Epilife keratinocyte media (Invitrogen) at 37°C. The other half was incubated with mouse mAb (IgG1) HBsAg (MyBiosource Inc., CA, USA) as a control. The levels of IL-8 or IL-1β in the supernatants of homogenates of ex vivo explants were measured by ELISA kits. The mRNA expression levels of *P. acnes* CAMP factor and Th1/Th2/Th17-related pro-inflammatory cytokines were determined by RT-qPCR analysis. The production of mAb to CAMP factor and RT-qPCR analysis were described above.

**3D culture of sebocytes**

The Matrigel (BD Biosciences) (100 µl) was placed onto 1 cm diameter glass-bottom for 20 min on ice. The immortalized human sebaceous gland cell line
SZ95 (10^5 cells) in 400 μl Dulbecco’s Modified Eagle’s Medium (DMEM) containing epidermal growth factor (EGF) and 2% Matrigel were added to the Matrigel layer as previously described (Yoshida et al., 2013). The media were replaced twice a week. Two weeks after culture, SZ95 sebocytes grown in a 3D model were incubated with *P. acnes* (ATCC 6919; 10^7 CFU) along with 5 μg mouse mAb (IgG1) to *P. acnes* CAMP factor or HBsAg for 24 h. The levels of IL-6 in the culture media were measured by ELISA kits.

**Statistics**

Experiments were repeated at least three times with similar results. Statistical significance was determined by Student’s unpaired two-tailed *t*-test, as indicated in the legend (*p* < 0.05, **p** < 0.01, ***p*** < 0.001, ****p*** < 0.0001).
Supplementary figure 1. The protective immunity in mice vaccinated with lower concentrations of recombinant CAMP factor.

ICR mice were subcutaneously vaccinated with 20 (a, b) or 10 (c, d) μg recombinant CAMP factor or GFP with 2% aluminum (alum) adjuvant according to a protocol as described in section of Materials and Methods. The levels of pro-inflammatory MIP-2 cytokines (a, c) and bacterial colonization (CFUs) (b, d) in the ear injected with *P. acnes-* or PBS were quantified by ELISA and plating serial dilutions on an agar plate, respectively. Error bars represent mean ± SD of five mice. *P < 0.05, **P < 0.01 and ***P < 0.001 were assessed by using Student’s t-test.
Supplementary figure 2. Images of non-lesional and lesional skins on the back of an acne patient.

The pictures of non-lesional (NAL) and lesional (AL) skins (seven days of onset of inflammatory papules) were taken from a male acne patient aged 22 years old without prior treatment. The non-lesional skin was collected 5 cm away from lesional skin. Scale bar = 5 mm.
Supplementary figure 3.

**Supplementary figure 3.** Reduction of IL-6 in acne lesions by mAb to *P. acnes* CAMP factor.

Human skin punch biopsies were obtained from non-lesional (NAL) (n=7) and acne lesional (AL) (n=10) skin in patients with acne vulgaris. Each skin biopsy was cut in half, and half was incubated with mAb (IgG1) to *P. acnes* CAMP factor (CAMP factor) for 24 h in antibiotic-free the Epilife keratinocyte media. The other half was incubated with HBsAg mAb as a control (C). The levels of IL-6 in skins after mAb incubation were measured by ELISA. *P <0.05, by Student’s *t*-test. n.s. = not significant.
Supplementary figure 4. Suppression of *P. acnes*-induced IL-6 secretion from sebocytes grown in a 3D model by mAb to *P. acnes* CAMP factor.

(a) Phase-contrast microscopy of SZ95 sebocyte cells after 14 days in a 3D culture. Scale bar = 100 µm. (b) *P. acnes* (ATCC 6919; 10^7 CFU) bacteria with mAb to *P. acnes* CAMP factor (CAMP factor) or HBsAg (C) were added into the 3D cultures of sebocyte cells for 24 h. The amounts of IL-6 in the culture media were quantified by ELISA. *P* < 0.05, by Student’s *t*-test.
Supplementary table 1. Differential expression of *P. acnes* proteins under aerobic vs anaerobic conditions of bacterial growth.

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* Proteins expressed differentially with ratios > 1.5-fold or < 0.6-fold under aerobic (+O<sub>2</sub>) vs anaerobic (-O<sub>2</sub>) conditions of *P. acnes* growth via quantification using ICPL in conjunction with nanoLC-LTQ analysis.
REFERENCES


