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[Continued on next page]

(54) Title: METHODS AND COMPOSITIONS FOR TREATING P.ACNES

(57) Abstract: The disclosure provides an antigenic composition useful for immunization against *P. acnes, K. pneumoniae, S. aureus*, or *Streptococcus pyogenes*. The disclosure provides a method for producing a vaccine for preventing infection and screening agents useful for preventing infection.

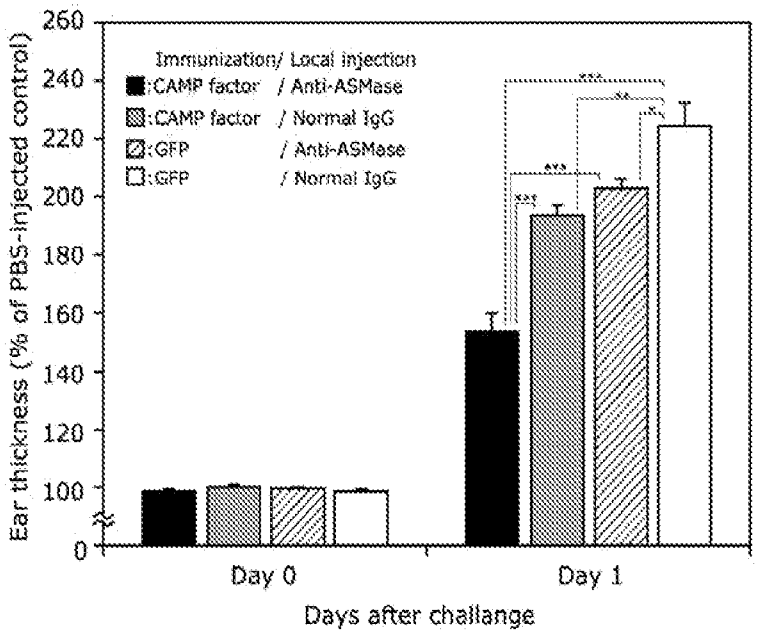

FIGURE 14

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METHODS AND COMPOSITIONS FOR TREATING *P.ACNES*

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. Provisional Application Serial No. 61/120,221, filed December 5, 2008, which is incorporated herein by reference.

TECHNICAL FIELD

[0002] The disclosure relates generally to an antigenic composition useful for immunization against *P. acnes*. The disclosure is a method for producing a vaccine for preventing *P. acnes* associated diseases and disorders including rosceacea in humans and animals, a vaccine against *P. acnes* in humans and animals, and an approach to producing vaccines against *P. acnes*.

BACKGROUND

[0003] As a member of the resident human microflora, the Gram-positive anaerobic coryneform bacterium *Propionibacterium acnes* (*P. acnes*) is found predominantly in the sebaceous gland of the skin. It can, however, also be isolated from the conjunctiva, the external ear canal, the mouth, the upper respiratory tract and, in some individuals, the intestine. *P. acnes* has an estimated skin density of 10^2 to 10^{5-6} cm^{-2}. *P. acnes* is a well-recognized opportunistic pathogen, especially in relation to medical implants such as central nervous system shunts, silicone implants and prosthetic hip joints. It is also responsible for ocular and periocular infections and endophthalmitis and has been implicated in periodontal and dental infections. Dental probing and treatment has lead to the dissemination of *P. acnes* in the bloodstream, which is a recognized cause of endocarditis in relation to damaged or prosthetic heart valves. *P. acnes* also plays a role in inflammatory acne, since antimicrobial therapy directed against *P. acnes* results in improvement, while the development of antibiotic resistance in *P. acnes* is associated with relapse. The common form of acne, known as acne vulgaris, affects up to 80% of the population at some time in their lives, making it the most common skin infection. There is

also a strong association between severe forms of acne and joint pain, inflammation of the bone (osteitis) and arthritis. In patients suffering from this condition, known as SAPHO (synovitis, acne, pustulosis, hyperostosis and osteitis) syndrome, isolates of *P. acnes* have been recovered from bone biopsy samples, as well as synovial fluid and tissue.

[0004] Two distinct phenotypes of *P. acnes*, types I and II, have been identified based on serological agglutination tests and cell-wall sugar analysis. Recently, recA-based sequence analysis has revealed that *P. acnes* types I and II represent phylogenetically distinct groups (McDowell *et al.*, 2005).

[0005] *P. acnes* produces a co-haemolytic reaction with both sheep and human erythrocytes (Choudhury, 1978) similar to the Christie-Atkins-Munch-Petersen (CAMP) reaction first demonstrated in 1944 (Christie *et al.*, 1944). The CAMP reaction describes the synergistic haemolysis of sheep erythrocytes by the CAMP factor from *Streptococcus agalactiae* and the toxin (sphingomyelinase C) from *Staphylococcus aureus*, with the CAMP factor demonstrating non-enzymic affinity for ceramide (Bernheimer *et al.*, 1979). Examination of sphingomyelinase-treated sheep erythrocytes has revealed the formation of discrete membrane pores by recombinant *Streptococcus agalactiae* CAMP factor (Lang & Palmer, 2003). In addition to the extensive study of the CAMP factor of *Streptococcus agalactiae* (Bernheimer *et al.*, 1979; Brown *et al.*, 1974; Jurgens *et al.*, 1985, 1987; Ruhlmann *et al.*, 1988; Skalka *et al.*, 1980), a number of other Gram-positive and Gram-negative bacteria are known to produce a positive CAMP reaction, including *Pasteurella haemolytica* (Fraser, 1962), *Aeromonas* species (Figura & Guglielmetti, 1987), some *Vibrio* species (Kohler, 1988) and group G streptococci (Soedermanto & Lammler, 1996). Some of these species can also use phospholipase C (α-toxin) from *Clostridium perfringens* or phospholipase D from *Corynebacterium pseudotuberculosis* as a co-factor for haemolysis in addition to the *Staphylococcus aureus* toxin (Frey *et al.*, 1989). The CAMP factor genes of *Actinobacillus pleuropneumoniae* and *Streptococcus uberis* have

been identified, cloned and expressed in *Escherichia coli* (Frey *et al.*, 1989; Jiang *et al.*, 1996).

[0006] The precise role of the CAMP molecule in bacterial virulence remains unclear. It is likely that the co-haemolytic reaction represents a laboratory phenotype, or epiphenomenon, that is convenient for CAMP factor detection, but which may not be directly related to the role of the molecule in colonization and pathogenesis. The CAMP factor from *Streptococcus agalactiae* binds to the Fc region of IgG and IgM molecules, similar to the binding of IgG by *Staphylococcus aureus* protein A (Jurgens *et al.*, 1987), and partial amino acid sequence similarity between the CAMP factor protein of *Streptococcus agalactiae* and *Staphylococcus aureus* protein A has been demonstrated (Ruhlmann *et al.*, 1988).

SUMMARY

[0007] The disclosure provides compositions and methods useful for treating or preventing P. acnes infection. In one embodiment, the methods and compositions comprise an ASMase inhibitor including, for example, small molecule inhibitors or anti-ASMase antibodies. In another embodiment, the composition and methods comprise a vaccine comprising a P. acnes CAMP factor. In yet another embodiment, the methods and compositions comprise an anti-P. acnes CAMP factor antibody. In yet a further embodiment, the methods and compositions comprise a combination of a vaccine, or antibody against CAMP Factor and an ASMase inhibitor or antibody.

[0008] The disclosure also provides an immunogenic composition comprising a substantially purified polypeptide comprising a sequence referred to in Table 1, an immunogenic fragment thereof, and any combination of the foregoing. In one embodiment, a CAMP Factor, lipase, or sailidase polypeptide or fragment thereof is use in the immunogenic composition. In yet another embodiment, a polypeptide comprising SEQ ID NO: 2, 3, 7, 9 or 11 or an immunogenic fragment thereof is used in the preparation of the immunogenic composition. In yet another embodiment, a vector comprising a

polynucleotide encoding a polypeptide of SEQ ID NO: 2, 3, 7, 9 or 11 or an antigenic fragment thereof is expressed in a vector that is administered to a subject. In one embodiment, the vector comprises an attenuated bacterial vector or an attenuated viral vector. In yet another embodiment, the antigen is expressed by a plant or plant cell.

[0009] The disclosure also provides a composition comprising at least one recombinant attenuated bacterial or viral vector comprising at least one polynucleotide encoding one or more *P. acnes* polypeptides selected from the group consisting of a CAMP Factor, a lipase, or a sialidase such that the polypeptide is expressed in the at least one recombinant attenuated vector and an inhibitor of ASMase activity.

[0010] The disclosure also provides a method of inducing protective immunity in a subject comprising administering a composition as described above to the subject and contacting the subject with an ASMase inhibitor.

[0011] The disclosure also provides an immunoprotective composition comprising at least one attenuated vector or plant preparation expressing an antigen useful for inducing an immunoprotective response against *Propionibacterium acnes* (*P. acnes*), said antigen comprising an extracellular or immunogenic protein of *P. acnes* or immunogenic fragment thereof linked to transcriptional promoter and termination signals. In one embodiment, the *P. acnes* protein or fragment thereof is selected from the group consisting of CAMP factor, a lipase, a sialidase, and any combination thereof.

[0012] The disclosure provides a composition useful for treating a *P. acnes* infection comprising an ASMase inhibitor. In yet a further embodiment, a CAMP antigen or vaccine may be used in combination with the ASMase inhibitor. In yet another embodiment, an antigenic composition comprising a disrupted non-infective *P. acnes* cell and further comprising an ASMase inhibitor is used.

[0013] The disclosure provides a method for treating *P. acnes* comprising administering to a subject a vaccine comprising a CAMP factor and a composition comprising an ASMase inihibitor.

BRIEF DESCRIPTION OF THE FIGURES

[0014] Figure 1A-B shows ear inflammation and thickness after injection with *P. acnes* and *S. epidermidis*. Ear inflammation was observed when an ICR mouse was subcutaneously injected with 25 µl of *P. acnes* (10^8 CFU) (A). Injection with 25 µl of PBS into the other ear of the same mouse did not cause visible inflammation. *P. acnes* (10^5 to 10^8 CFU) and *S. epidermidis* (10^8) were subcutaneously injected into mouse skins. Ear thickness was measured everyday for 3 days using Peacock Thickness gauge (B). The ears of two mice per group were measured.

[0015] Figure 2A-F show *P. acnes* induces gramulomatous response and colonizes in the root of hair follicle. H&E staining demonstrated that injection with *P. acnes* (10^8 CFU) into mouse ears for one day increased ear thickness and caused a granulomatouse response (arrowhead) (B). PBS injection serves as a control (A). The area of granulomatouse response was stained with Accustain Gram stain (a gram-positive bacteria staining kit) (Sigma, St. Louis, MO). *P. acnes* (circle; stained in purple) was surrounded by a densely packed granulomatous infiltrate (C) one day after injection. There is no P acnes accumulated in hair follicle (D) one day after injection. However, two days after injection, *P. acnes* (circles and arrows) migrated to hair follicle and colonized in the root follicle (F). Histology of hair follicle from mice injected with PBS for two days were illustrated as a control (E). Bars: 100µm.

[0016] Figure 3A-C shows implantation of tissue chambers and phagocytes in tissue chamber fluids. A tissue chamber (internal and external diameters, 1.5 and 3 mm, respectively, length, 1 cm; internal volume, 80 µl) was subcutaneously implanted into abdominal skin (A) of ICR mice for 7 days before bacteria injection. The tissue chamber consisted of closed ploytetrafluoroethylene Teflon cylinders with 12 regularly spaced 0.1mm holes. Bar: 1 cm. H&E staining showed that mouse tissues wrapped a tissue chamber after 7-day implantation (B). Bar: 1.0 mm. Tissue chamber fluids were drawn by pecutaneous aspiration. After centrifugation,

infiltrated cells (phagocytes) were stained with nucleus dye Hoechst 33258 (C). Arrows indicated phagocytes in tissue chamber fluids. Bar: 5μm.

[0017] Figure 4A-B shows detection of macrophage-inflammatory protein (MIP)-2 concentration and *P. acnes* growth in tissue chamber fluids. After implantation of tissue chambers for 7 days, *P. acnes*, *S. epidermidis* (20 μl; 10^7 CFU) or PBS (20 μl) were injected into tissue chambers. Sampling tissue chamber fluids was performed 3 days after bacterial injection. Measurement of MIP-2 in the supernatants of fluids was carried out by sandwich ELISA that used the Quantikine M mouse MIP-2 set (R&D System, Minneapolis, MN) (A). In vivo *P. acnes* growth was detected by spreading the tissue chamber fluids on MHB agar plates to quantify CFU (B).

[0018] Figure 5A-D shows quantitative analysis of *P. acnes* proteome alterations using isotope-coded protein labels (ICPL): Identification of CAMP factor and lipase. *P. acnes* was grown under aerobic and anaerobic conditions. Lysates (1 mg) of *P. acnes* from aerobic and anaerobic growth were labeled with ICPL tags, C^{12}-N-nicotinoyloxy-succinimide (Nic-NHS) and C^{13}-Nic-NHS, respectively. All lysine side chains of proteins in lysates were modified selectively. After mixing C^{12}-Nic-NHS-with C^{13}-Nic-NHS-labeled samples, the mixture was subjected to a LTQ mass spectrometer (Thermo Electron Corp. Waltham, MA) for protein identification and quantification. Two-tag labeling introduced a mass difference of 6 Da per labeled site in mass spectra. More than 300 proteins of *P. acnes* were identified. 23 proteins were either up- or down-regulated under anaerobic or aerobic conditions (Table 1). Two secretory virulence factors (lipase and CAMP factor) with double charges and 3Da mass differences were shown (A and B). Both of the virulence factors have a higher expression in *P. acnes* under anaerobic conditions. Two peptides (SYSEKHLGVAFR (SEQ ID NO:1) and DLLKAAFDLR (SEQ ID NO:2)) were sequenced and assigned to the internal peptides of lipase (C) and CAMP factor (D), respectively.

[0019] Figure 6A-G shows removal of sialic acids by sialidase increases the susceptibility of human sebocytes to *P. acnes*. Sialic acids on the cell surface of immortalized human sebocytes (SZ95) were detected by the reaction with biotinylated Maackia Amurensis (MAA) lectin I (10 μg/ml) and streptavidin-FITC conjugate. The FITC-fluorescence intensity was counted by flow cytomtery to reflect the level of sialic acids (A). The sebocytes were pre-treated with PBS (vehicle), sialidase (10 μg/ml) (grean, A), or GFP (10 μg/ml) (B) at pH 6 for 2 h. The decrease of the FITC-fluorescence intensity in sialidase-treated sebocytes inidcated that puried sialidase is an effective enzyme. After pretreatment of sialidase (10 μg/ml, 2h), sebocytes were co-cultured with *P. acnes* (10^7 CFU/10^6 cells) for 24 h. *P. acnes*-induced cell death in vehicle-, sialidase- or GFP-treated sebocytes were counted by trypan blue staining (C). After washing out with suspended *P. acnes*, the number of *P. acnes* adhered to sebocytes was calculated by spreading trypsinized sebocytes on MHB agar plates to quantify CFU/cells (D). The adherence of *P. acnes* into vehicle (E), sialidase (F, arrows) or GFP (G)-treated sebocytes was visualized by staining with Accustain Gram stain kit.

[0020] Figure 7A-C shows that sialidase is immunogenic when mice were immunized with an E. coil vector-based vaccine or recombinant protein/Freund (in)complete adjuvants. The irradiated *E. coli* vector-based vaccine (*E. coli* BL21 (DE3) T7/lacO sialidase) was constructed by inserting PCR products of sialidase into the pEcoli-Nterm 6xHN vector (Clontech). The production of antibody *E. coli* vector (10^9 CFU)-immunized mice was detected by western blot analysis 6 weeks after vaccination (A). Mice immunized with *E. coli*-empty vector (lacZ) serve as negative controls. ICR mice were also immunized with a recombinant sialidase-6xNH fusion protein or GFP using Freund/(in)complete adjuvants. For the subcutaneous vaccination at first injection, mice were inoculated with 200 μg of the sialidase-6xNH fusion protein or GFP which was emulsified with a complete Freund adjuvant. Two weeks after

injection, the second injection was performed. Mice were intramuscularly injected with the same amount of antigens which were mixed well with an incomplete Freund adjuvant. Anti-sialidase antibody was detected by western blot (B) and antigen microarrays (C) one week after second vaccination. 0.35 µg of purified sialidase-6xNH fusion protein and IgG indicated were spotted twice on antigen microarrays. Data is representative of three separate experiments with similar results. Sialidase antibodies can be provoked when mice were immunized with both *E. coli*-vector-based vaccines and recombinant proteins/Freund adjuvants.

[0021] Figure 8 shows protective immunity of a sialidase-based vaccine to *P. acnes*-induced ear thickness. ICR mice were immunized with recombinant sialidase-6xNH fusion protein or GFP using Freund (In)complete adjuvants. After confirmation of antibody production by western blot, *P. acnes* (10^7 CFU, 25µl) was subcutaneously injected into ears of sialidase- and GFP-immunized mice. Injection of PBS (25µl) served as a control. Ear thickness was measured for 9 days after injection and calculated as % of ear thickness in PBS-injected ears.

[0022] Figure 9 shows anti-sialidase antiserum in vitro. *P. acnes* was pre-incubated with anti-sialidase antiserum for 2 h. The immortalized human sebocytes (SZ-95) were co-cultured with the antiserum-treated *P. acnes* 18 hr. After incubation, the cell death of sebocytes induced by cytotoxicity of *P. acnes* was determined with pNPP. The immortalized human sebocyte line, SZ95, was cultured on a 96-well plate until a density of $2x10^5$ cells/well in Sebomed basal medium (Biochrom, Berlin, Germany) supplemented with 5 ng/ ml human recombinant epidermal growth factor (Sigma, St. Louis, MO), 10% (v/v) heat-inactivated fetal bovine serum (Mediatech Inc., Herndon, VA), at 37 °C under atmosphere of 5% (v/v) CO_2 in air. *P. acnes* were cultured as described above, washed with PBS by centrifuging. *P. acnes* were suspended to Sebomed basal medium containing 2.5% (v/v) anti-sialidase or anti-GFP (control) antiserum, and incubated at 37°C for 2 h. The sebocytes were washed with PBS two times and then incubated with 100 µl of

the neutralization reaction mixtures containing $2x10^6$ CFU *P. acnes* and 2.5 µl antiserum for 18 h. As a control, an equal amount of PBS was added instead of *P. acnes*. As a background, Triton-X was added to get a final concentration of 0.1% (v/v) to kill sebocytes. After incubation, cytotoxicity of neutralizing mixture was determined with p-Nitrophenyl phosphate disodium (pNPP). The sebocytes were washed with PBS three times and incubated with 100 µl of 2.5% (w/v) pNPP in ACPI for 1 hr at 37°C. After incubation, 10 µl of 1N NaOH was added to stop the reaction and absorbance at 405 nm was measured. Cytotoxicity of neutralizing mixture was calculated as (no *P. acnes* group-*P. acnes* added group)÷(no *P. acnes* group-back ground group) x100.

[0023] Figure 10A-F shows protective immunity of an inactivated *P. acnes* vaccine. ICR mice were immunized with heat-killed *P. acnes* (10^8 CFU) and boosted twice at the three week intervals. Ten weeks (one week after second boost) after immunization, live *P. acnes* (10^7 CFU, 25µl) or PBS (25µl) was subcutaneously injected into ears of killed *P. acnes* - immunized and PBS-inoculated mice. Ear thickness was calculated as % of ear thickness in PBS-injected ears (A). 24 (B, C) and 72 h (D, E) after live *P. acnes* injection, the ear redness in killed *P. acnes* -immunized (B, D) and PBS-inoculated (C, E) mice was shown. Measurement of MIP-2 in the supernatants of fluids was conducted by sandwich ELISA. The elevation of MIP-2 induced by *P. acnes* (20 µl; 10^7 CFU) injection was considerably suppressed in killed *P. acnes*-immunized mice (F).

[0024] Figure 11 shows characterization of *P. acnes* CAMP factor. (A) Recombinant *P. acnes* CAMP factor was expressed in E. coli (arrowhead). E. coli transformed with pEcoli-Nterm 6×HN vector containing a cDNA insert encoding CAMP factor was incubated without (lane 1) or with (lane 2) IPTG, disrupted, and separated by SDS-PAGE (10% acrylamide). Purified CAMP factor is shown on the right panel. (B) The expression and purity of CAMP factor was confirmed by NanoLTQ MS/MS mass spectrometry. A sequenced internal peptide (AVLLTANPASTAK; SEQ

ID NO:3)) of CAMP factor is presented. (C) Co-hemolytic activity of recombinant CAMP factor was examined on a sheep blood agar plate. S. aureus strain 113 (2×10^5 CFU/10 ul) was streaked on agar plate. Ten ul of recombinant CAMP factor (250 ug/ml) or GFP as a control protein (250 ug/ml) was spotted beside the S. aureus streak. (D) Immunogenicity of CAMP factor in ICR mice was evaluated by Western blot. Mice were intranasally vaccinated with UV-killed E. coli over-expressing CAMP factor or GFP. The mice were bled 14 days after the vaccination. Anti-CAMP factor (1:2,000 dilution; lanes 1 and 2) or anti-GFP antiserum (lanes 3 and 4) was reacted with recombinant CAMP factor (0.2 µg; lanes 1 and 3) or GFP (lanes 2 and 4). The immunoreactivity was detected with goat anti-mouse IgG (H+L)-HRP conjugate. (E) The titer of CAMP factor antibodies was determined by ELISA. The mice were bled 14, and 21 days after the vaccination with CAMP factor or GFP (n=10). The antisera (1:10,000 dilution) were reacted with CAMP factor immobilized on a microtiter ELISA plate. The captured antibodies were detected with goat-anti-mouse IgG (H+L)-HRP conjugate and OptEIA™ Reagent Set. The optical density of each well was measured at 450 nm. Horizontal bar represents average of 10 individual assays. (F) CAMP factor was detected in the supernatant of *P. acnes* culture by Western blotting. Recombinant CAMP factor (0.2 µg; lane 1) as a positive control, 10-fold concentrate of *P. acnes* culture supernatant (70 µg total protein; lane 2), and 10-fold concentrate of RCM (70 µg total protein; lane 3) as a negative control were separated by SDS-PAGE (10% acrylamide), transferred to a polyvinylidene fluoride membrane and reacted with mouse anti-CAMP factor antiserum (1:1,000 dilution, left panel) or anti-GFP antiserum (right panel). The 6×HN tag of recombinant CAMP factor was removed by enterokinase before loading into a SDS-PAGE. (G) Cytotoxicity of recombinant CAMP factor was examined in the human keratinocyte cell line (HaCaT) or murine macrophage cell line (RAW264.7). The cells (1×10^5/well) were incubated with the indicated concentration of recombinant CAMP factor or GFP at 37oC for 18 hr. After the incubation, cell

viability was determined and cytotoxicity was calculated as described in Methods. The data represent mean ± SE (n=6, p< 0.005** and p< 0.0005*** by Student's t-test, vs. GFP control). (H) Intradermal injection with CAMP factor induced inflammatory reaction in ICR mouse ear. The left ear was intradermally injected with recombinant CAMP factor (10 μg/20 μl) or GFP (10 μg/20 μl) in PBS. Right ear received an equal amount of PBS (20 μl). The ear thickness was measured using a micro caliper 24 hr after the injection and changes reported as % of ear thickness in PBS-injected ears. The data represented as mean ± SE (n=4, p< 0.005** by Student's t-test).

[0025] Figure 12A-C shows the involvements of bacterial CAMP factor and host ASMase in *P. acnes* pathogenicity in vitro. (A) CAMP factor and ASMase were detected in the supernatant of cell culture by Western blot following co-cultured with *P. acnes*. The HaCaT (lanes 1 and 3) or RAW264.7 (lanes 2 and 4) (5×10^5/well) were co-cultured with *P. acnes* (5×10^6 CFU/well; MOI=1: 10) (lanes 1 and 2) or without *P. acnes* (lanes 3 and 4) in serum-free medium at 37°C for 14 hr. The concentrates of cell culture supernatant (10 μg total protein) were subjected to Western blotting. CAMP factor and ASMase were detected with mouse anti-CAMP factor antiserum and goat anti-ASMase IgG, respectively. (B) *P. acnes*-mediated cell death was neutralized by anti-CMAP factor antiserum in vitro. HaCaT or RAW264.7 cells (1×10^5/well) were co-cultured with *P. acnes* (1×10^6 CFU/well; MOI=1: 10) for 14 hr in the presence of mouse anti-CAMP factor or anti-GFP antiserum (2.5% v/v). (C) Including ASMase inhibitor decreased *P. acnes*-mediated cell death in vitro. HaCaT or RAW264.7 cells (1×10^5/well) were cultured without or with *P. acnes* (1×10^6 CFU/well, MOI=1: 10) in medium containing desipramine (10 μM), a selective ASMase inhibitor, or the equal amount of PBS (vehicle) at 37°C for 14 hr. After incubation, the cell viability was determined and the cytotoxicity was calculated as described in Materials and Methods. The data represent as mean ± SE (n=10, p< 0.05* and p< 0.0005*** by Student's t-test).

[0026] Figure 13A-D shows the possible involvement of host ASMase in pathogenicity of *P. acnes* in vivo. (A) Amount of soluble ASMase in mouse ear increased 24 hr after bacteria challenge. Ears of ICR mice were intradermally injected with of *P. acnes* in PBS (1×10^7 CFU/20 µl; left ear) or PBS (20 µl; right ear), and excised after 24 hr. Ear tissue was obtained with a 8 mm biopsy and homogenized in PBS. The supernatant (1 ug of total protein) was subjected to Western blotting. ASMase (upper panels) and GAPDH (lower panels) were detected with goat anti-ASMase IgG followed by anti-GAPDH IgG (left panels). Normal goat or mouse IgG was used as a negative control for the detection (right panels). (B) *P. acnes* challenge into mouse ear attracted CD11b+ macrophages which highly expressed ASMase. Frozen sections of mouse ear obtained 24 after bacteria challenge were stained with biotinylated anti-mouse CD11b IgG, a conventional macrophage marker, and TRITC-streptavidin conjugate, followed by Goat anti-ASMase IgG and anti-goat IgG-TRITC conjugate. The nuclei were stained with DAPI (blue). Bar=200 µm.(C) Transmission electron microscopy (10,000× magnification) was used to visualize colonized *P. acnes* and ruptured cell membrane in mouse ears injected with *P. acnes* or PBS. PA, *P. acnes*; CM, cell membrane; NC, nucleus. Bar=1 µm. (D) Systemic pre-treatment of ICR mice with selective ASMase inhibitor relieved *P. acnes*-induced inflammation. ICR mice were intraperitoneally injected with desipramine (20 mg/kg mouse) or an equal amount of PBS (vehicle) 30 min prior to the bacterial challenge. After pretreatment, live *P. acnes* (1×10^7 CFU/20 µl) in PBS or an equal amount of PBS (control) was intradermally injected into left ear or right ear, respectively. The ear thickness was measured using a micro caliper before and 24 hr after the bacterial challenge and changes reported as % of ear thickness in PBS-injected ears. The data represent as mean ± SE (n=3, $p< 0.005$** by Student's t-test).

[0027] Figure 14 shows a combination of CAMP factor vaccine and local injection with anti-ASMase IgG synergistically suppressed *P. acnes*-induced inflammation. ICR mice were

vaccinated with UV-killed E. coli over-expressing CAMP factor or GFP in a 3-week interval. Two weeks after the second boost, *P. acnes* was intradermally injected into the ear of vaccinated mice in the same manner as described above. Within 30 min, the left ear (received *P. acnes*) was injected with goat anti-ASMase IgG (4 µg/20 µl) or normal goat IgG (control) in PBS, and the right ear was injected with an equal volume of PBS (n=8). Ear thickness was measured 24 hr after the bacteria challenge and changes reported as % of ear thickness in PBS-injected ears. The data represent as mean ± SE ($p< 0.05$*, $p<0.005$**, $p< 0.0005$*** by Student's t-test).

[0028] Figure 15A-C shows the effects of CAMP factor-based vaccine on *P. acnes*-induced inflammation on mice. (A) Intradermal injection with CAMP factor induced inflammatory reaction in ICR mouse ear. The left ear was intradermally injected with recombinant CAMP factor (10 µg/ 20 µl) or GFP (10 µg/ 20 µl) in PBS. Right ear received an equal amount of PBS (20 µl). The ear thickness was measured using a micro caliper 24 hr after the injection and changes reported as % of ear thickness in PBS-injected ears. The data represented as mean ± SE (n=4, $P< 0.005$** by Student's t-test). (B) The titer of CAMP factor antibodies was determined by ELISA. The mice were bled 14, and 21 days after the vaccination with CAMP factor (n=10). The antisera (1: 10,000 dilution) were reacted with CAMP factor immobilized on a microtiter ELISA plate. The captured antibodies were detected with goat-anti-mouse IgG (H+L)-HRP conjugate and OptEIA™ Reagent Set. The optical density of each well was measured at 450 nm. Bar represents average of 10 individual assays. (C) Sole immunization of ICR mice with CAMP factor provided therapeutic immunity against *P. acnes*-induced inflammation. Live *P. acnes* ($1x10^7$ CFU/ 20 µl) in PBS or an equal amount of PBS (control) was intradermally injected into left ear or right ear, respectively, of naïve mice. After 24 hr, the mice were intranasally immunized with UV-killed E. coli over-expressing CAMP factor or GFP (arrow). Ear thickness was measured at the indicated times after bacterial challenge and changes reported as % of ear thickness

in PBS-injected ears. Data represent mean ± SE (n=10, P<0.05*, P<0.005**, P< 0.0005*** by Student's t-test).

[0029] Figure 16A-B shows co-cytotoxic properties of CAMP factor and bacterial SMase in vitro. The HaCaT (A) or RAW264.7 cells (B) were pretreated with SMase from S. aureus (350 mU/ml) or an equal amount of vehicle for 15 min, washed three times to remove the enzyme, and then incubated with recombinant CAMP factor (25 ug/ml) or GFP at 37°C for 18 hr. After the incubation, cell viability was determined and cytotoxicity was calculated. The data are presented as mean ± SE (n=6, p< 0.0005*** by Student's t-test).

[0030] Figure 17A-B shows *P. acnes* CAMP factor exerted virulence activity. Ears of ICR mice were injected intradermally with recombinant GFP (left ear) and CAMP factors (right ear). (A) Inflammation-induced ear redness (arrow) was visualized 24 h after injection. (B) Ear swelling was observed in an H&E-stained frozen tissue section of GFP- (I, iii) or CAMP factor (ii, iv)-injected ear. The magnified images [4× (i, iii) and 20× (ii, iv)] indicated the deposits of ruptured erythrocytes (arrowheads). Bars (a)= 1 cm. Bars [b(I, iii)]= 2 mm. Bars [b(ii, iv)]= 0.5 mm.

[0031] Figure 18A-D shows transiently express CAMP factors and GUS in radish leaves. (a) Leaves of radish (*Raphanw sativus L.*) were infiltrated with *A. tumefaciens* (LBA4404 strains) transforming a 35S::*GUS* construct (right). Leaves infiltrated with non-transformed LBA4404 cells (left) served as negative controls. Dotted circles indicate locations of syringe infiltration with *A. tumefaciens.* Blue stained areas indicate the GUS expression. The dynamic pattern of GUS expression in radish leaves from 1 to 5 days after infiltration was analyzed by (b) histochemical and (c) GUS activity assays. (*P<0.05 and **P<0.005, by Student's *t*-test). (d) Detection of CAMP factor expression by Western blot analysis. Ground radish leaves (20 µg) infiltrated with *A. tumefaciens* carrying a 35S::*CAMP factor-His* (CAMP factor-His), a 35S::*SCAP-MBP-His* (SCAP-MBP-His) or recombinant GUS (rGUS) were run on a 10% (w/v) SDS-PAGE and blotted onto a

nitrocellulose membrane. The membranes were then probed with anti-CAMP factor serum produced by mice immunized with UV-irradiated *E. coli*, BL21 (DE3) over-expressing CAMP factor. An arrow indicates CAMP factor appearing at a molecular weight of 29 kDa. Bar = 6 mm.

[0032] Figure 19 shows mice immunized with CAMP factor-encapsulated leaves produced CAMP factor specific antibodies. Purified CAMP factor (65 µg) run on a 10% (w/v) SDS-PAGE was blotted onto a nitrocellulose membrane and immuno-reacted to sera obtained from mice immunized with leaves encapsulating GUS (left) or CAMP factors (right). A single band with 29 kDa indicates the purified CAMP factor reactive to serum from CAMP factor-immunized mice, verifying the immunogenicity of CAMP factor.

[0033] Figure 20A-C shows that passive immunization of mice with neutralizing antibody to CAMP factor diminished *P. acnes*-induced inflammation. (A) 5 % (v/v) anti-GUS (open circles) or anti-CAMP factor (solid circles) serum-treated *P. acnes* (1 x 10^7 CFUs) was inoculated into the right ears of ICR mice to induce an increase in ear thickness as described in the "Materials and Methods". As a control, an equal volume of PBS was injected into the left ears of the same mice. Ear thickness was measured with a micro-caliper at the indicated times after bacterial injection. The ear thickness of *P. acnes*-injected ear was calculated as % of a PBS-injected control. Error bars represented mean ± SE of four mice (**P<0.005, by Student's *t*-test). (B) Ear redness (arrows) was visualized 3 days after injection with anti-GUS serum (i) or anti-CAMP factor (ii) serum treated-*P. acnes* (10^7 CFUs). Bar = 1 cm. (C) Ear inflammation was observed in an H&E-stained frozen tissue section of ear injected with PBS alone (i, iv) or *P. acnes* treated with anti-GUS (ii, v) or anti-CAMP factor (iii, vi) serum. The granulamatous reactions (arrowheads) were visualized under magnification 4× (i, ii, iii; bars= 2 mm) and 20× (iv, v, vi; bars= 0.5 mm).

[0034] Figure 21A-C shows passive neutralization of *P. acnes* CAMP factor reduced the production of pro-inflammatory MIP-2

cytokine and bacterial colonization without altering *P. acnes* survival at other body sites. (A) Measurement of pro-inflammatory MIP-2 cytokine was carried out by a sandwich ELISA using a Quantikine M mouse MIP-2 set. Compared to the neutralization with anti-GUS serum (open bar), passive neutralization with anti-CAMP factor serum (solid bar) markedly suppressed the *P. acnes*-induced increase in MIP-2. (B) The left ears of mice were injected with *P. acnes* (1×10^7 CFUs) in the presence of anti-GUS serum (open bar) or anti-CAMP factor serum (solid bar). (C) The right ears were injected with live *P. acnes* (1×10^7 CFUs) alone. Bacterial colonization (CFUs) was quantified in agar plates as described in "Materials and Methods. Error bars represent mean ± SE of four mice (*$P<0.05$, by Student's *t*-test).

[0035] Figure 22 shows that vaccination with CAMP factor conferred protective effect on P. acnes-induced ear swelling. Seven weeks after vaccinated with GUS- (open bar) and CAMP factor (solid bar), mice were challenged intradermally with an amount of 25 µl aliquots of live P. acnes (1×10^7 CFUs) suspended in PBS overnight to right ears. As a control, 25 µl of PBS was injected into the left ear of the same mice. The increase in ear thickness was measured using a micro caliper after the bacterial challenge. The increase in ear thickness of P. acnes challenged ear was calculated as % of a PBS-injected control.

DETAILED DESCRIPTION

[0036] The exemplary descriptions provided herein are exemplary and explanatory only and are not restrictive of the invention, as claimed. Moreover, the invention is not limited to the particular embodiments described, as such may, of course, vary. Further, the terminology used to describe particular embodiments is not intended to be limiting.

[0037] With respect to ranges of values, the invention encompasses each intervening value between the upper and lower limits of the range to at least a tenth of the lower limit's unit, unless the context clearly indicates otherwise. Further, the invention encompasses any other stated intervening values.

Moreover, the invention also encompasses ranges excluding either or both of the upper and lower limits of the range, unless specifically excluded from the stated range.

[0038] Unless defined otherwise, the meanings of all technical and scientific terms used herein are those commonly understood by one of ordinary skill in the art to which this invention belongs. One of ordinary skill in the art will also appreciate that any methods and materials similar or equivalent to those described herein can also be used to practice or test the invention. Further, all publications mentioned herein are incorporated by reference.

[0039] It must be noted that, as used herein and in the appended claims, the singular forms "a" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a polypeptide" includes a plurality of such polypeptides and reference to "the bacteria" includes reference to one or more bacteria and equivalents thereof known to those skilled in the art, and so forth.

[0040] Also, the use of "or" means "and/or" unless stated otherwise. Similarly, "comprise," "comprises," "comprising" "include," "includes," and "including" are interchangeable and not intended to be limiting.

[0041] It is to be further understood that where descriptions of various embodiments use the term "comprising," those skilled in the art would understand that in some specific instances, an embodiment can be alternatively described using language "consisting essentially of" or "consisting of."

[0042] *Propionibacterium acnes* (*P. acnes*) is involved in many human polymicrobial diseases including acne vulgaris, endocarditis, endophthalmitis, osteomyelitis, joint, nervous system, and cranial neurosurgery infections, and implanted biomaterial contamination. More than fifty million people in the U.S have acne vulgaris. In addition, acne vulgaris is the most common skin disease that affects 85-100% of people at some time during their lives. Systemic antibiotic therapy for

acne lesions non-specifically kills the majority of skin bacteria, which impacts the homeostasis of skin resident flora. Vaccines against acne vulgaris and *P. acnes*-induced diseases were not available prior to this disclosure. The disclosure provides anti-*P. acnes* vaccines to suppress *P. acnes*-induced skin inflammation.

[0043] Proliferation of *P. acnes* starts in the microcomedone, which is the precursor of acne lesion characterized by hyperkeratinization, formation of a keratin plug, and increase in sebum secretion by sebaceous gland. The microcomedo provides an anaerobic, sebum-rich microenvironment in the hair follicle, which promotes overgrowth of *P. acnes*. The initial event in acne inflammation is the disruption of follicular epithelium by this overgrowth of *P. acnes*, allowing the bacteria in the comedo to come in contact with the host immune systems, triggering granulomatous inflammation (typical inflammatory acne). *P. acnes* stimulates the production of pro-inflammatory cytokines, including interleukins -1β, -8, -12, and tumor necrosis factor-α, via toll-like receptor 2.

[0044] Acne vulgaris is one of the most common skin diseases that can result in severe inflammatory lesions that are highly associated with *P. acnes* infection. *S. epidermidis* and *P. acnes* have been recognized as major skin bacteria that cause the formation of acne vulgaris. In addition, these bacteria have the ability to synthesize lipases that degrade sebum triglycerides into free fatty acids which trigger inflammatory responses. Treatment of acne should be started as early as possible to minimize the risk of scarring and adverse psychological effects. Many antibiotics have been used for acne treatment, but these antibiotics in general are non-specific, short lasting and normally are applied when acne lesions have already occurred (such as in late stages of acne). Development of anti-acne vaccines can prevent acne progression from the early stages and increase the specificity of treatments as described herein.

[0045] Acne vulgaris is a multi-factorial disease associated with polymicrobial infection, hormone regulation and immune

responses. The inflammatory stage of acne vulgaris is usually of greatest concern to the patient. Inflammatory lesions may lead to scarring and adverse psychological effects. Vaccines, which selectively suppress the *P. acnes*-induced inflammation, will minimize the risk of changing the homeostasis of body hormones and resident skin microbes.

[0046] Hemolysis is a virulence factor employed by numerous bacterial pathogens to degrade, invade host cells, and resist the host immune attack. This is achieved through various mechanisms targeting the cell membrane: enzymatic, pore-forming, or surfactant. When *P. acnes* is grown on a sheep blood agar plate in close proximity to beta-hemolytic microorganisms, such as *Staphylococcus aureus* (*S. aureus*) and *Clostridium perfringens*, it synergistically enhances hemolysis similar to the classical Christie, Atkins, Munch-Peterson (CAMP). CAMP reactions are induced by the combination of CAMP factor co-hemolysin, which is a pore-forming toxin, and sphingomyelinase (SMase) derived from the other bacterial partner. CAMP factor itself has only weak hemolytic activity on the erythrocytes, but pretreating the cells with SMase enhances its activity. SMase initially hydrolyzes sphingomyelin on the cell membrane of erythrocytes to ceramide, which renders the cells susceptible to the hemolytic activity of CAMP factor. The entire genomic sequence of *P. acnes* includes numerous genes whose products are involved in degrading host molecules, and five genes encoding CAMP factor homologs of *Streptococcus agalactiae* (*S. agalactiae*) have been found in the genome information. This comprehensive analysis of *P. acnes* proteins by a proteomic technique utilizing isotope-coded protein labels coupled to NanoLC-MS analysis revealed that one of the CAMP factor homologs (accession number: gi/50842175, incorporated herein by reference), showing 42% identity in nucleotide sequence to the *S. agalactiae* CAMP factor, is produced at higher concentrations by bacteria cultured under anaerobic condition than under aerobic conditions. These data suggest a physiological significance for the CAMP factor for *P. acnes*.

[0047] The CAMP factor from *Streptococcus agalactiae* binds to the Fc region of IgG and IgM molecules, similar to the binding of IgG by *Staphylococcus aureus* protein A (Jurgens *et al.*, 1987), and partial amino acid sequence similarity between the CAMP factor protein of *Streptococcus agalactiae* and *Staphylococcus aureus* protein A has been demonstrated (Ruhlmann *et al.*, 1988). Evidence is presented of differences amongst *P. acnes* types IA, IB and II in the expression of proteins with sequence similarity to the CAMP co-haemolysin.

[0048] The disclosure demonstrates that *P. acnes* secretes CAMP factor, which is shown to act synergistically with bacterial sphingomyelinase (SMase) to lyse erythrocytes. Furthermore, the disclosure demonstates that recombinant *P. acnes* CAMP factor alone induced cell death in human keratinocyte (HaCaT) and murine macrophage (RAW264.7) cell lines in a dose-dependent manner. For example, intradermal injection of mouse ears with CAMP factor induced significant ear swelling. In addition, host acid SMase (ASMase) was released/secreted from HaCaT and RAW264.7 cells when the cells were co-cultured with *P. acnes*. *P. acnes*-induced cytotoxicity in both cell lines was significantly neutralized by including either a selective ASMase inhibitor, anti-ASMase antibodies or anti-CAMP factor antiserum. Intradermal injection of mouse ears with live *P. acnes* attracted numerous macrophages, which strongly express ASMase, resulting in an increase in soluble ASMase in the ear after *P. acnes* challenge. Most notably, vaccination of ICR mice with CAMP factor yielded protective immunities against *P. acnes*-induced inflammation and the development of skin lesions. In addition, the combination of vaccinating with CAMP factor and locally injecting anti-ASMase IgG synergistically reduced *P. acnes*-induced ear swelling. These data demonstrate that *P. acnes* benefits from host enzymes that amplify its pathogenicity; *P. acnes* may utilize host ASMase to enhance the toxicity of its CAMP-factor, which may contribute to its evasion of host immune defenses, degrade host tissues, and spread the pathogen.

[0049] The complete genome sequence of *P. acnes* is known and incorporated herein by reference in its entirety. The disclosure identifies virulence factors and provides a set of vaccines capable of providing protective immunity against *P. acnes*. Other virulence factors and antigenic compositions will be readily apparent and are encompassed by the disclosure. Specifically, the antigens upregulated in anaerobic conditions as identified in Table 1 are targets for the development of vaccines based upon the teachings herein. The disclosure estabilshes a proteome of *P. acnes* by comparing the differential expression of *P. acnes* proteins between anaerobic and aerobic conditions (see examples and Table 1). This analysis revealed a number of genes that were upregulated. The disclosure further provides additional data on three secretory virulence factors (CAMP factor, lipase, and sialidase) associated with *P. acnes*-induced host cell damage and inflammation.

[0050] The anti-*P. acnes* vaccines provided by the disclosure benefit subjects suffering from polymicrobial *P. acnes* associated diseases including acne vulgaris, endocarditis, endophthalmitis, osteomyelitis, joint, nervous system, and cranial neurosurgery infections, and implanted biomaterial contamination.

[0051] In addition to vaccine targets, three secretory virulence factors (CAMP factor, lipase, and sialidase) as well as killed-*P. acnes* serve as candidates for development of anti-*P. acnes* drugs. For example, based upon the disclosure methods of identifying therapeutics for the treatment of microbial infections can include approaches from proteomic protein identification to vaccine evaluation. The disclosure provides a platform for the studies of functional proteomics and vaccine/drug creation using the identified secretory virulence factors.

[0052] The disclosure provides, for example, anti-*P. acnes* vaccines targeting secreted CAMP factor, lipase and sialidase as well as antigens from Killed-*P. acnes*. The vaccines of the

disclosure were demonstrated both in vitro and in vivo. The vaccines reduced inflammation caused by *P. acnes*.

[0053] The disclosure further demonstrates that targeting secretory virulence factors is an effective strategy to decrease *P. acnes*-induced inflammation.

[0054] The vaccines of the disclosure can be used alone or with systemic antibiotic therapy.

[0055] A "polynucleotide" generally refers to any polyribonucleotide (RNA) or polydeoxyribonucleotide (DNA), which may be unmodified or modified RNA or DNA. Polynucleotides include, without limitation, single-stranded and double-stranded DNA, DNA that is a mixture of single-stranded and double-stranded regions, single-stranded and double-stranded RNA, and RNA that is a mixture of single-stranded and double-stranded regions. Polynucleotides also include hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single-stranded and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. Polynucleotides also include DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications may be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. Oligonucleotides are relatively short polynucleotides. Examples of polynucleotides useful in the methods of the disclosure to produce antigen to induce an immune reaction include those set forth in Table 1, including fragments thereof encoding antigenic epitopes.

Table 1. Protein differential expression with/without oxygen			
Accession #	Proteins	$+O_2$	$-O_2$
Secretory virulence factors			
gi/50842175	CAMP factor		*
gi/50843543	lipase		*
Membrane proteins			
gi/50841878	ABC transporter ATP-binding protein	*	
gi/50843296	Extracellular solute-binding transport protein, putative oligopeptide-binding protein		*
gi/50843565	S-layer protein		*
Enzymes			
gi/50843566	Arginyl-tRNA synthetase		*
gi/50842182	Aminopeptidase		*
gi/50843224	Biofunctional GMP synthase	*	
gi/50842971	Carbamate kinase		*
gi/50841588	Catalase		*
gi/50842082	Methylmalonyl-CoA mutase	*	
gi/50842890	Phenylalanyl-tRNA synthetase beta chain		*
gi/50842304	Phosphoglycerate kinase		*
gi/50842950	Polyribonucleotide nucleotidyltransferase		*
gi/50841767	Putative Clp-family ATP-binding protease	*	
gi/50841972	UTP-glucose-1-phosphate uridylyltransferase		*
Others			
gi/50843142	Conserved protein (DUF174)		*
gi/50843329	Elongation factor G	*	
gi/50843484	Molecular chaperone DnaK		*
gi/50841600	Myosin-crossreactive antigen		*
gi/50843708	Rare lipoprotein A, RlpA family	*	
gi/50843315	50S Ribosomal protein L2	*	
gi/50843340	50S Ribosomal protein L10	*	

The sequences associated with the foregoing accession numbers are incorporated herein by reference in their entirety.

[0056] A "polypeptide" refers to any polypeptide comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than those normally encoded by a codon.

[0057] Polypeptides include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques that are well known in the art. Such modifications are well described in the literature and are known in the art. Modifications may occur anywhere in a polypeptide, including the peptide

backbone, the amino acid side-chains and the amino or carboxyl termini. Such modifications may be present to the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from post-translation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, biotinylation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. Examples of polypeptides useful in the methods and compositions of the disclosure comprise the *P. acnes* polypeptides set forth in SEQ ID Nos: 2, 3, 7 and 9 and human ASMase as set forth in SEQ ID NO: 11, antigenic fragment thereof, and antigenic fragments comprising 80%, 85%, 90%, 95%, 98%, or 99% identity to SEQ ID NO: 2, 3, 7, 9 and 11. Such polypeptides and fragments thereof are useful for immunization and to raise antibodies. Antigens comprising polypeptides of the disclosure are useful for generating an immune response in a subject.

[0058] An immune response is generated to an antigen through the interaction of the antigen with the cells of the immune system. The resultant immune response may be broadly distinguished into two extreme categories, being humoral or cell mediated immune responses (traditionally characterized by antibody and cellular effector mechanisms of protection,

respectively). These categories of response have been termed Th1-type responses (cell-mediated response), and Th2-type immune responses (humoral response). Extreme Th1-type immune responses may be characterized by the generation of antigen-specific, haplotype-restricted CTLs, and natural killer cell responses. In mice, Th1-type responses are often characterized by the generation of antibodies of the IgG2a subtype, while in the human these correspond to IgG1 type antibodies. Th2-type immune responses are characterized by the generation of a broad range of immunoglobulin isotypes including in mice IgG1, IgA, and IgM.

[0059] The driving force behind the development of these two types of immune responses is cytokines, a number of identified protein messengers which serve to help the cells of the immune system and steer the eventual immune response to either a Th1 or Th2 response. Thus, high levels of Th1-type cytokines tend to favor the induction of cell mediated immune responses to the given antigen, while high levels of Th2-type cytokines tend to favor the induction of humoral immune responses to the antigen. It is important to remember that the distinction of Th1 and Th2-type immune responses is not absolute. In reality, an individual will support an immune response which is described as being predominantly Th1 or predominantly Th2. Traditionally, Th1-type responses are associated with the production of the INF-γ and IL-2 cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of Th1-type immune responses are not produced by T-cells, such as IL-12. In contrast, Th2-type responses are associated with the secretion of IL-4, IL-5, IL-6, IL-10 and tumor necrosis factor-β(TNF-β).

[0060] The disclosure provides *P. acnes* antigens that are immunoprotective. Such antigens can be delivered in a number of ways to the host so as to stimulate a protective immune response against *P. acnes*. The antigens can be delivered via an attenuated vector that results in presentation via MHC class I (*e.g.*, such vectors include *L. monocytogenes, E. coli, non-virulent P. acnes* or another attenuated bacterial vector

such as *Mycobacterium bovis* BCG, *Shigela flexneri*). The term "attenuated," when used with respect to a bacteria, means that the bacteria has lost some or all of its ability to proliferate and/or cause disease or other adverse effect when the bacteria infects an organism. For example, an "attenuated" bacterium may be unable to replicate at all, or be limited to one or a few rounds of replication, when present in an organism in which a wild-type or other pathogenic version of the attenuated bacteria can replicate. Alternatively or additionally, an "attenuated" bacterium might have one or more mutations in a gene or genes that are involved in pathogenicity of the bacteria. Many genes, loci, or operons are known, mutations in which will result in an attenuated bacteria. Examples of attenuated bacteria used as live vaccines include *S. typhi* carrying a mutation in its galE or htrA gene, and *V. cholerae* carrying mutations in its ctxA gene.

[0061] Microorganisms which are used to express the *P. acnes* for use in immunoprotective compositions include, without limitation, *Campylobacter sp., Yersinia sp., Helicobacter sp., Gastrospirillum sp., Bacteroides sp., Klebsiella sp., Lactobacillis sp., Streptococcus gordonii, Enterobacter sp., Salmonella sp., Shigella sp., Aeromonas sp., Vibrio sp., Clostridium sp., Enterococcus sp. and Escherichia coli* (see *e.g.* U.S. Pat. Nos. 5,858,352, and 6,051,416, and Levine *et al.*, in "New Generation Vaccines Second Edition" ed. Levine *et al.*, Marcel Dekker, Inc. pp 351-361 (1997), Levine *et al.*, in "New Generation Vaccines Second Edition" ed. Levine *et al.*, Marcel Dekker, Inc. pp 437-446 (1997), Butterton *et al.*, in "New Generation Vaccines Second Edition" ed. Levine *et al.*, Marcel Dekker, Inc. pp 379-385 (1997) and Fennelly *et al.*, in "New Generation Vaccines Second Edition" ed. Levine *et al.*, Marcel Dekker, Inc. pp 363-377 (1997)). For example, *Campylobacter jejuni, Campylobacter coli, Listeria monocytogenes, Yersinia enterocolitica, Yersinia pestis, Yersinia pseudotuberculosis, Escherichia coli, Shigella flexneri, Shigella sonnei, Shigella dysenteriae, Shigella*

boydii, *Helicobacter pylori*, *Helicobacter felis*, *Gastrospirillum hominus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Bacteroides fragilis*, *Clostridium difficile*, *Salmonella typhimurium*, *Salmonella typhi*, *Salmonella gallinarum*, *Salmonella pullorum*, *Salmonella choleraesuis*, *Salmonella enteritidis*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Enterococcus faecalis* can be used. *Escherichia coli* include, but are not limited to, entero-toxic, entero-hemorrhagic, entero-invasive, entero-pathogenic or other strains can be used in the disclosure.

[0062] Alternatively, or in addition to the above, a non-bacterial attenuated vector such as a replication-deficient viral vectors may be used in the methods and compositions of the disclosure. Such viral vectors useful in the methods and compositions of the disclosure include, but are not limited to, Vaccinia, Avipox, Adenovirus, AAV, Vaccinia virus NYVAC, Modified vaccinia strain Ankara (MVA), Semliki Forest virus, Venezuelan equine encephalitis virus, and herpes viruses. Naked DNA vectors can also be used in addition to antigenic proteins alone or in combination with an adjuvant. The naked DNA can be taken up and expressed by cells of the vaccinated subject resulting in the induction of an immune reaction to the expressed polypeptide.

[0063] Examples of suitable viral vectors include herpes simplex viral vectors, vaccinia or alpha-virus vectors and retroviruses, including lentiviruses, adenoviruses and adeno-associated viruses. In one embodiment, these vectors are replication defective virus vectors. Gene transfer techniques using these viruses are known to those skilled in the art. Retrovirus vectors, for example, may be used to stably integrate the polynucleotide of the disclosure into the host genome, although such recombination may not be advisable. Replication-defective adenovirus vectors by contrast remain episomal and therefore allow transient expression.

[0064] In one embodiment, the adenovirus used as a live vector is a replication defective human or simian adenovirus. Typically these viruses contain an E1 deletion and may be

grown on cell lines that are transformed with an E1 gene. Suitable Simian adenoviruses are, for example, viruses isolated from Chimpanzee. Examples of viruses suitable for use in the disclosure include C68 (also known as Pan 9) (U.S. Pat. No. 6,083,716, incorporated herein by reference) and Pan 5, 6 and Pan 7 (WO 03/046124 incorporated herein by reference). Thus, these vectors can be manipulated to insert a heterologous polynucleotide coding for an antigen such that the product is expressed. The use formulation and manufacture of such recombinant adenoviral vectors is set forth in detail in WO 03/046142, which is incorporated by reference.

[0065] The disclosure also contemplates the use of plant systems for expression of antigens of the disclosure. The plant tissue or purified polypeptide may be used for immunization. For example, agroinfiltration is a method by which transient expression of genes or production or protein is accomplished in a plant. In the method a suspension of Agrobacterium is delivered to a plant leaf, where the agrobacterium transfers a desired coding sequence (e.g., a CAMP polynucleotide) to plant cells. A strain of Agrobacterium is transformed with a polynucleotide comprising the coding sequence of interest. Subsequently the strain is grown in culture the agrobacterium is then injected into a plant tissue (e.g., the airspaces inside the leaf through stomata. Once inside the leaf polynucleotide is then transiently expressed. Many plants can be transformed by this method, but the most common ones are *Nicotiana benthamiana* and *Nicotiana tabacum*.

[0066] In addition, the disclosure envisions immunization utilizing a combination of antigens and/or vectors. The disclosure contemplates a homologous or heterologous prime-boost vaccination strategy. The heterologous strategy may include priming with one vector, *e.g. L. monocytogenes* expressing one or more proteins, and boosting with another vector, *e.g.*, adenovirus expressing the same protein or proteins, or vice versa. Boosting may also include immunizing with a *P. acnes* protein or proteins or fragments thereof in an adjuvant. The specific examples provided herein demonstrate

the delivery of the antigens to an animal host utilizing various vaccination strategies and the resulting immunoprotection against *P. acnes* challenge.

[0067] The disclosure comprises several types of vaccines. One group of vaccines comprises an attenuated bacterial vector expressing one or more *P. acnes* antigens. Other vaccines of the disclosure comprise *P. acnes* antigens (*e.g.*, polypeptide or fusion polypeptides) in a suitable adjuvant. Another group of vaccines of the disclosure comprise a viral vector (*e.g.*, adenovirus) expressing one or more *P. acnes* antigens.

[0068] Each vaccine is administered, *e.g.* transdermally, subcutaneously, intramuscularly, intranasally, inhaled, or even orally to a mammalian host. The vaccine can be administered as part of a homologous or heterologous prime-boost strategy. Most importantly, the vaccine protects the mammalian hosts against infection with *P. acnes*.

[0069] A "vaccine" as used herein refers to a composition of matter comprising a molecule that, when administered to a subject, induces an immune response. Vaccines can comprise polynucleotide molecules, polypeptide molecules, and carbohydrate molecules, as well as derivatives and combinations of each, such as glycoproteins, lipoproteins, carbohydrate-protein conjugates, fusions between two or more polypeptides or polynucleotides, and the like. A vaccine may further comprise a diluent, an adjuvant, a carrier, or combinations thereof, as would be readily understood by those in the art.

[0070] A vaccine may be comprised of separate components. As used herein, "separate components" refers to a situation wherein the vaccine actually comprises two discrete vaccines to be administered separately to a subject. In that sense, a vaccine comprised of separate components may be viewed as a kit or a package comprising separate vaccine components. For example, in the context of the disclosure, a package may comprise a first immunogenic composition comprising an attenuated bacterial vector and a second antigenic composition comprising an attenuated viral vector comprising the same or

different *P. acnes* antigens (*e.g.*, CAMP factor, a lipase, or a sialidase).

[0071] A vaccine "induces" an immune response when the antigen or antigens present in the immunogenic compositions/vaccine cause the vaccinated subject to mount an immune response to that antigen or antigens. The vaccinated subject will generate an immune response, as evidenced by activation of the immune system, which includes the production of vaccine antigen-specific T cells, vaccine antigen-specific B cells, vaccine antigen-specific antibodies, and cytokines. The resulting immune response may be measured by several methods including ELISPOT, ELISA, chromium release assays, intracellular cytokine staining, FACS analysis, and MHC tetramer staining (to identify peptide-specific cells). A skilled artisan may also use these methods to measure a primary immune response or a secondary immune response.

[0072] An "antigen" is a substance capable of generating an immune response in a subject exposed to the antigen. Antigens are usually polypeptides and are the focus of the host's immune response. An "epitope" or "antigenic determinant" is that part of an antigen to which T cells and antibodies specifically bind. An antigen may contain multiple epitopes. Antigens of the disclosure comprise *P. acnes* extracellular or immunogenic polypeptides (*e.g.*, lipase, CAMP Factor and those set forth in Table 1). In specific embodiments, the *P. acnes* polypeptides comprises an antigenic fragment of a polypeptide comprising the sequence as set forth in SEQ ID NO: 2, 3, 7 or 9 and antigenic fragments of human ASMase of SEQ ID NO:11 or fragments that have at least 80%-99% identity to an antigenic fragment of SEQ ID NO: 2, 3, 7, 9 and 11.

[0073] In one embodiment, the vaccine comprises an antigenic fragment of a *P.acnes* CAMP factor. An antigenic fragment typically comprises at least 6 amino acid (e.g., 6-10, 10-12, 12-20, 30-50 or more amino acids). Typically the antigenic fragment is a fragment of the protein found on the surface of the protein and is comprises a soluble domain. In one embodiment, the antigenic fragment may be part of a fusion

protein comprising one or more non-contiguous sequence of the protein (e.g., non-contiguous sequence of CAMP factor of *P.acnes*). In one embodiment, the vaccine comprises an antigenic domain of a polypeptide comprising SEQ ID NO:7. In one embodiment, the vaccine comprises a polypeptide comprising a sequence at least 80%, 90%, 95%, 98% or 99% identical to SEQ ID NO:7. In yet another embodiment, the vaccine comprises a polypeptide having a sequence as set forth in SEQ ID NO:7 or a fragment thereof of at least 6 amino acids and which is capable of producing antibodies that specifically bind to a P.acnes CAMP factor (e.g., a polypeptide consisting of SEQ ID NO:7).

[0074] A vaccine can include killed and disrupted *P. acnes*. As described more fully below the killing and disruption results in the release of various antigens that may not be secreted unless cultured in an anaerobic environment. Thus, in one embodiment, the disclosure contemplates culturing *P. acnes* under anaerobic conditions followed by killing and/or disruption of the bacterial and preparing a vaccine from the disrupted *P. acnes* preparation. The disrupted preparation may be further purified to enrich an antigen or antigens in the immunogenic compositions. In another embodiment, the anaerobically cultured *P. acnes* are killed by gamma irradiation or other methods known to those of skill in the art and the whole bacterial cell used in an immunogenic preparation.

[0075] A priming vaccine used in some embodiments of the disclosure comprises a *P. acnes* CAMP Factor, lipase or sialidase antigen. The priming vaccine comprises an antigenic epitope of a *P. acnes* antigen, the full length antigen, a vector comprising a polynucleotide encoding the antigen and the like. In one embodiment, the priming vaccine comprises a polynucleotide encoding an antigen under control of a foreign promoter within a bacterium, plant cell or virus. The polynucleotide of the priming vaccine is present in a suitable delivery vector such as a plasmid or other vector such as a bacterial, plant cell or viral vector. The polynucleotide may

be under the control of a suitable promoter such as a promoter derived from the HCMV IE gene. The priming vaccine is administered in an amount effective for priming an immune response to the *P. acnes* antigen. As used herein, "priming" of an immune response occurs when an antigen is presented to T cells or B cells. As a result, primed cells can respond to the same antigen again as memory cells in a second, subsequent immune response. Thus, priming generates both the primary immune response and establishes immunological memory. One skilled in this art appreciates that a primary immune response represents the adaptive immune response upon initial exposure to an antigen in a particular context such as in the pathogen or in a vaccine. However, it will also be appreciated that the disclosure is not limited to use of the priming vaccine in the context of immunologically naive individuals. Rather, priming may also occur in individuals who have been exposed to the antigen but who have not received the priming vaccine.

[0076] The priming immunogenic (vaccine) composition may be administered once prior to administration of the boosting immunogenic (vaccine) composition. In another embodiment, the priming vaccine may be administered several times.

[0077] The boosting vaccine used in the method of the disclosure may comprise at least one *P. acnes* antigen (e.g., CAMP Factor, lipase or sialidase antigen polypeptide or fragment thereof) corresponding to the antigen of the priming vaccine. In addition, the boosting vaccine may comprise (in addition to the priming antigen) a different antigen or vector comprising the antigen or coding region thereof. In one embodiment, the boosting vaccine comprises a *P. acnes* polypeptide antigen to enhance the immunogenicity of the subject to *P. acnes*. For example in one embodiment, the boosting vaccine comprises a *P. acnes* antigen expressed in a viral vector. The *P. acnes* antigen can be selected from the group of antigen listed as upregulated in Table 1 including, but not limited to, CAMP Factor, lipase, sialidase antigens, fragments or combinations thereof.

[0078] The boosting vaccine is administered in an amount effective for "boosting" a primed immune response to the *P. acnes* antigen. As used herein, "boosting" an immune response means to induce a secondary immune response in a subject that has been primed (*i.e.*, already exposed) by an initial exposure to an antigen. A secondary immune response is characterized by the activation and expansion of specific memory T cells and B cells. Thus, boosting a specific immune response augments the primed immune response by inducing immune cells to proliferate and differentiate upon subsequent exposure to that antigen. The boosting vaccine may achieve one or more of the following effects: induces CD4+ T cells, induces anti-*P. acnes* antibodies (e.g., antibodies to the antigen in the vaccine), boosts the activity of the CD8+ T cells primed by the priming vaccine, and induces additional CD8+ T cells not originally identified in the initially primed immune response. The boosting vaccine may also induce CD4+ T cells and induce anti-*P. acnes* antibodies (e.g., anti-CAMP factor antibodies).

[0079] The existence of an immune response to the first dose of the immunoprotective composition may be determined by known methods (*e.g.*, by obtaining serum from the individual before and after the initial immunization, and demonstrating a change in the individual's immune status, for example an immunoprecipitation assay, or an ELISA, or a bactericidal assay, or a Western blot, or flow cytometric assay, or the like) prior to administering a subsequent dose. The existence of an immune response to the first dose may also be assumed by waiting for a period of time after the first immunization that, based on previous experience, is a sufficient time for an immune response and/or priming to have taken place. Boosting dosages of an immunoprotective composition can be administered as needed.

[0080] Certain vaccine adjuvants are particularly suited to the stimulation of either Th1 or Th2-type cytokine responses. Traditionally, the best indicators of the Th1:Th2 balance of the immune response after a vaccination or infection includes direct measurement of the production of Th1 or Th2 cytokines

by T lymphocytes *in vitro* after restimulation with antigen, and/or the measurement of the IgG1:IgG2a ratio of antigen specific antibody responses. Thus, a Th1-type adjuvant is one which stimulates isolated T-cell populations to produce high levels of Th1-type cytokines when re-stimulated with antigen *in vitro*, and induces antigen specific immunoglobulin responses associated with Th1-type isotype.

[0081] The disclosure further relates to antibodies for the prevention and/or treatment of a *P. acnes* infection. In a first embodiment, an antibody is raised against a *P. acnes* antigen of the disclosure. Such antibodies are produced by administering an antigenic composition comprising an antigenic polypeptide (e.g., a *P. acnes* CAMP Factor), a vector expressing an antigenic polypeptide or a purified preparation of a *P. acnes* antigen as a vaccine.

[0082] Antibodies (such as anti-CAMP Factor or anti-ASMase antibodies) according to the disclosure will be administered in one or more dosages, and the amount needed will depend on during which phase of the disease the therapy is given as well as on other factors. In order to produce such antibodies, the antigenic composition (such as an antigenic fragment of an CAMP factor or ASMase) according to the disclosure will be administered to a subject in order to induce the production of the above described antibodies. The antibodies can be monoclonal antibodies. Once obtained, such novel antibodies may be produced by conventional techniques and used in therapy. In general, a monoclonal antibody to an epitope of an antigen can be prepared by using a technique which provides for the production of antibody molecules from continuous cell lines in culture and methods of preparing antibodies are well known to the skilled in this field (see *e.g.* Coligan (1991) Current Protocols in Immunology, Wiley/Greene, NY; Harlow and Lane (1989) Antibodies: A Laboratory Manual, Cold Spring Harbor Press, NY; and Goding (1986) Monoclonal Antibodies: Principles and Practice (2nd ed) Academic Press, New York, N.Y.). In addition, such antibodies may be humanized using

techniques known in the art. Furthermore, the antibody may include antibody fragments known in the art.

[0083] Passive immunization is the induction of immunity acquired by the transfer of antibodies from another individual (A Keller and Stiehm, 2000). There are many advantages of passive immunization. (a) Unlike active immunization (vaccines), biological effects of passive immunization are immediate and can be of value, as in cases where symptoms have already occurred. Thus, the modality using passive neutralization of *P. acnes* CAMP factor may benefit patients who have already developed acne. (b) No cell-mediated immunity and no direct bactericidal effect that will have low impact on microbe commensalisms. (c) No adjuvant-derived side effects are induced. (d) The administered dose can be adjusted based on the severity of disease. (e) It can be easily combined with other therapies. Additionally, unlike active immunization, which requires time to induce protective immunity and depends on the host's ability to mount an immune response, passive antibody can theoretically confer protection regardless of the immune status of the host (Casadevall, 2002). The disclosure demonstrates that passive immunization targeting secretory CAMP factors instead of bacterial surface proteins can neutralize the *P. acnes* virulence without directly killing bacteria, lowering the risk of creating drug-resistant *P. acnes* and altering the commensalisms of *P. acnes*.

[0084] As demonstrated herein, a synergistic effect can be seen in the treatment of a *P. acnes* infection by immunizing with an antigenic CAMP factor peptide or polypeptide and also contacting the subject with an antibody to ASMase or an ASMase inhibitor. For example, the disclosure contemplate treating or preventing a P. acnes infection using (a) a vaccine comprising a *P. acnes* CAMP factor peptide or polypeptide, (b) an anti-ASMase antibody, (c) an ASMase inhibitor, and (d) any combination of the foregoing. Again, as used herein a CAMP factor peptide or polypeptide does not necessarily refer to the origin of the peptide or polypeptide but rather to (a) the sequence, which will have some degree of identity to the wild-

type CAMP factor; and (b) the ability of an antibody developed against such a sequence to recognize

[0085] For therapeutic purposes, the antibody is formulated with conventional pharmaceutically or pharmacologically acceptable vehicles for administration, conveniently by injection. Vehicles include deionized water, saline, phosphate-buffered saline, Ringer's solution, dextrose solution, Hank's solution, and the like. Other additives may include additives to provide isotonicity, buffers, preservatives, and the like. The antibody may be administered parenterally, typically intravenously or intramuscularly, as a bolus, intermittently or in a continuous regimen.

[0086] Methods for ameliorating *P. acnes* in a subject by administering to the subject a *P. acnes* antigen(s) (e.g., a P. acnes CAMP Factor protein, polypeptide, peptide) or a vector comprising a *P. acnes* antigen alone or in combination with an anti-SMase (e.g., an anti-ASMase) antibody or inhibitor thereof, in a pharmaceutically acceptable carrier, are also provided. In addition, methods for ameliorating *P. acnes* in a subject, by administering to the subject antibodies that bind to *P. acnes* antigens or to an ASMase, in a pharmaceutically acceptable carrier, are also provided.

[0087] Attenuated vaccines can be administered directly to the mammal. The immunogenic compositions and vaccines obtained using the methods of the disclosure can be formulated as pharmaceutical compositions for administration in any suitable manner. One route of administration is oral. Other routes of administration include rectal, intrathecal, buccal (*e.g.*, sublingual) inhalation, intranasal, and transdermal and the like (see *e.g.* U.S. Pat. No. 6,126,938). Although more than one route can be used to administer a particular composition, a particular route can often provide a more immediate and more effective reaction than another route.

[0088] The immunoprotective compositions to be administered are provided in a pharmaceutically acceptable solution such as an aqueous solution, often a saline or buffered solution, or they can be provided in powder form. There is a wide variety

of suitable formulations of pharmaceutical compositions of the disclosure. See, *e.g.*, Lieberman, Pharmaceutical Dosage Forms, Marcel Dekker, Vols. 1-3 (1998); Remington's Pharmaceutical Science, 17th ed., Mack Publishing Company, Easton, Pa. (1985) and similar publications. The compositions may also include an adjuvant. Examples of known suitable adjuvants include alum, aluminum phosphate, aluminum hydroxide, and MF59 (4.3% w/v squalene, 0.5% w/v Tween 80, 0.5% w/v Span 85)--these are the only ones currently licensed for use in humans. For experimental animals, one can use Freund's, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dip-almitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion, or Bacille Calmette-Guerin (BCG). The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogenic antigen.

[0089] The concentration of immunogenic antigens of the disclosure in the pharmaceutical formulations can vary widely, *e.g.*, from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, and the like, in accordance with the particular mode of administration selected.

[0090] Formulations suitable for oral administration can comprise (a) liquid solutions, such as an effective amount of the recombinant bacteria or polypeptide suspended in diluents, such as buffered water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as lyophilized powder, liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, sucrose, mannitol, sorbitol, calcium

phosphates, corn starch, potato starch, tragacanth, microcrystalline cellulose, acacia, gelatin, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening agents, preservatives, flavoring agents, dyes, disintegrating agents, and pharmaceutically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or sucrose and acacia emulsions, gels, and the like containing, in addition to the active ingredient, carriers known in the art. It is recognized that the attenuated vaccines, when administered orally, must be protected from digestion. This is typically accomplished either by complexing the vaccines with a composition to render it resistant to acidic and enzymatic hydrolysis or by packaging the vaccines in an appropriately resistant carrier such as a liposome or enteric coated capsules. Means of protecting the attenuated bacteria or antigen from digestion are well known in the art. The pharmaceutical compositions can be encapsulated, *e.g.*, in liposomes, or in a formulation that provides for slow release of the active ingredient.

[0091] The vaccines, alone or in combination with other suitable components, can be made into aerosol formulations (*e.g.*, they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

[0092] The dose administered to a subject, in the context of the disclosure should be sufficient to effect a beneficial therapeutic and/or prophylactic response in the subject over time. The dose will be determined by the efficacy of the particular vaccine employed and the condition of the subject, as well as the body weight or vascular surface area of the subject to be treated. The size of the dose also will be determined by the existence, nature, and extent of any adverse

side-effects that accompany the administration of a particular vaccine in a particular subject.

[0093] In determining the effective amount of the vaccine to be administered in the treatment or prophylaxis of an infection or other condition, the physician evaluates vaccine toxicities, progression of the disease, and the production of anti-vaccine antibodies, if any.

[0094] The compositions are administered to a subject that is at risk from acquiring an infection caused by *P. acnes* or to prevent or at least partially arrest the development of the infection and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose" or "therapeutic effective amount." Amounts effective for therapeutic use will depend on, *e.g.*, the antigen composition, the manner of administration, the weight and general state of health of the subject, and the judgment of the prescribing physician. Single or multiple doses of the antigen compositions may be administered depending on the dosage and frequency required and tolerated by the subject, and route of administration. In addition, a booster may be administered in the same or different formulation. For example, the method contemplates administration of a first composition comprising a *P. acnes* antigen in an attenuated bacterial vector and a second composition comprising a *P. acnes* antigen in an attenuated non-bacterial vector. The second composition may be administered simultaneously or subsequent to administration of the first immunogenic composition.

[0095] In particular embodiments, a therapeutically effective dose of the immunoprotective composition is administered to a subject. Amounts of live attenuated bacteria or non-bacteria expressing the *P. acnes* or other antigens present in the initial immunization generally range from about $5x10^5$ to $5x10^{11}$ organisms per subject, and more commonly from about $5x10^8$ to $5x10^9$ organisms per subject.

[0096] The immunoprotective compositions are typically administered to an individual that is immunologically naive

with respect to *P. acnes*. Usually, 2-4 doses of an immunological composition of the disclosure may be sufficient, however additional doses may be required to achieve a high level of immunity. Additional booster doses may be given every 1-5 years, as necessary, to maintain a high level of immunity.

[0097] In general, administration to any individual should begin prior to the first sign of disease, or possibly at the first sign of possible or actual exposure to *P. acnes*.

[0098] The vaccines of the disclosure can be packaged in packs, dispenser devices, and kits for administering vaccines to a mammal. For example, packs or dispenser devices that contain one or more unit dosage forms are provided. Typically, instructions for administration of the compounds will be provided with the packaging, along with a suitable indication on the label that the compound is suitable for treatment of an indicated condition. For example, the label may state that the active compound within the packaging is useful for treating a particular infectious disease or for preventing or treating other diseases or conditions that are mediated by, or potentially susceptible to, a mammalian immune response.

[0099] The following specific examples are meant to be illustrative and non-limiting. Those of skill in the art will recognize various modification and substitutions that can be made in the compositions and methods that follow. Such modification and substitutions do not depart from the disclosure and are encompassed herein.

EXAMPLES

[00100] The disclosure indicates that injection of *P. acnes* (ATCC 6919; 10^8 CFU) into ICR mouse ears induced an increase in the ear thickness (Fig. 1) and gramulomatous response (Fig. 2A, B). One day after injection, *P. acnes* was surrounded by a densely packed granulomatous infiltrate (Fig. 2C). *P. acnes* immigrated to hair follicles and aggregated in the sebaceous glands two days after injection (Fig. 2 D, E). Although ears injected with *S. epidermidis* (ATCC 12228; 10^8 CFU) had a minor swelling, this swelling can be rapidly subsided within four days. Thus, the disclosure provides a model for measurement

and testing of agents that effect *P. acnes*. For example, the mouse ear model of the disclosure can be used to test the cytotoxicities of virulence factors of *P. acnes*. Additionally, the model is useful for evaluating the anti-inflammatory effects of *P.acnes* vaccines.

[00101] When *P. acnes* is grown on a sheep blood agar plate in close proximity to beta-hemolytic microorganisms, such as *Staphylococcus aureus* (*S. aureus*) and *Clostridium perfringens*, it synergistically enhances hemolysis similar to the classical Christie, Atkins, Munch-Peterson (CAMP). CAMP reactions are induced by the combination of CAMP factor co-hemolysin, which is a pore-forming toxin, and sphingomyelinase (SMase) derived from the other bacterial partner. CAMP factor itself has only weak hemolytic activity on the erythrocytes, but pretreating the cells with SMase enhances its activity. The entire genomic sequence of *P. acnes* includes numerous genes whose products are involved in degrading host molecules, and five genes encoding CAMP factor homologs of *Streptococcus agalactiae* (*S. agalactiae*) have been found in the genome information. This analysis of *P. acnes* proteins by a proteomic technique utilizing isotope-coded protein labels coupled to NanoLC-MS analysis revealed that one of the CAMP factor homologs (accession number: gi/50842175, incorporated herein by reference), showing 42% identity in nucleotide sequence to the *S. agalactiae* CAMP factor, is produced at higher concentrations by bacteria cultured under anaerobic condition than under aerobic conditions. These data suggest a physiological significance for the CAMP factor for *P. acnes*.

[00102] SMases have been widely isolated and characterized from bacteria, yeast, and various tissues and biological fluids of mammalians. In spite of low identity between bacterial and mammalian SMases, the amino acid sequences share a number of conserved residues, suggesting a common catalytic mechanism. The disclosure demonstrates that *P. acnes* benefits from a host SMase that amplifies its CAMP factor-mediated pathogenicity. The disclosure show a host SMase in CAMP factor-mediated pathogenicity of *P. acnes* both in vitro and in

vivo, and the synergistic potential of a vaccine treatment targeting CAMP factor and local injection with IgG against a host SMase in *P. acnes*-associated inflammatory acne vulgaris.

[00103] A tissue chamber model was used to detect pro-inflammatory cytokines and bacterial growth. The tissue chamber model was first described and extensively characterized in the guinea pig and then adapted to the mouse. This model accurately mimics bacterial infections *in vivo*. Because bacteria are inoculated directly into the chamber, with no adherence and invasion step through epithelia, the minimal infective dose of *P. acnes* which is required for a persistent infection reflects virulence. The host response is mediated exclusively by phagocytes and comprises antimicrobial peptides, cytokines, chemokines, leukocyte infiltration, and apoptosis. A tissue chamber model was utilized to evaluate the efficacy of anti-*P. acnes* vaccines. A tissue chamber (Fig. 3) was subcutaneously implanted into abdominal skin of ICR mice for 7 days.

[00104] After implantation of tissue chambers, *P. acnes*, *S. epidermidis* (20 μl; 10^7 CFU) or PBS (50 μl) were injected into tissue chambers. Three days after bacterial injection, tissue chamber fluids were harvested for detection of macrophage-inflammatory protein (MIP)-2. Compared with PBS-injected mice, the level of MIP-2 was significantly increased by three and two folds in *P. acnes*- and *S. epidermidis*-injected mice, respectively (Fig. 4A). *In vivo* survival (colonies) of *P. acnes* can be detected on MHB agar plates after spreading the tissue chamber fluids on plates (Fig. 4B).

[00105] In humans *P. acnes* multiplies in anaerobic environment where sebum accumulate in clogged follicles. It has been demonstrated that the level of lipase production was increased markedly by *P. acnes* in the absence of oxygen. In an effort to identify the virulence factors which were highly expressed under anaerobic conditions, a comprehensive quantitative proteome analysis was performed on *P. acnes* in the presence or absence of oxygen. Identified virulence factors were selected as *P. acnes* vaccine candidates. Changes in the abundances of

individual protein species in O_2-grown *P. acnes* as compared to no-O_2-grown *P. acnes* were analyzed using a nongel-based isotope-coded protein label (ICPL) method. 342 *P. acnes* proteins were identified and sequenced by LTQ MS/MS. 152 of 342 proteins were successfully labeled with ICPL. 23 proteins were identified as up- or down- regulated under anaerobic or aerobic conditions (Table 1). Two secretory virulence factors (CAMP factor and lipase) were highly expressed under anaerobic conditions (Fig. 5A and B). Two internal peptides (SYSEKHLGVAFR (SEQ ID NO:1) and DLLKAAFDLR (SEQ ID NO:2)) of lipase and CAMP factor were presented, respectively (Fig. 5C and D). Although it is well known that lipase is involved in the pathogenesis of acne lesions, the role of CAMP factor in acne development is totally unknown. More recently, it was reported that CAMP factor of *Streptococcus agalactiae* behaved as a pore-forming toxin. Thus CAMP factor was selected as a target for the development of *P. acnes* vaccines.

[00106] Treatment of sialidase increases the susceptibility of human sebocytes to *P. acnes*. A variety of sialidases have been uncovered in the genome of *P. acnes*. Three sialidases were selected from *P. acnes* genome for cloning, including a cell wall-anchored sialidase (accession # gi|50843035; (SEQ ID NO:12 and 13, polynucleotide and polypeptide, respectively), a secreted sialidase B (accession# gi|50842171; SEQ ID NO:14 and 15, polynucleotide and polypeptide, respectively) and a sialidase-like protein (accession# gi|50843043; SEQ ID NO:16 and 17, polynucleotide and polypeptide, respectively). The cell wall-anchored sialidase (accession # gi|50843035) exhibits the strongest enzyme activity in removing the sialic acids from the surface of human SZ95 sebocytes. In addition, the selected *P. acnes* sialidase is a surface protein that potentially serves as an excellent target for vaccine development. Thus, this protein was selected to investigate immunogenicity. The cell wall-anchored sialidase contains an LPXTG cell wall-anchoring motif in the C-terminal domain. Although it is known that sialidase of *P. acnes* shares identities (~ 30%) with sialidase (EC 3.2.1.18) (accession#

Q02834) of *Micromonospora viridifaciens* and a cell wall surface anchor family protein (accession# Q04M99) of *Streptococcus pneumoniae* serotype 2, the immunogenicity of sialidase of *P. acnes* was unexplored.

[00107] A gene encoding sialidase was PCR amplified from template DNA prepared from *P. acnes.* Specific primers including the sense and anti-sense primer were designed. The PCR products were inserted into a pEcoli-Nterm 6xHN plasmid and expressed in *E. coli* [*E. coli BL21 (DE3)*]. After IPTG induction, over-expressed sialidase-6xNH fusion protein from *E. coli* was detected in a Coomassie blue stained SDS-PAGE gel at approximately 53.1 kDa molecular weight. The sialidase-6xNH fusion protein was purified using a TALON resin column. The sialidase expression was confirmed by Matrix Assisted Laser Desorption Ionisation - Time of Flight (MALDI-TOF MS) as well as NanoLC-MS/MS sequencing. Purified sialidase-6xNH fusion protein was in-gel digested with trypsin prior to NanoLC-MS/MS analysis. Nineteen internal peptides derived from sialidase were fully sequenced by NanoLC-MS/MS analysis via an HCTultra PTM system ion trap mass spectrometer. The MS/MS spectra of sequenced peptides matched well with those of sialidase (accession # gi|50843035) of *P. acnes*. An internal peptide (VVELSDGTLMLNSR; 316-329 amino acid residue of SEQ ID NO:13) of sialidase was present. These results indicate that sialidase was expressed in *E. coli BL21 (DE3)* and suggest that mass spectrometry provided a powerful modality to confirm the expression of a protein that has no antibody available for western blot.

[00108] To determine the enzyme activity, purified sialidase-6xNH fusion protein (10 µg/ml) was added to human SZ95 sebocyte culture for 2 h to remove the sialic acids on the surface of sebocytes. The amount of surface sialic acids was determined by flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA) using the reaction of biotinylated *Maackia Amurensis* lectin I and streptavidin-FITC conjugate. The fluorescence of MAA labeled-sailic acids in sialidase-treated sebocytes was dramatically decreased by 63% (Fig. 6A), whereas the

fluorescence in GFP-treated sebocytes was unchanged (Fig. 6B). The data indicated that purified sialidase retained an enzyme activity. After treatment with sialidase (10 μg/ml) for 2h, sebocytes (10^6 cells) were exposed to live *P. acnes* (10^7 CUF) overnight. Live *P. acnes* induced an approximately 15~20% of cell death in untreated or GFP-treated sebocytes. However, the *P. acnes*-induced cell death in sialidase-treated sebocytes was significantly increased by 35% (Fig. 6C), demonstrating that the treatment of sialidase increases the susceptibility of sebocytes to *P. acnes*. It has been demonstrated that incubation of human buccal epithelial cells with the sialidase significantly increased *Pseudomonas aeruginosa* adherence. The adherence of *P. acnes* into sialidase-treated sebocytes was examined. The results showed that pre-treatment with sialidase (10 μg/ml for 2h), but not GFP, considerably increased the adherence of *P. acnes* into sebocytes (Fig. 6D). Accustain Gram stains indicated that the number of *P. acnes* interacted with sebocytes was increased once surface sialic acids of sebocytes were removed by sialidase (Fig. 6E-G). Therefore inhibiting the activity of sialidase or neutralizing sialidase by antibodies, siRNA, small molecules inhibitors the bind the active site, antisense and the like can be useful in providing protection against *P. acnes* infection. Antisense molecules can be generated based upon the polynucleotide sequences provided herein.

[00109] The intact particle of *E. coli* has been used as a vector for intranasal and epicutaneous vaccination. To engineer *E. coli* vector-based vaccines targeting an anthrax spore coat associated protein (SCAP), a gene encoded SCAP was constructed into the pET15b vector (EMD Biosciences, Inc.). After IPTG induction, *E. coli* carrying either an empty expression vector or SCAP expression plasmid were killed by UV irradiation. For immunization, the UV-irradiated *E. coli* vector-based vaccine, not mixed with exogenous adjuvants, was then directly administered into the nasal cavity of mice. Sera harvested from each group (n = 4) of mice three weeks after immunization was pooled. The production of anti-SCAP IgG was

detected by an antigen array. The antigen micrparray was created by spotting with recombinant SCAP, maltose binding protein (MBP)-tagged SCAP, and mouse IgG (positive controls). The sera from mice immunized with an empty expression vector and an *E. coli* vector with SCAP over-expression were hybridized on arrays. While the negative control yielded only background signals, the positive control (IgG) generated a dilution-dependent signal reduction. The experiment showed that anti-SCAP antibody was produced after immunization with *E. coli* vector-based vaccine (*E. coli BL21 (DE3) T7/lacO SCAP*) by comparing to control serum. More importantly, the anti-SCAP antibody can be produced without boosting.

[00110] A gene encoded SCAP was also constructed into the pCAL-n-FLAG vector (Fig. 7C) followed by transformation into the *E. coli* BL21 strain which served as the antigen carrier. For safety concerns, transformed *E. coli* was destructed by gamma irradiation. ICR mice were immunized via intranasal administration with irradiated *E. coli* vectors encoding SCAP. Antibody production was measured via western blot by reaction with serum obtained from one month post-immunized mice. Mice were able to produce detectable levels of antibody to SCAP without any boost. Pretreatment of a nonionic surfactant (tetradecyl-β-D-maltoside; TDM) (Antatrace Inc., Maumee, OH) on the skin surface of ICR mice at a concentration of 0.125 % (in sterile water) for 15 min slightly disrupts the stratum corneum barrier but greatly enhances the epicutaneous immunization of *E.coli* based-tetanus toxin C fragment vaccine (*E.coli* BL21 *nir/B* tetC). Pretreatment of TDM (0.125%) on the skin surface of ICR mice for 15 min, followed by washing and then the epicutaneous application of irradiated *E.coli* vectoring SCAP, these mice can produce an antibody response to SCAP. Antibody production was measured via western blot by reaction with serum obtained from one month post-immunized mice. Mice were able to produce detectable levels of antibody to SCAP without any boost. These results demonstrate that SCAP is an immunogenic anthrax protein when mice were non-invasively immunized with *E. coli* vector-based vaccines. The

results demonstrated that TDM can disrupt the stratum corneum barrier of the skin which significantly potentiates skin immunity. The disclosure thus provides an immunization protocol to immunize with CAMP factor and sialidase.

[00111] A UV-irradiated *E. coli* vector-based vaccine [*E. coli BL21 (DE3) T7/lacO Sialidase*] was used to test the immunogenicity of *P. acnes* sialidase (accession # gi|50843035). A dose of UV (4,500 J/m^2) was given to irradiate all *E. coli*, both expressing (a *Sialidase-vector*) and not expressing *Sialidase* genes (a LacZ-*empty vector*), as demonstrated by the inability to form colonies on LB agar plates. The amount of sialidase in *E. coli* vectors was not changed after UV-irradiation. ICR mice were intranasally immunized with UV-irradiated *E. coli BL21 (DE3) T7/lacO Sialidase* and boosted 3 weeks after the first nasal inoculation. The production of antibody (IgG) in mouse sera was detected 3 and 6 weeks after immunization by western blot. A strong band appearing at approximately 53.1 kDa was visualized when a sialidase-6xNH fusion protein transferred membrane was reacted with mouse serum harvested 6 weeks after immunization. No sialidase-reacted antibody production was found in LacZ-empty vector-immunized mice. ICR mice were also immunized with sialidase-6xNH fusion protein or GFP using Freund/(in)complete adjuvants. The antibody production was detected by western blot analysis three weeks after immunization. A strong band appearing at 53.1 kDa was visualized when the sialidase-6xNH fusion protein was reacted with serum from sialidase-immunized mice. The antibody production was also confirmed by antigen microarray. An antigen microarray was created by printing with sialidase-6xNH fusion protein and mouse IgG (a positive control). After hybridization with mouse sera, fluorescent signals displayed in antigen microarrays indicated sialidase antibody production. The sialidase antibody was detectable in the serum harvested from mice three week after immunization. No sialidase-reacted antibody production was found in GFP-

immunized mice. Data from both antigen microarray and western blot confirmed that sialidase was an immunogenic protein.

[00112] Protective immunity of sialidase-based vaccines to *P. acnes*-induced inflammation. ICR mice immunized with recombinant proteins (sialidase or GFP) using Freund/(in)complete adjuvants (Fig. 8) were challenged with live *P. acnes* (10^7 CFU). Three weeks after vaccination, one ear of mouse was subcutaneously injected with 25 μl of *P. acnes* (10^7 CFU) and the other ear was injected with 25 μl of PBS as a control. Injection of *P. acnes* induced ear thickness and redness. Ear thickness was measured every day for 9 days. Ear thickness in GFP-immunized mice was rapidly elevated by more than two folds one day after *P. acnes* challenge. The elevation of ear thickness was significantly reduced by more than 50% when mice were immunized with sialidase (Fig. 8). The ear redness in GFP-immunized mice was subsided 7 days after *P. acnes* challenge, whereas the recovery of ear redness in sialidase-immunized mice was occurred 3 days after *P. acnes* challenge. These results indicated that sialidase-immunized mice suppressed *P. acnes*-induced ear inflammation. The production of *P. acnes*-induced pro-inflammatory cytokines were also measured after vaccination. A tissue chamber model (Fig. 3) was employed to detect the level of *in vivo* pro-inflammatory cytokines. A tissue chamber was subcutaneously implanted into abdominal skin of ICR mice 7 days before *P. acnes* (10^7 CFU) injection. The data indicated that tissue chamber fluid contains various immune cells including macrophages ($CD11b^+$), neutrophils ($Gr\text{-}1^+$), NK cells ($CD49b^+$) and T cells ($CD3^+$), suggesting an influx of immune cells into a tissue chamber. Three days after *P. acnes* injection, tissue chamber fluids containing pro-inflammatory cytokines were drawn by pecutaneous aspiration. The level of MIP-2 cytokine in immunized mice was measured by ELISA. In the GFP-immunized mice, a significant increase in MIP-2 level was observed 3 days after *P. acnes* injection. Importantly, the *P. acnes*-induced increase of MIP-2 cytokine was reduced by 61% in the sialidase-immunized mice. These results demonstrate that

sialidase-based vaccines effectively decrease ear thickness and the production of pro-inflammatory cytokines in the mice.

[00113] Pretreatment of *P. acnes* with serum from sialidase-immunized mice significantly decreased the cytotoxicity of *P. acnes* to human sebocytes (Fig. 9). The culture of sebocytes with anti-GFP serum-treated *P. acnes* causes an approximately 30% of cell death, whereas the cell death of sebocytes was decreased to nearly 5% when cells were co-cultured with anti-sialidase serum-treated *P. acnes*. The results indicated that sialidase-immunized mice provoked antibodies that can effectively neutralize the cytotoxicity of *P. acnes* to human sebocytes.

[00114] The CAMP factor was up-regulated in *P. acnes* under anaerobic or aerobic conditions. Thus, whether CAMP factor exerts a toxic effect on skin cells was examined. Keratinocytes have been known to be one of the primary targets of *P. acnes* during acne lesions. Therefore, whether CAMP factor exerts a detrimental effect on keratinocytes in the ears of ICR mice was tested. Purified recombinant CAMP factor was obtained by using the same protocol used to clone and purify sialidase. The PCR products of CAMP factor were inserted into a pEcoli-Nterm 6xHN plasmid and expressed in *E. coli* [*E. coli BL21 (DE3 CAMP factor)*]. After IPTG induction, the expression of CAMP factor-6xNH fusion protein was detected in a Coomassie blue stained SDS-PAGE gel at approximately 36 kDa molecular weight (Fig. 11, lanes 1 and 2). The CAMP factor-6xNH fusion protein was purified using a TALON resin column and confirmed by NanoLC-MS/MS sequencing. Sixteen internal peptides derived from CAMP factor were fully sequenced by NanoLC-MS/MS analysis via an HCTultra PTM system ion trap mass spectrometer. The MS/MS spectra of sequenced peptides matched well with those of CAMP factor (accession # gi|50842175) of *P. acnes*. An internal peptide (AVLLTANPASTAK (SEQ ID NO:3); 147-159 amino acid of SEQ ID NO:11) of CAMP factor was presented (Fig. 11B). Purified CAMP factor and GFP (1 µg/µl) were subcutaneously injected into the ears of ICR mice. After one-day injection, apoptotic cells were examined

by terminal deoxynucleotidyltransferase dUTP nick end labeling (TUNEL) assay. Injection of recombinant CAMP factor did not induce detectable ear thickness. No apoptotic cells were detected from mice injected with GFP. Apoptotic cells are only detectable in the CAMP factor-injected mice, suggesting that CAMP factor is a toxic protein. During TUNEL assay, tissue sections of CAMP factor-injected ears were double-stained with a differentiated keratinocyte marker K10. The localization of apoptotic cells in keratinocytes suggested that CAMP factor is harmful to skin keratinocytes. To test the immunogenicity of CAMP factor, mice were immunized with a UV-irradiated *E. coli* vector-based vaccine [*E. coli BL21 (DE3) T7/lacO CAMP factor*]. A dose of UV (4,500 J/m^2) was given to irradiate all *E. coli*, both expressing (a CAMP factor-vector) and not expressing CAMP factor genes (a LacZ-empty vector). ICR mice were intranasally immunized with UV-irradiated *E. coli BL21 (DE3) T7/lacO* CAMP factor. The anti-CAMP factor antibody (IgG) in mouse sera was detectable 3 weeks after immunization. The result suggested that anti-CAMP factor antibody can be produced without boosting if mice were immunized with an irradiated *E. coli*-vector-based vaccine [*E. coli BL21 (DE3 CAMP factor)*]. No CAMP factor-reacted antibody production was found in the LacZ-empty vector-immunized mice. The anti-CAMP factor antibody can be also produced when mice were immunized with recombinant proteins/(in)complete adjuvants. ICR mice were immunized with CAMP factor-6xNH fusion protein or GFP using Freund/(in)complete adjuvants following the protocols as described above. The anti-CAMP factor antibody was detectable in the CAMP factor-, but not GFP-immunized mice. These results indicated that CAMP factor is immunogenic when mice were immunized with *E. coil*-vector based vaccines or recombinant proteins/(in)complete adjuvants. However, it is worthwhile to note that mice cannot produce anti-CAMP factor antibody if they were immunized with whole organism *P. acnes*.

[00115] Protective immunity in heat-killed *P. acnes*-immunized mice. The heat-killed *P. acnes* was used as an inactivated anti-*P. acnes* vaccines. After inactivating *P. acnes* at 65°C

for 30 min, inactivated *P. acnes* was spread on LB agar plates. The inability to form colonies indicated that the inactivation of *P. acnes* was completed. For immunization, heat-killed *P. acnes* (10^8 CFU; 50µl) was intranasally inoculated into ICR mice for three times (first inoculation, a first boost at third week, and a second boost at the sixth week). Mice inoculated with 50µl PBS serve as controls. The antibody production was detected by western blot ten weeks after immunization. For detection of protective immunity, ears of ICR mice immunized with heat-killed *P. acnes* were subcutaneously challenged with live *P. acnes* (10^7 CFU). Ear thickness was measured for three days. The challenge of *P. acnes* to PBS-inoculated mice induced a 1.8-fold increase in ear thickness. Importantly, the increase of *P. acnes*-induced ear thickness was decreased by 40% in the killed-*P. acnes*-immunized mice. Similarly, the *P. acnes*-induced ear redness was significantly suppressed in killed-*P. acnes*-immunized mice, suggesting that mice immunized with killed-*P. acnes* produced a protective immunity to *P. acnes* infection. The change in the level of pro-inflammatory cytokines was determined after immunization. A tissue chamber was subcutaneously implanted into abdominal skin of ICR mice for 7 days, and then *P. acnes* (10^7 CFU) was injected into the implanted tissue chamber. The level of MIP-2 cytokine in tissue chamber fluid was measured by ELISA three days after *P. acnes* injection. In the PBS-inoculated mice, a significant increase in MIP-2 level was observed 3 days after *P. acnes* injection. The increase of MIP-2 cytokine was reduced by more than 50% when mice were immunized with killed-*P. acnes*.

[00116] The methods next examined if the antibodies against sialidase and CAMP factor can be produced in killed *P. acnes*-immunized mice. 50 µg of purified recombinant sialidase, CAMP factor as well as lysates of *P. acnes* were subjected to a 12.5% SDS-PAGE gel and transferred to a PDVF membrane. The membrane was incubated overnight with mouse serum obtained from killed *P. acnes*-immunized mice. Many proteins with molecular weights greater than 50 kDa reacted positively to serum obtained from the killed *P. acnes*-immunized mice.

However, neither sialidase nor CAMP factor reacted to the serum, indicating that neither sialidase nor CAMP factor is not immunogenic if mice were immunized with whole organism *P. acnes*.

[00117] Overall, the data indicated that sialidase and CAMP factor are immunogenic when mice were immunized with *E. coli*-vector-based vaccines or recombinant proteins/(in)complete Freund adjuvants. Mice immunized with killed *P. acnes* produced antibodies against several proteins (>50 kDa), but not sialidase and CAMP factor. Mice immunized with a sialidase-based vaccine or killed *P. acnes* produced a significant protection against live *P. acne* challenge.

[00118] Bacteria culture. *P. acnes* (ATCC 6919) was cultured on Brucella broth agar, supplemented with 5% (v/v) defibrinated sheep blood, vitamin K, and hemin under anaerobic conditions using Gas-Pak (BD Biosciences, San Jose, CA) or aerobic conditions at 37°C. Bacteria isolated from a single colony were inoculated in Reinforced Clostridium Medium (RCM) (Oxford, Hampshire, England) and cultured at 37°C until logarithmic growth phase (OD600=0.7-2.0). *S. aureus* 113 (ATCC 35556) was cultured on Tryptic soy broth (TSB) agar plates. Bacteria isolated from a single colony were inoculated in TSB at 37oC overnight. Bacterial pellets were harvested by centrifugation at 5,000 g for 10 min.

[00119] Molecular Cloning and Expression of Recombinant CAMP factor. A polymerase chain reaction (PCR) product encoding a putative protein (29-267 amino acid residues) of CAMP factor (accession number: gi/50842175) was generated using gene-specific primers designed based on the complete genome sequence of *P. acnes*. The forward PCR primer (5'-TAAGGCCTCTGTCGACGTCGAGCCGACGACGACCATCTCG-3'; SEQ ID NO:4) included 16 nucleotides containing a SalI site to match the end of the In-Fusion Ready pEcoli-Nterm 6×HN vector (Clontech Laboratories, Inc., Mountain View, CA), and 26 nucleotides encoding the N-terminal of CAMP factor. The reverse PCR primer (5'- CAGAATTCGCAAGCTTGGCAGCCTTCTTGACATCGGGGGAG-3'; SEQ ID NO:5) consisted of 16 nucleotides containing a HindIII site to

match the end of the vector and 23 nucleotides encoding the C-terminal of the protein. PCR was performed by using *P. acnes* genomic DNA as a template. The amplified DNA products were inserted at the restriction enzyme sites into an In-Fusion Ready pEcoli-Nterm expression plasmid and transformed into competent cells [*Escherichia coli* (*E. coli*), BL21 (DE3), Invitrogen, Carlsbad, CA], which were subsequently selected on Luria-Bertani (LB) plates containing ampicillin (50 µg/ml) and cultured overnight at 37°C. For green fluorescence protein (GFP) expression as a control, pEcoli-Nterm-GFP vector (Clontech Laboratories) supplied with the kit as a positive control was used for transformation. An aliquot of the overnight culture was diluted 1:20 with LB medium and incubated at 37oC until reaching OD_{600}=0.7. Isopropyl-ß-D-thiogalactoside (IPTG) (1 mM) was added into the culture and incubated for 4 hr to induce protein synthesis. Bacteria were harvested by centrifugation and disrupted by sonication on ice for 5 min and lysed by centrifuging at 3,000 g for 30 min. The pellet was washed with PBS and dissolved in 50 mM sodium phosphate buffer containing 6 M guanidine HCl and 300 mM NaCl. The expressed protein possessing 6×HN tag was purified in denaturing condition with a TALON Express Purification Kit (Clontech Laboratories). The purified protein was dialyzed against H_2O, lyophilized, dissolved in ethylene glycol (1 mg/1.2 ml), and then refolded in 10 ml of 250 mM Tris-HCl buffer, pH 8.4, containing 5 mM cysteine, 0.5 mM cystine, and 1.5 M urea at 4°C overnight. The refolded protein was dialyzed against PBS and concentrated. A 10% SDS-polyacrylamide gel electrophoresis (PAGE) and coomassie blue staining were utilized to detect the protein expression.

[00120] Protein Identification via NanoLC- LTQ MS/MS Analysis. In-gel digestion with trypsin and protein identification via a NanoLC-LTQ mass spectrometry (MS) analysis were performed. The automated NanoLC-LTQ MS/MS setup consisted of an Eksigent Nano 2D LC system, a switch valve, a C18 trap column (Agilent, Santa Clara, CA), and a capillary reversed phased column (10 cm in length, 75 µm i.d.) packed with 5 µm, C18 AQUASIL resin

with an integral spray tip (Picofrit, 15 µm tip, New Objective, Woburn, MA). A reversed-phase LC directly coupled to a LTQ ion trap mass spectrometer (Thermo Electron, Waltham, MA) was run using a linear gradient elution from buffer A (H2O plus 0.1% formic acid) to 50% buffer A plus 50% buffer B (acetonitrile plus 0.1% formic acid) in 100 min. The instruments were operated in the data dependent mode. Data on the four strongest ions above an intensity of 2×10^5 were collected with dynamic exclusion enabled and the collision energy set at 35%. Large-scale MS/MS spectra were extracted using default value by Bioworks® 3.2 (Thermo Scientific, San Jose, CA). Charge state deconvolution and deisotoping were not performed. All MS/MS spectra were analyzed using in-house Sorcerer™ 2 system with SEQUEST (v.27, rev. 11) as the search program for protein identification. SEQUEST was set up to search the target-decoy ipi.MOUSE.v3.14 database containing protein sequences in both forward and reverse orientations (68627 entries in each orientation) using trypsin as the digestion enzyme with the allowance of up to five missed cleavages. The false positive rates were roughly determined by doubling the ratio between the number of decoy hits and the total number of hits. SEQUEST was searched with a fragment ion mass tolerance of 0.5 Da and a parent ion tolerance of 1.0 Da.

[00121] Co-hemolytic Activity of CAMP factor. Co-hemolytic reaction of recombinant CAMP factor was detected on a sheep blood agar. *S. aureus* 113 (ATCC 35556) (10 µl, 2×10^7 CFU/ml in PBS), used as a source of SMase, was streaked on an agar plate. Ten µl of recombinant CAMP factor (250 µg/ml) or GFP as a control protein (250 µg/ml) was spotted beside the *S. aureus* streak grown at 37°C for 18 hr under an aerobic condition.

[00122] Vaccination and titration of antibodies to CAMP factor. Female 8-week-old ICR mice were used in all experiments. ICR mice were housed according to institutional guidelines. The mice were intranasally vaccinated with E. coli [BL21 (DE3)] over-expressing CAMP factor or GFP that were inactivated by UV (3500 J/m^2) irradiation. Irradiated E. coli was unable to grow on a LB agar plate (data not shown). Twenty

five µl of E. coli suspension (1×10^9 CFU) was inoculated into the nasal cavity. Sera were individually collected for detection and titration of antibody to CAMP factor 2 and 3 weeks after vaccination.

[00123] To determine antibody titers to CAMP factor by enzyme-linked immunosorbent assay (ELISA), purified CAMP factor (5 ug/ml) was diluted with a coating buffer (0.015 M Na_2CO_2, 0.35 M $NaHCO_2$ and 0.05% NaN_3), and coated onto a 96-well ELISA plate (Corning, Lowell, MA) at 4°C overnight. The plates were washed with PBS containing 0.1% (w/v) Tween-20, and blocked with PBS containing 1% (w/v) bovine-serum albumin (BSA) and 0.1% (w/v) Tween-20 for 2 hr at room temperature. Antisera (1:10,000 dilution) obtained from mice vaccinated with E. coli over-expressing CAMP factor or GFP were added to the wells and incubated for 2 hr. Goat anti-mouse IgG (H+L) IgG-horseradish peroxidase (HRP) conjugate (Promega, Madison, WI) (1:5,000 dilution) was added, incubated for 2 hr, and then washed. HRP activity was determined with a OptEIA™ Reagent Set (BD Biosciences, San Diego, CA). The optical density (OD) of each well was measured at 450 nm.

[00124] Cell Culture, Determination of Cytotoxicity and Neutralization assay. A human keratinocyte cell line, HaCaT, or a murine macrophage cell line, RAW264.7, was cultured in Dulbecco's Modified Eagle's Medium (DMEM) or Roswell Park Memorial Institute (RPMI) 1640 medium, respectively, supplemented with 10% heat-inactivated fetal bovine serum (FBS) at 37°C with 5% (v/v) CO_2. For determination of cytotoxicity of CAMP factor, cells (1×10^5/well) were incubated in a 96-well micro titer plate with recombinant CAMP factor or GFP in a 1% FBS-medium for 18 hr. After incubation, cell viability was determined with an acid phosphatase (ACP) assay. Cells were washed with PBS three times and incubated with 100 µl of 10 mM p-nitrophenyl phosphate (pNPP) in an ACP assay buffer [1 M sodium acetate buffer, pH5.5, containing 0.1% (w/v) triton-X] for 1 hr at 37°C. Ten µl of 1N NaOH was then added to stop the reaction and OD at 405 nm was measured.

Cytotoxicity was calculated as the percentage of cell death caused by triton-X (0.1%, v/v).

[00125] To detect secretion and/ or release of CAMP factor and acid SMase (ASMase), HaCaT or RAW264.7 cells ($5x10^5$) were co-cultured with [$5x10^6$ CFU/well; multiplicity of infection (MOI)=1:10] or without *P. acnes* in a serum-free medium in a 24-well plate at 37oC for 14 hr. The supernatant was centrifuged and filtrated with a 0.22 um pore-size filter to remove cell debris and bacteria, and then concentrated 10 folds using a 10 kDa cut-off ultrafiltration membrane (Amicon Inc., Beverly, MA). The concentrated supernatant (10 µg) was subjected to a 10% SDS-PAGE for Western blot analysis using mouse anti-CAMP factor antiserum and goat anti-ASMase IgG (Santa Cruz Biotechnology, Inc., Santa Cruz, CA).

[00126] For neutralization assay, cells (1×10^5/well) were co-cultured with *P. acnes* (1×10^6 CFU/well; MOI=1:10) in the presence of anti-CAMP factor or anti-GFP antiserum (2.5%, v/v), in which complements had been deactivated by heating at 56oC for 30 min, for 14 hr. To examine involvement of host ASMase in *P. acnes* pathogenicity, cells were co-cultured with *P. acnes* in the presence or absence of a cell-permeable selective ASMase inhibitor, desipramine (10 µM) (Sigma, St. Louis, MO) for 14 hr. After incubation, cell viability was determined and cytotoxicity was calculated as described above.

[00127] Intradermal injection of mouse ear with recombinant CAMP factor. To examine involvement of CAMP factor to *P. acnes*-inflammation in vivo, the ear of Imprinting Control Region (ICR) mice (Harlan, Indianapolis, IN) was intradermally injected with recombinant CAMP factor (10 µg/20 µl) or GFP (10 µg/20 µl) in PBS. The contralateral ear received an equal amount of PBS (20 µl). The ear thickness was measured using a micro caliper (Mitutoyo, Kanagawa, Japan) 24 hr after injection and a CAMP factor-induced increase in ear thickness reported as % of ear thickness in PBS-injected ears.

[00128] Detection of ASMase in ICR mouse ear. Ears of ICR mice were intradermally injected with of live *P. acnes* (1×10^7 CFU/20 µl) in PBS. The contralateral ear received an equal amount of

PBS (20 µl). Twenty four hr after bacterial challenge, the ear was excised, punched with an 8 mm biopsy and homogenized in 200 µl of PBS with a tissue grinder. The supernatant (1 ug of total protein) was subjected to Western blotting using goat anti-ASMase IgG (0.2 µg/ml) (Santa Cruz Biotechnology, Inc.) followed by monoclonal anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) IgG (2 µg/ml) (Fitzerald Inc., Concord, MA). Normal goat or mouse IgG was used as a negative control for the detection.

[00129] Transmission electron microscopy and fluorescence immunohistochemistry. ICR mouse ears were intradermally injected with live *P. acnes* or PBS as described above. Twenty four hr after bacterial challenge, the ear was excised and fixed in Karnovsky's fixative (4% paraformaldehyde, 2.5% glutaraldehyde, 5mM $CaCl_2$ in 0.1M Na Cacodylate buffer, pH 7.4) overnight at 4°C followed by 1% OsO_4 in 0.1M Na Cacodylate buffer, pH 7.4, en bloc staining with 4% uranyl acetate in 50% ethanol, and subsequently dehydrated using a graded series of ethanol solutions followed by propylene oxide and inflitration with epoxy resin (Scipoxy 812, Energy Beam Sciences, Agawam, MA). After polymerization at 65°C overnight, thin sections were cut and stained with uranyl acetate (4% uranyl acetate in 50% ethanol) followed by bismuth subnitrate. Sections were examined at an accelerating voltage of 60kV using a Zeiss EM10C electron microscope (Oberkochen, Germany).

[00130] ICR mouse ears were intradermally injected with live *P. acnes* or PBS as described above. Twenty four hr after bacterial challenge, the ear was excised, fixed in an optimum cutting temperature (OCT) compound (Sakura Finetek, Torrance, CA) and frozen at -80°C. Sections (7 µm) were fixed in 10% formamide in PBS. After blocking with PBS containing 5% BSA and anti-mouse cluster of differentiation (CD) 16/CD32 IgG (5 ug/ml) (BD Biosciences Pharmingen, Sparks, MD) for 30 min, sections were then incubated with biotinylated anti-mouse CD11b IgG (5 µg/ml) (BD Biosciences Pharmingen), a macrophage marker, followed by goat anti-ASMase IgG (5 µg/ml). Tetramethylrhodamine isothiocyanate (TRITC)-streptavidin

conjugate (5 ug/ml) (ZYMED, Carlsbad, CA) and fluorescein isothiocyanate (FITC)-labeled anti-goat IgG (5 µg/ ml) (Santa Cruz Biotechnology, Inc.) were applied to the sections, incubated for 30 min at room temperature, and followed by 4'-6-Diamidino-2-phenylindole (DAPI) staining (Sigma). Images were obtained using an Olympus BX41 fluorescent microscope (Olympus, Center Valley, PA).

[00131] Effect of desipramine on *P. acnes*-induced inflammation in vivo. To examine involvement of host ASMase in *P. acnes* pathogenicity, the ICR mice were intraperitoneally injected with a selective inhibitor of ASMase (20 mg/kg mouse) in PBS or an equal amount of PBS as a control. The ears of ICR mice were intradermally injected with live *P. acnes* or PBS as described above 30 min after desipramine treatment. Ear thickness was measured 24 hr after injection and a *P. acnes*-induced increase in mouse ear reported as % of ear thickness in PBS-injected ears.

[00132] Combination effect of CAMP factor vaccine and local injection with anti-ASMase IgG on *P. acnes*-induced inflammation. ICR mice were vaccinated with inactivated E. coli over-expressing CAMP factor or GFP as described above in 3-week interval. Two weeks after the second boost, live *P. acnes* was intradermally injected into the ear of vaccinated mice in the same manner as described above. Within 30 min, the left ear (received *P. acnes*) was injected with goat anti-ASMase IgG (4 µg/20 µl) or normal goat IgG (control) in PBS, and the right ear (received PBS) was injected with an equal volume of PBS. Ear thickness was measured 24 hr after injection and a *P. acnes*-induced increase in mouse ear reported as % of ear thickness in PBS-injected ears.

[00133] CAMP factor Expression, Mass Spectrometric Identity, and Biological Activity. To assess the expression of CAMP factor, *E. coli* transformed with an expression plasmid containing an insert encoding CAMP factor were incubated with or without IPTG. A prominent band of the expected size (the deduced molecular mass of CAMP factor-6×NH fusion protein is 32.4 kDa) was detected in the insoluble fraction from IPTG-

induced cells. The CAMP factor was purified using a TALON resin column. CAMP factor expression was confirmed by NanoLC-LTQ MS/MS mass spectrometric sequencing after in-gel trypsin digestion. Nine internal peptides derived from CAMP factor were fully sequenced by NanoLC-LTQ MS/MS mass spectrometry, matching well with those from *P. acnes* CAMP factor (accession number: gi/50842175). An internal peptide (AVLLTANPASTAK (SEQ ID NO:3); 147-158 amino acid residues) of CAMP factor was identified, validating the expression of recombinant CAMP factor.

[00134] A conventional CAMP test was utilized to examine biological activity of the recombinant CAMP factor, which demonstrated a co-hemolytic CAMP reaction when spotted adjacent to SMase-expressing *S. aureus* on a sheep blood agar plate. The data indicates that the recombinant CAMP factor is biologically active.

[00135] Immunogenicity of recombinant CAMP factor. To examine a potential of CAMP factor as a target of vaccine, ICR mice were immunized with CAMP factor, or a GFP control protein, using an E. coli-based delivery system. IgG against CAMP factor was detected 14 days after immunization by Western blot. No immunoreactivity against CAMP factor was detectable in GFP-immunized mice. ELISA analysis showed a significant increase in antibody titer 14 days after immunization, increasing at 21 days after immunization. Anti-CAMP factor IgG titers in the antiserum from CAMP factor-immunized mice was >100,000 21 days after immunization, respectively, while the titer from GFP-immunized mice was <100 (antiserum dilution curves not shown). These data indicate that CAMP factor is highly immunogenic.

[00136] Identification of CAMP factor in the supernatant of *P. acnes* cultures. To identify secreted CAMP factor, the supernatant of *P. acnes* cultures from logarithmic growth phase was concentrated and subjected to Western blotting with a mouse anti-CAMP factor antiserum. A single band was detected in the *P. acnes* culture supernatant (Fig. 11F, left panel, lane 2) at the position corresponding to recombinant CAMP

factor that had been treated with enterokinase to remove 6×NH tag (Fig. 11F, left panel, lane 1), but was not detected in concentrated RCM used for *P. acnes* culture (Fig. 11F, left panel, lane 3). No bands were detected with anti-GFP control antiserum (Fig. 11F, right panel). These data suggest that CAMP factor is a secreted protein.

[00137] Involvement of CAMP factor in pathogenicity of *P. acnes*. To explore the CAMP factor cytotoxicity in vitro, the human keratinocyte cell line, HaCaT, or the murine macrophage cell line, RAW264.7, was treated with recombinant CAMP factor or GFP control protein. Treatment with CAMP factor resulted in dose-dependent cytotoxicity in both HaCaT and RAW264.7 cells (Fig. 11G). To examine involvement of CAMP factor in *P. acnes*-induced inflammation, mouse ear was intradermally injected with recombinant CAMP factor or GFP. A significant increase in ear thickness was observed in CAMP factor-injected ear 24 hr after the injection, but no increase induced by GFP injection (Fig. 11H). These data suggest the involvement of CAMP factor in *P. acnes*-induced inflammatory reaction.

[00138] The involvement of bacterial CAMP factor and host ASMase in *P. acnes* pathogenicity in vitro. To examine whether ASMase is released from host cells when co-cultured with *P. acnes*, HaCaT or RAW264.7 cells were cultured with and without *P. acnes* for 14 hr. After incubation, the cell culture supernatant was subjected to Western blotting, probing with a mouse anti-CAMP factor antiserum and goat anti-ASMase IgG. CAMP factor and ASMase were detected in the cell culture supernatant only when the cells were co-cultured with *P. acnes* (Fig. 12A, lanes 1 and 2). The homology between human and mouse ASMases (mature protein) is greater than 90% and their molecular weights are almost identical. Neither of these ASMases were detected in the cell cultures without *P. acnes* (Fig. 12A, lanes 3 and 4), and ASMase was not detected in the supernatant of *P. acnes* culture. These data suggest that ASMase is released and/or secreted from the host cells co-cultured with *P. acnes*.

[00139] To examine the effect of neutralization of CAMP factor on *P. acnes*-induced cytotoxicity, HaCaT and RAW264.7 cells were co-cultured with *P. acnes* in the presence of anti-CAMP factor or anti-GFP antiserum (Fig. 12B). *P. acnes* induced 29.3% and 44.0% cell death on HaCaT cells, respectively, in the presence of anti-GFP control antiserum. The addition of anti-CAMP factor antiserum reduced *P. acnes*-induced cell death by 18.2% and 2.1%, respectively. To examine the involvement of host ASMase in the pathogenicity of *P. acnes*, cells were co-cultured with *P. acnes* in the presence of desipramine, a selective ASMase inhibitor, or an equal amount of PBS (vehicle) (Fig. 12C). *P. acnes* induced 43.4% and 45.4% cell death on HaCaT and RAW264.7 cells, respectively. The addition of desipramine significantly reduced *P. acnes*-induced cell death on the both cells by 21.9% and 30.6%, respectively. These data suggest the involvements of CAMP factor and ASMase in *P. acnes*-induced cytotoxicity.

[00140] Possible involvement of host ASMase in *P. acnes* pathogenicity in vivo. To examine involvement of host ASMase in *P. acnes* pathogenicity in vivo, the ears of ICR mice were injected intradermally with *P. acnes* or PBS, excised 24 hr after bacterial challenge, and used for the following experiments. First, the mouse ears were homogenized and the homogenate supernatant was subjected to Western blot using anti-ASMase IgG (Fig. 13A). A single band was detected at the expected molecular weight for ASMase (~60 kDa). *P. acnes* injection increased the amount of ASMase in the ear relative to PBS injection. To examine the localization of ASMase in the mouse ear, frozen sections were co-stained with anti-mouse CD11b IgG, a conventional macrophage marker, followed by goat anti-ASMase IgG (Fig. 13B). This double immunofluorescent staining revealed infiltration of macrophages into the site of *P. acnes* administration 24 hr after bacterial challenge; no CD11b+ macrophages were observed in PBS-injected control ears. ASMase was highly expressed in the infiltrating CD11b+ macrophages. Transmission electron microscopy showed colonizing and/ or phagocytosed *P. acnes* in macrophage-like

cells and in the extracellular space 24 hr after bacterial challenge (Fig. 13C); no bacteria was observed in PBS injected ears. In addition, ruptured cell membranes were observed in the *P. acnes*-injected ear, whereas the cell membranes of the PBS-injected ear appeared intact. These data suggest that intradermal *P. acnes* challenge induces the infiltration of macrophages, which highly express ASMase. In addition, *P. acnes*-induced ear swelling was significantly relieved when mice were systemically pretreated with desipramine 30 min before the bacterial challenge (Fig. 13D), suggesting the involvement of host ASMase in *P. acnes*-induced inflammation and the development of skin lesions.

[00141] The effect of combining CAMP factor vaccination with local injection of anti-ASMase IgG on *P. acnes*-induced inflammation ICR mice were vaccinated with CAMP factor or GFP control. *P. acnes* or PBS was injected intradermally into the ears of vaccinated mice. Thirty min after challenge, the left ear (received *P. acnes*) was locally injected with goat anti-ASMase IgG or normal goat control IgG, while the right ear was injected with an equal volume of PBS. Both the combination of GFP vaccination with local injection of anti-ASMase IgG and CAMP factor vaccination with local injection of normal IgG reduced *P. acnes*-induced ear swelling to 202.8% and 193.5% 24 hr after bacterial challenge, respectively, in comparison with GFP vaccination combined with normal IgG injection (224.5%). By contrast, the combination of CAMP factor vaccination with local injection with anti-ASMase IgG synergistically decreased *P. acnes*-induced ear swelling to 153.7% (Fig. 14). These data indicate that suppression of both of bacterial CAMP factor and host ASMase synergistically reduced *P. acnes*-induced inflammation and skin lesions.

[00142] To determine antibody titers to CAMP factor by enzyme-linked immunosorbent assay (ELISA), purified CAMP factor (5 µg/ml) was diluted with a coating buffer (0.015 M Na2CO2, 0.35 M $NaHCO_2$ and 0.05% NaN_3), and coated onto a 96-well ELISA plate (Corning, Lowell, MA) at 4°C overnight. The plates were washed with PBS containing 0.1% (w/v) Tween-20, and blocked with PBS

containing 1% (w/v) BSA and 0.1% (w/v) Tween-20 for 2 hr at room temperature. Antisera (10,000 dilutions) obtained from mice vaccinated with E. coli over-expressing CAMP factor or GFP were added to the wells and incubated for 2 hr. Goat anti-mouse IgG (H+L) IgG-horseradish peroxidase (HRP) conjugate (Promega, Madison, WI) (1:5,000 dilution) was added, incubated for 2 hr, and then washed. HRP activity was determined with a OptEIA™ Reagent Set (BD Biosciences, San Diego, CA). The OD of each well was measured at 450 nm.

[00143] Therapeutic effects of vaccination with CAMP factor on *P. acnes*-induced inflammation. Live *P. acnes* (1 x 10^7 CFU/ 20 ul) in PBS were intradermally injected in the central portion of the left ear. Twenty µl of PBS was injected into the right ear of the same mouse as a control. To examine in vivo therapeutic effects of vaccination, ICR mice were vaccinated with E. coli over-expressing CAMP factor or GFP 24 hr after bacterial challenge as described above. The ear thickness was measured using a micro caliper for 98 days and a *P. acnes*-induced increase in ear thickness reported as % of ear thickness in PBS-injected ears.

[00144] Co-cytotoxic activity of CAMP factor and bacterial SMase. The human keratinocyte cell line, HaCaT, or murine macrophage cell line, RAW264.7, was cultured in DMEM or RPMI1640 medium, respectively, supplemented with 10% heat-inactivated FBS, at 37°C under atmosphere of 5% (v/v) CO2 in air. For determination of co-cytotoxic activity of SMase and CAMP factor, cells (1×10^5/well) were preincubated in a 96-well plate at with SMase from *S. aureus* (350 mU/ml, Sigma) or an equal amount of PBS (vehicle control) in serum-free medium containing 10 mM $MgCl_2$ for 15 min. After the pretreatment, the cells were washed with PBS and then incubated with CAMP factor (25 µg/ml) or GFP as a control in 1% serum-medium for 18 hr. As a positive control for 100% cytotoxicity, triton-X was added to get a final concentration of 0.1% (v/v) for cell lysis. After the incubation, cell viability was determined as described in Experimental procedures and cytotoxicity was calculated as the percentage of cell death caused by triton-X.

[00145] The hemolytic power is thought to be a virulence factor for numerous microbial pathogens to degrade tissue, invade host cells, disseminate themselves, and escape from the host immune attack. Microbial hemolysins generally possess the capacity to lyse erythrocytes in vitro, but many are toxic to other cell types as well. *P. acnes* secretes CAMP factor as an exotoxin (Fig. 11). Although the hemolytic action of CAMP factor has been demonstrated on erythrocytes and artificial plasma membranes, little attention has been paid to the cytotoxicity of CAMP factor on other cell types. Therefore, the cytotoxic activity of CAMP factor on host cells was examined, and its physiologic significance to the pathogenicity of *P. acnes*, which is relevant to inflammatory acne vulgaris.

[00146] The human keratinocyte is one of the major targets of *P. acnes*. In addition, intradermal injection of mouse ears with live *P. acnes* induces infiltration of numerous CD11b+ macrophages (Fig. 13B and C). The tissue chamber model data integrated with a dermis-based cell-trapped system was used to mimic the in vivo microenvironment of acne lesions, injection of live *P. acnes* into the intradermally-implanted tissue chamber attracts Gr-1+ neutrophils and CD11b+ macrophages into the chamber. Thus, the interaction between murine macrophage and *P. acnes* is investigated in our model of *P. acnes*-induced inflammation in mice. Therefore, the human keratinocyte cell line, HaCaT, and the murine macrophage cell line, RAW264.7, were employed to determine cytotoxic activity of CAMP factor from *P. acnes* in vitro. The data indicate that CAMP factor is an important virulence factor for *P. acnes* to degrade host cells (Fig. 17G).

[00147] Evidences obtained from a number of in vitro experiments suggest only weak hemolytic activities of CAMP factor co-hemolysin itself without SMases. CAMP factor does not have significant homology to any other pore-forming toxins. Only the full-length recombinant CAMP factor has been found to exert co-hemolytic activity on a sheep blood agar plate, but the structure-function relationship remains

unclear. Lang and co-authors indicate that GBS CAMP factor binds to glycosylphosphatidylinositol (GPI)-anchored proteins on the cell membrane of erythrocytes, which act as cell surface receptors for this toxin, and that the interaction supports its ability to form oligomeric pores in sheep erythrocyte membranes. Amount of GPI-anchored proteins is augmented by the reduction of sphingolipid levels on the cell membrane. Since GPI-anchored proteins are found ubiquitously in mammalian cells, the same mechanism may be involved in the cytotoxic reaction of CAMP factor on keratinocytes and macrophages. Indeed, the removal of sphingomyelin on the cell membrane by pretreating with bacterial SMase increased the cell susceptibility to CAMP factor (Fig. 17).

[00148] Several different forms of mammalian SMases have been identified, including endosomal/ lysosomal ASMase (a soluble enzyme), which is ubiquitous in mammalian tissues, and plasma membrane-associated, or cytosolic, neutral SMase, which is located mostly in the central nervous system. These enzymes catalyze the hydrolytic cleavage of sphingomyelin on the cell membrane to ceramide in the same catalytic mechanism as bacterial SMases. The released ceramide, in turn, can act as a cellular signal for various activities, including apoptosis, differentiation, and proliferation. The activity of the SMases are regulated by a wide range of extracellular signaling; growth factors, cytokines, neurotransmitters, hormones, and stresses, such as ultraviolet and reactive oxygen species. Ubiquitously-expressed ASMase exerts important functions during the innate immune response to infectious pathogens. Therefore, focus was put on the interaction of host ASMase and bacterial CAMP factor as related to pathogenicity of *P. acnes*. ASMase was released and/or secreted from the host cells when the cells were co-cultured with *P. acnes* (Fig. 18A). The cytotoxicity of *P. acnes* was neutralized in the presence of mouse anti-CAMP factor antiserum in vitro (Fig. 18B). In addition, adding the specific ASMase inhibitor, desipramine, to co-cultured cells and *P. acnes* significantly reduced the cytotoxicity. The data from the in vitro experiments suggest

that CAMP factor is a potential virulence factor for *P. acnes* and involvement of host ASMase in the virulence of *P. acnes*.

[00149] There have been only a few studies showing that CAMP factor is a potential virulence of pathogen in vivo. A high dose of partially purified CAMP factor from GBS was lethal to rabbits and mice when injected intravenously. Mice that had been infected with sublethal doses of GBS developed fetal septicemia after receiving repeated injections with purified CAMP factor. The disclosure demonstrates that intradermal injection of the mouse ears with recombinant CAMP factor induced ear swelling, indicating that CAMP factor is involved in *P. acnes*-induced inflammation and skin lesion in vivo.

[00150] To examine whether host ASMase is involved in *P. acnes* pathogenicity and *P. acnes*-induced inflammation, the ears of ICR mice were injected intradermally with *P. acnes* according to a rat ear model previously described. The amount of soluble ASMase increased in the ear after injection with *P. acnes* (Fig. 13A). The profile of the granulomatous inflammation in the mouse ear model (Fig. 13B and C) is similar to that of inflammatory acne in the human hair follicle; numerous *P. acnes* were observed inside phagosomes of an infiltrating macrophage in an inflammatory acne lesion in the hair follicle. *P. acnes* resists killing by phagocytes and is able to survive in macrophages. GBS beta-hemolysin/ cytolysin, a pore-forming exotoxin, was demonstrated to contribute to the subversion of phagocytic host immune defenses. During the intracellular life cycle of Listeria monocytogenes, a pore-forming toxin named listeriolysin O is largely responsible for mediating rupture of the phagosomal membrane to allow its escape from the phagosome into the host cytosol, its replicative niche. Lysosomal ASMase is known to contribute to macrophage killing of bacteria in the early stage of phagocytosis, and is required for the proper fusion of late phagosomes with lysosomes, which is crucial for efficient transfer of lysosomal antibacterial hydrolases into phagosomes. Taken together, phagocytosed *P. acnes* in the macrophage may take advantage of the host lysosomal ASMase to

enhance the toxicity of CAMP factor to escape from phagosomes, an interaction which may be involved in the *P. acnes* resistance against phagocytosis. Indeed, we observed a number of macrophages in the *P. acnes*-challenged ear, many of which had cell membrane that were ruptured by colonizing *P. acnes* (Fig. 19D). Infection by Salmonella or E. coli triggered an early surge in the extracellular secretion and/ or release of ASMase activity from macrophages. *P. acnes* may shrewdly utilize released and/ or secreted ASMase from macrophages to invade or to escape cells to spread cell-to-cell.

[00151] An effective vaccines for *P. acnes*-associated inflammation as an alternative treatment for acne consist of killed-whole organism *P. acnes* and a *P. acnes* cell wall-anchored sialidase. Thus, the potential of CAMP factor as a target of acne vaccine development was examined. *P. acnes* CAMP factor was highly immunogenic when vaccination was performed by an E. coli-based vaccine delivery system (Fig. 11D and E). The vaccination with *P. acnes* CAMP factor elicited protective immunities to *P. acnes*-induced inflammation. In addition, a local injection mouse ear with anti-ASMase IgG reduced *P. acnes*-induced inflammation. The combination of CAMP factor vaccination with a local injection of anti-ASMase IgG synergistically reduced *P. acnes*-induced ear swelling (Fig. 14). The data suggests the synergistic interaction of CAMP factor and host ASMase in vivo.

[00152] ***P. acnes* utilized host ASMase to amplify its CAMP factor-mediated pathogenicity.** Lysosomal ASMase in macrophages play important roles during the innate immune response to infectious pathogens; the enzyme contributes to macrophage mediated killing of pathogens and lysosomal fusion with phagosomes. However, *P. acnes* may hijack ASMase released and/ or secreted from host cells to enhance its CAMP factor-delivered pathogenicity. The synergism may contribute to its evasion of host immune defenses, degrade host tissues and spread the pathogen cell-to-cell. Recent studies have afforded abundant evidences showing that *P. acnes* is involved not only in acne vulgaris, but also in many diseases, including

endocarditis, endophthalmitis, osteomyelitis, joint, nervous system, cranial neurosurgery infections, and implanted biomaterial contamination. Treatment targeting *P. acnes* CAMP factors and host ASMase may have a potential to be widely applied for these *P. acnes*-associated diseases to suppress the pathogen expansion.

[00153] Plant materials. Japanese radish sprouts (Kaiware-daikon) (*Raphanus sativus L.*) was obtained from a commercial supplier (ICREST International, JCP, Carson, CA). Japanese radish sprouts that were 9 cm in length with two leaflets were used and grown at room temperature under a 23 Watt fluorescent bulb (Philips, Portland, OR), and were sprayed with water daily.

[00154] Vector construction. The binary vector pBI121 harboring the reporter GUS driven by the cauliflower mosaic virus 35S promoter was used for gene construction (Jefferson, 1987). The open reading frames of CAMP factor cDNA cloned in a pEcoli-Nterm 6×HN vector was amplified by PCR using a forward primer (5′- CCTTCTAGAGGAGATATACCATGGGTCATAATCAT-3′; SEQ ID NO:18) and a reverse primer (5′-TCCCCCGGGTTAATTAATTAAGCGGCCGCC-3′ (SEQ ID NO:19). The SCAP cDNA cloned in a pIVEX-MBP vector (Liu *et al.*, 2008) was amplified using a forward primer (5′-AGATCTAGAATGTCTGGTTCTCATCATCATCATC-3′; SEQ ID NO:20) and a reverse primer (5′-GCCCCCGGGTTAGCCTTCGATCCCGAGGTT-3′ (SEQ ID NO:21). The primers were designed to add restriction sites to the ends of PCR products. Specifically, the restriction sites XbaI and SmaI were encoded into the forward and reverse primers, respectively. PCR products were treated with XbaI and SmaI then cloned into polylinker sites of pBI121 vectors to generate 35S::*CAMP factor-His* and 35S::*SCAP-MBP-His* constructs.

[00155] Agrobacterium tumefaciens transformation. All constructs were transformed into an *Agrobacterium tumefaciens* strain LBA4404 using a liquid nitrogen freeze-thaw method (An *et al.*, 1988). A single colony of LBA4404 cells was inoculated in 5 ml of YEP medium [10 mg/ml bacto-trypton (DIFCO, Detroit,

MI), 10 mg/ml yeast extract (DIFCO, Detroit, MI), and 5 mg/ml NaCl (Sigma, St. Louis, MO; pH 7.5)] with 250 rpm shaking at 28°C overnight. Subsequently, 50 ml of fresh YEP was inoculated with 2 ml of liquid culture and incubated with 250 rpm shaking at 28°C until the OD_{600} reached 0.8. The bacteria were centrifuged at 3,000 ×*g* for 5 min at 4°C and the pellet was resuspended in 1 ml of 20 mM calcium chloride. The bacteria (0.2 ml) were transferred to a 1.5 ml microfuge tube and 1 µg of gene constructs was added. The mixture was frozen in liquid nitrogen for 5 min then thawed at 37°C water bath for 5 min. One ml of YEP medium was added to the mixture and incubated with 150 rpm shaking at 28°C for 2 to 4 h. The bacteria were centrifuged at 3,000 ×*g* for 5 min then resuspended in 0.1 ml of YEP. Transformants were selected by plating bacteria on YEP-agar medium (YEP medium containing 1.5% agar) containing antibiotics (50 µg/ml kanamycin and 50 µg/ml streptomycin) and incubating at 28°C for 2 to 3 days.

[00156] Agroinfiltration of gene constructs into leaves and protein extraction. A single colony of *A. tumefaciens* transformants was cultured in 2 ml of YEP medium containing 50 g/ml kanamycin and 50 g/ml streptomycin with 250 rpm shaking at 28°C until OD_{600} reached approximately 0.5. Afterward, the bacteria were collected by centrifugation at 1,300 ×*g* for 5 min, and resuspended in 2 ml sterile ddH_2O. All bacterial suspensions were maintained at room temperature for 30 min until agroinfiltration. Non-transformed *Agrobacterium* served as a negative control and was cultured under the same conditions as the transformants without adding kanamycin in the medium. For syringe infiltration, the central lower epidermises (i.e., the centermost 25 mm^2 area) of potted seedlings leaves were wounded with a sterile scalpel (number 15, Feather Safety Razor Co., Osaka, Japan) and 0.1 ml of *Agrobacterium* bacterial suspension (5 x 10^7 CFUs) was injected into the wound site, which was positioned between a finger and a 1 ml syringe (BD, Bioscience, San Diego, CA). Infiltration was confirmed by visually monitoring the diffusion of bacterial suspension toward the leaf margin (Schob *et al.*,

1997). Agroinfiltrated leaves were grown for five days before GUS assays and immunization was performed. Agroinfiltrated leaves were stained using a histochemical GUS assay solution consisting of 0.1 M $NaPO_4$ (pH 7.0), 0.5 mM $K_3Fe(CN)_6$, 0.5 mM $K_4Fe(CN)_6$, 0.1% (v/v) Triton X-100, and 0.05% (w/v) 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid, cyclohexylammonium salt (Sigma, St. Louis, MO). Leaves were submerged in the staining solution and incubated at 37°C in the dark overnight. After incubation, leaves were removed from the staining solution and immersed in a stop solution containing 42.5% (v/v) ethanol, 10% (v/v) formaldehyde, and 5% (v/v) acetic acid (Jefferson, 1987). Quantitative determination of GUS activity was accomplished by the fluorometric assay. Whole leaves were grounded with 200 µl of 1 × CCLR [100 mM K-phosphate (pH 7.8), 1 mM EDTA, 10% (v/v) glycerol, 1% (v/v) Triton X-100 and 7 mM β-mercaptoethanol]. The mixture was centrifuged at 10,000 ×*g* for 5 min at 4°C and 200 µl supernatant was removed to a new microtube on ice following by mixing with 1 mM 4-Methylumbelliferyl-D-glucuronide buffer at 37°C for 1 h (Jefferson, 1987). The enzymatic reaction was measured spectrofluorometrically with excitation at OD_{365} and emission at OD_{455} by SpectraMAX GeminiEM spectrofluorometer (Molecular Devices, Sunnyvale, CA). To investigate the dynamic expression of antigen in radish leaves, leaves were removed at 0, 1, 3, and 5 days to quantify the level of GUS.

[00157] Purification of CAMP factor and SCAP from leaf tissues were carried out by affinity chromatography on a Ni-NTA agarose column (Qiagen, Valencia, CA) with certain modifications. The column was washed with water and equilibrated with buffer A (8 M guanidine, 100 mM NaH_2PO_4, 10 mM Tris-HCl, pH 8.0). Leaf material (1 g) was ground under liquid nitrogen using mortar and pestle in 15 ml ice-cold extraction buffer A. Guanidine-solubilized proteins were centrifuged at 12,000 ×*g* for 20 min to remove the debris and insoluble material and the supernatant was gently stirred with 1.6 ml Ni-NTA agarose resin for 1 h at room temperature. The mixture was loaded onto a column previously equilibrated with

buffer A. Briefly, the column was washed with buffer B (8 M urea, 100 mM NaH_2PO_4, 10 mM Tris-HCl, pH 6.8). Finally, proteins were eluted with buffer C (8 M urea, 100 mM NaH_2PO_4, 10 mM Tris-HCl, pH 6.3), D (8 M urea, 100 mM NaH_2PO_4, 10 mM Tris-HCl, pH 5.9), and buffer E (8 M urea, 100 mM NaH_2PO_4, 10 mM Tris-HCl, pH 4.5).

[00158] Intranasal immunization with whole leaves containing recombinant CAMP factors. Female ICR mice (3 to 6 weeks old; Harlan, Indianapolis, IN) were utilized for intranasal immunization that holds the potential to induce a mucosal immune response (Mantis, 2005). Mice were maintained in accordance to institutional guidelines. The central areas (25 mm^2) of five radish leaves expressing GUS or CAMP factors alone were excised using a sterile scalpel. To avoid *Agrobacterium* transgene introgression, leaf sections were pooled and ground in 700 µl ddH_2O and then sterilized by an UV crosslinker (Spectronics, Westbury, NY) at 7,000 J/m^2 for 30 min. Inactivation of sterilized *Agrobacterium* was confirmed by their inability to form colonies on YEP agar plates (data not shown). Whole leaves containing either CAMP factor or GUS alone (as a negative control) without adjuvants were then intranasally inoculated into the nasal cavities of ICR mice (25 µl/mouse). Three boosts at the same dose were performed at 1, 2, and 4 weeks after the first immunization.

[00159] To detect antigen expression, 15 µg recombinant GUS and 15 µg of whole leaves expressing CAMP factors or SCAP alone were separated using 10% SDS-PAGE. Bands were electrophoretically transferred to nitrocellulose membranes (Gil *et al.*, 2006). Membranes were probed with anti-CAMP factor serum obtained from mice immunized with UV-irradiated *E. coli*, BL21 (DE3) (Liu *et al.*, 2008) over-expressing *P. acnes* CAMP factors. To confirm antibody production in the immunized mice, purified CAMP factor (65 µg) was loaded into a 10% SDS-PAGE and transferred to a nitrocellulose membrane. The blot was immuno-reacted to serum (1:500 dilution) obtained from mice immunized for four weeks with whole leaves containing CAMP factor. Antibodies [Immunoglobulin G (IgG)]

were detected with anti-mouse horseradish peroxidase-conjugated IgG (1:5,000 dilution, Promega, Madison, WI). Peroxidase activity was visualized with a western lighting chemiluminescence kit (PerkinElmer, Boston, MA).

[00160] Passive immunization of anti-CAMP factor serum against *P. acnes*-induced inflammation. Complements in the serum were inactivated by heating at 56°C for 30 min. *P. acnes* was pre-treated with 5 % (v/v) inactivated anti-GUS serum or anti-CAMP factor serum in the medium at 37°C for 2 h. The 2 h incubation of anti-GUS serum (3.63 ± 1.47 x 10^8 CFUs) and anti-CAMP factor serum (3.3 ± 1.2 x 10^8 CFUs), respectively, did not significantly influence the growth of *P. acnes*. ICR mice were injected intradermally with an amount of 25 l aliquots of anti-GUS or anti-CAMP neutralized *P. acnes* (1 x 10^7 CFUs) suspended in PBS overnight. As a control, 25 l of PBS was injected into the right ear of the same mice. The increase in ear thickness was measured using a micro caliper (Mitutoyo, Japan) after the bacterial injection, the increase in ear thickness of *P. acnes* injected ear was calculated as % of a PBS-injected control. For histological observation, the ear on the day 3 after injection was excised, cross-sectioned, stained with H&E, and viewed on a Zeiss Axioskop2 plus microscope. To count the bacterial colonization, the bacteria-injected ears were homogenized in 1 ml of sterile PBS for 1 min on a vibrating homogenizer (mini-beadbeater, Biospec Products, Bartlesville, OK) in the presence of 0.5 ml of 2.0 mm zirconia beads (Biospec Products, Bartlesville, OK). The bacterial number in homogenates was quantified by serially diluting the bacteria and plating them on a RCM plate. After centrifugation at 1,300 ×*g*, MIP-2 in supernatants was measured by an ELISA kit as directed by the manufacturer (BD Biosciences, San Diego, CA).

[00161] To investigate whether passive administration of neutralizing antiserum influences the survival of *P. acnes* at other sites, the left ears of ICR mice were injected intradermally with an amount of 25 l aliquots of anti-GUS serum or anti-CAMP factor serum neutralized *P. acnes* (1 x 10^7

CFUs). The same amount of live *P. acnes* (1×10^7 CFUs) alone was injected into the right ears of the same mice overnight. The bacteria number was calculated by counting colonies on RCM plates.

[00162] Compared to active immunization of a CAMP factor-targeted vaccine, passive neutralization of CAMP factor displays roughly equal potency with respect to suppression of *P. acnes*-induced ear inflammation. The therapeutic antibodies to CAMP factors described herein can be extended for treatment of various *P. acnes*-associates human diseases including implant infections, pulmonary sarcoidosis, osteomyelitis, and endocarditis (Nakatsuji *et al.*, 2008c; Nishiwaki *et al.*, 2004; Zouboulis, 2004). With an eye toward human use, future studies will include generating the therapeutic monoclonal antibodies to *P. acnes* CAMP factor. Epicutaneous application of a human monoclonal antibody to CAMP factor onto the skins of patients with severe acne may locally eradicate *P. acnes* without interrupting the residence of *P. acnes* and other commensals in other locations of our body.

[00163] The following sequences are referenced herein. GenBank accession no. NC_006085 (the full genome of *P. acnes*, the sequence of which is incorporated herein by reference. The polypeptide sequence of *P. acnes* CAMP Factor identified as SEQ ID NO:7 and the corresponding coding polynucleotide identified as SEQ ID NO:6; and the polypeptide for lipase identified herein as SEQ ID NO:9 and the corresponding coding polynucleotide sequence identified as SEQ ID NO:8. Human ASMase is identified as SEQ ID NO:11; and the corresponding coding polynucleotide sequence as SEQ ID NO:10. Homologs and variants of ASMase's are known. For example, sphingomyelin phosphodiesterase 1, acid lysosomal isoform 2 precursor [Homo sapiens] gi|56117842|ref|NP_001007594.1|[56117842]; sphingomyelin phosphodiesterase 1, acid lysosomal isoform 1 precursor [Homo sapiens] gi|56117840|ref|NP_000534.3|[56117840]; sphingomyelin phosphodiesterase 1, acid lysosomal [Mus musculus]gi|6755582|ref|NP_035551.1|[6755582]; Sphingomyelin phosphodiesterase 1, acid lysosomal [Mus

musculus] gi|21961231|gb|AAH34515.1|[21961231]; Sphingomyelin phosphodiesterase 1, acid lysosomal [Mus musculus] gi|15030106|gb|AAH11304.1|[15030106], the sequences associated with the accession number are incorporated herein by reference. P. acnes sialidase sequence is provided in SEQ ID NO:13 and the corresponding polynucleotide in SEQ ID NO:12. P. acnes sialidiase B is provided in SEQ ID NO:15 and the corresponding polynucleotide in SEQ ID NO:14. A sialidase-like polypeptide and coding sequence are provided in SEQ ID NOs: 17 and 16, respectively.

What is claimed is:

1. An immunogenic composition comprising a substantially purified polypeptide comprising a sequence referred to in Table 1, an immunogenic fragment thereof, and any combination of the foregoing.

2. The immunogenic composition of claim 1, comprising a CAMP Factor, lipase, or sailidase polypeptide or fragment thereof.

3. The immunogenic composition of claim 1, wherein the polypeptide comprises SEQ ID NO: 2, 3, 7, 9 and 11 or an immunogenic fragment thereof.

4. The immunogenic composition of claim 1, 2, or 3, wherein the polypeptide is expressed in a vector.

5. The immunogenic composition of claim 4, wherein the vector comprises an attenuated bacterial vector or an attenuated viral vector.

6. The immunogenic composition of claim 5, wherein the vector comprises an *E. coli* or an adenovirus.

7. The immunogenic composition of claim 1, wherein the composition comprises at least one attenuated bacterial vector expressing or comprising at least one polypeptide selected from the group consisting of CAMP Factor, lipase or sialidase.

8. A composition comprising at least one recombinant attenuated bacterial or viral vector comprising at least one polynucleotide encoding one or more *P. acnes* polypeptides selected from the group consisting of a CAMP Factor, a lipase, or a sialidase such that the polypeptide is expressed in the at least one recombinant attenuated vector and an inhibitor of ASMase activity.

9. The composition of claim 1, wherein the ASMase activity

inhibitor comprises an antibody.

10. The composition of claim 1, wherein the ASMase inhibitor comprises a small molecule inhibitor.

11. A method of inducing protective immunity in a subject comprising administering the composition of any one of claims 1-3 or 8 to the subject and contacting the subject with an ASMase inhibitor.

12. The method of claim 10 or 11, further comprising boosting the immunity of the subject comprising administering an immunogenic composition comprising the same composition or a different composition comprising the same antigenic polypeptide.

13. An immunoprotective composition comprising at least one attenuated vector expressing an antigen useful for inducing an immunoprotective response against *Propionibacterium acnes* (*P. acnes*), said antigen comprising an extracellular or immunogenic protein of *P. acnes* or immunogenic fragment thereof linked to transcriptional promoter and termination signals.

14. The immunoprotective composition of claim 13, wherein the *P. acnes* protein or fragment thereof is selected from the group consisting of CAMP factor, a lipase, a sialidase, and any combination thereof.

15. The immunoprotective composition of claim 10 or 11, wherein composition comprises an attenuated vector selected from the group consisting of *Campylobacter jejuni, Campylobacter coli, Listeria monocytogenes, Yersinia enterocolitica, Yersinia pestis, Yersinia pseudotuberculosis, Escherichia coli, Shigella flexneri, Propionibacterium acnes, Shigella dysenteriae, Shigella boydii, Helicobacter pylori, Helicobacter felis, Gastrospirillum hominus, Vibrio cholerae,*

Vibrio parahaemolyticus, *Vibrio vulnificus*, *Bacteroides fragilis*, *Clostridium difficile*, *Salmonella typhimurium*, *Salmonella typhi*, *Salmonella gallinarum*, *Salmonella pullorum*, *Salmonella choleraesuis*, *Salmonella enteritidis*, *Streptococcus gordonii*, *Lactobacillis sp.*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Enterococcus faecalis*.

16. The immunoprotective composition of claim 13, further comprising a pharmaceutical diluent.

17. The immunoprotective composition of claim 13, wherein the composition further provides protective immunity against an infection by *K. pneumoniae*, *S. Aureus and/or S. pyogenes*.

18. A method of protecting a susceptible host against an infection of *Propionibacterium acnes* (*P. acnes*) comprising administering to said host an amount of the immunoprotective composition of claim 1 or claim 13 sufficient to invoke an immunoprotective response in the host and administering an ASMase inhibitor.

19. The method of claim 18, wherein the composition is in a pharmaceutically acceptable carrier.

20. A recombinant attenuated bacterial vector or viral vector comprising a polynucleotide encoding at least one antigenic polypeptide selected from a CAMP factor, a lipase or a sialidase from *P. acnes*.

21. A method of providing protective immunity to a subject, comprising administering the recombinant attenuated vector of claim 18 to a subject.

22. A composition useful for treating a *P. acnes* infection comprising an ASMase inhibitor.

23. The composition of claim 22, further comprising a CAMP

antigen or vaccine.

24. An antigenic composition, comprising a disrupted non-infective *P. acnes* cell and further comprising an ASMase inhibitor.

25. A method for treating *P. acnes* comprising administering to a subject a vaccine comprising a CAMP factor and a composition comprising an ASMase inihibitor.

26. The method of claim 25, wherein the vaccine and composition are administered simultaneously.

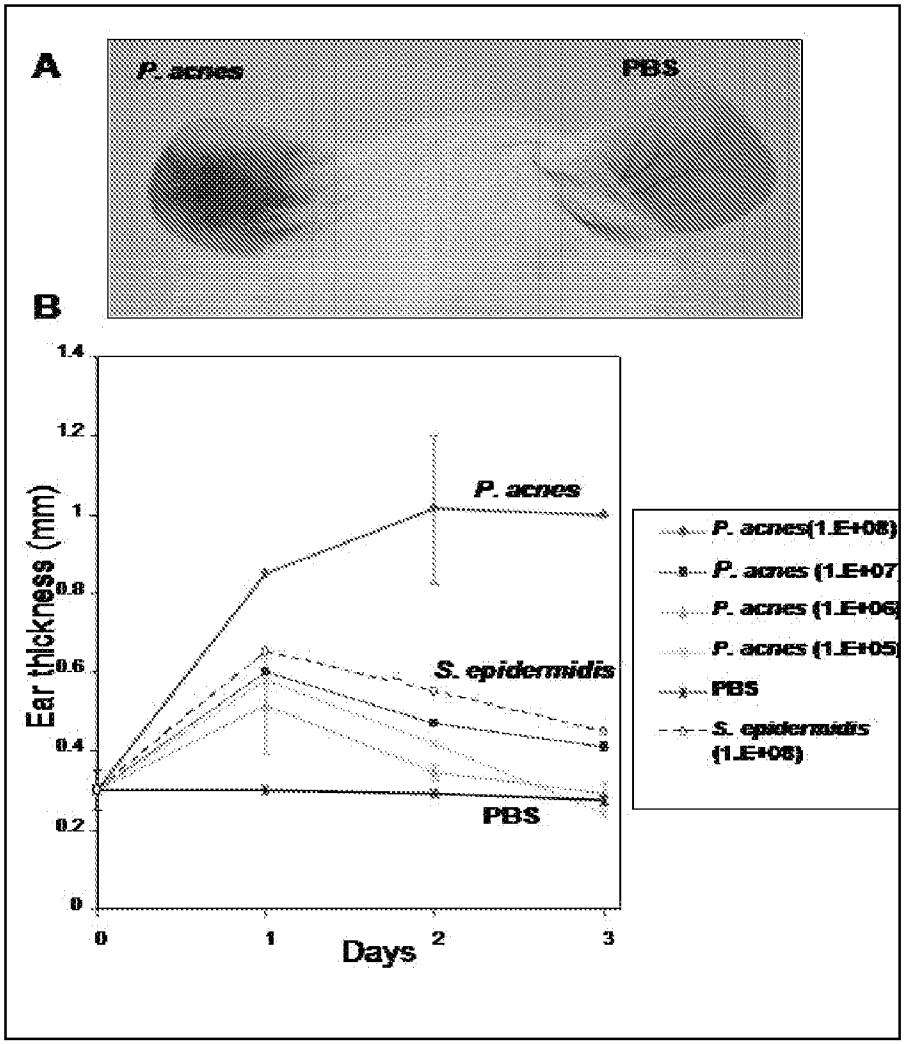

A
P. acnes
PBS
B
Ear thickness (mm)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
3
Days
P. acnes
S. epidermidis
PBS
P. acnes(1.E+08)
P. acnes (1.E+07
P. acnes (1.E+06)
P. acnes (1.E+05)
PBS
S. epidermidis (1.E+08)

FIGURE 1

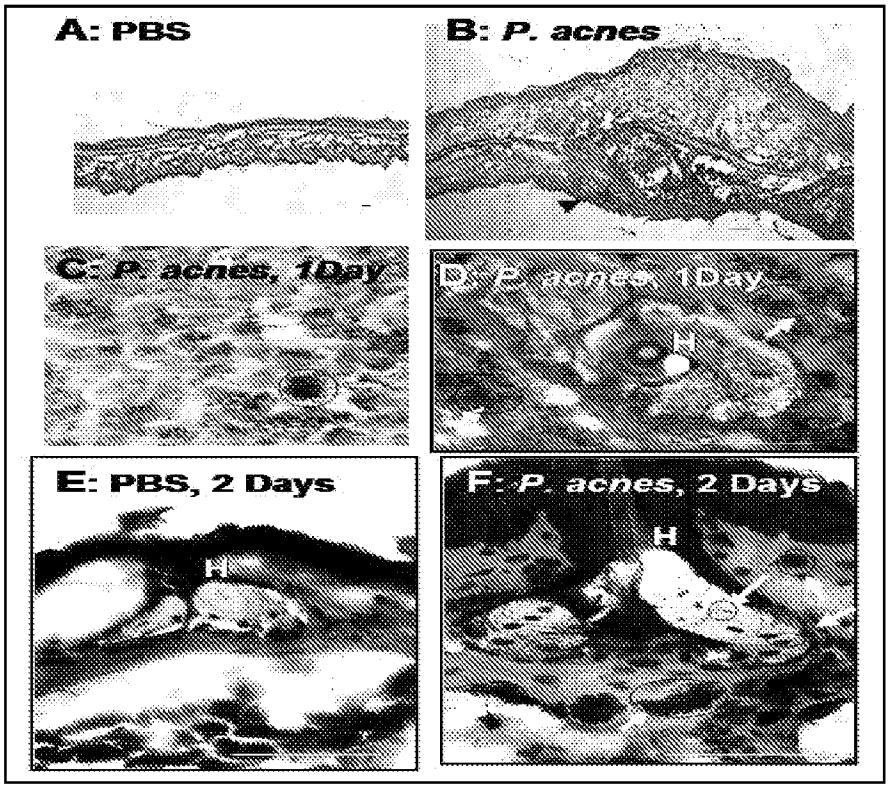


FIGURE 2

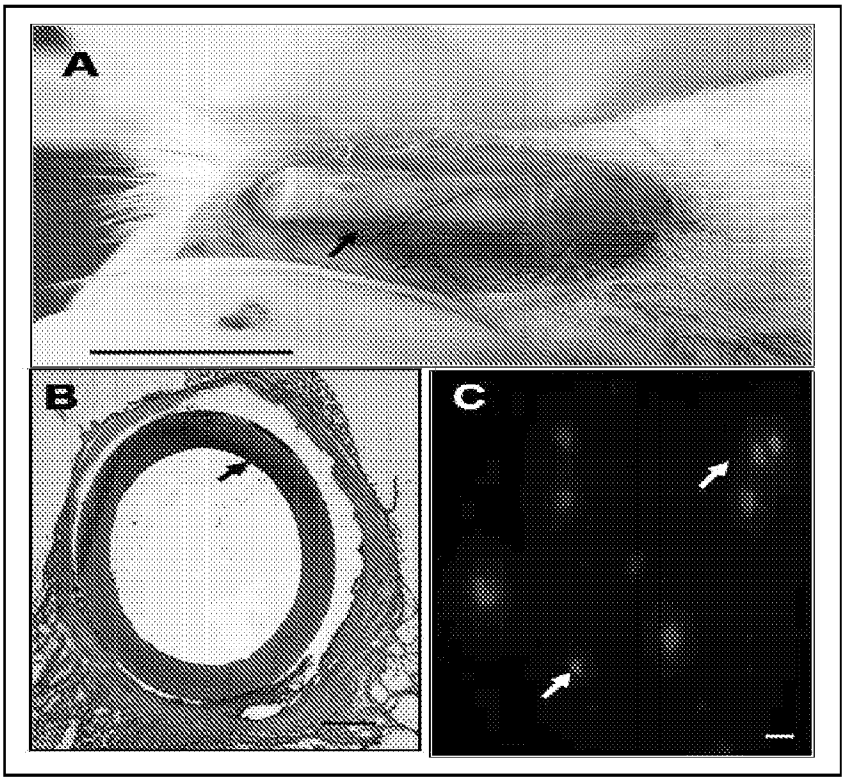


FIGURE 3

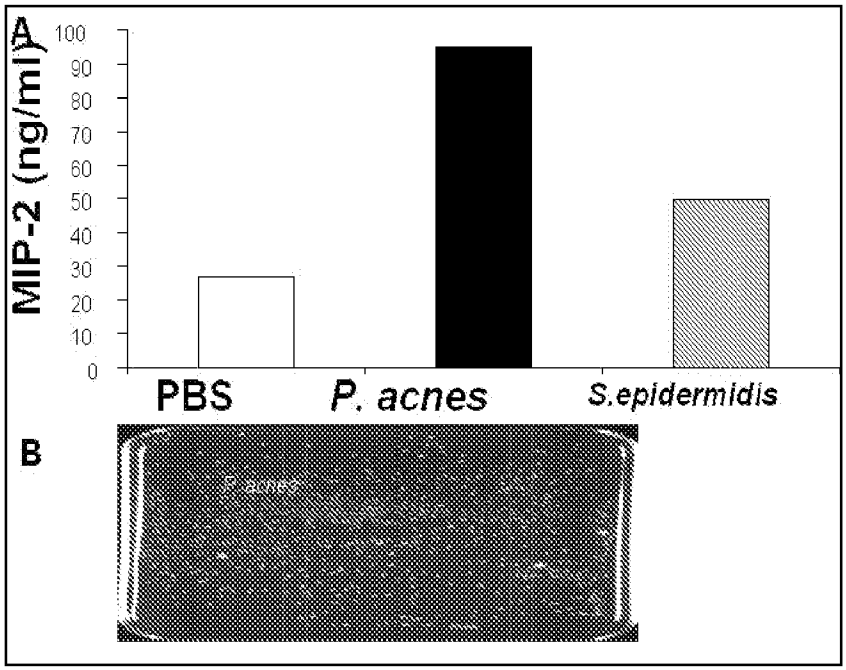

A
MIP-2 (ng/ml)
100
90
80
70
60
50
40
30
20
10
0
PBS
P. acnes
S.epidermidis
B
P. acnes

FIGURE 4

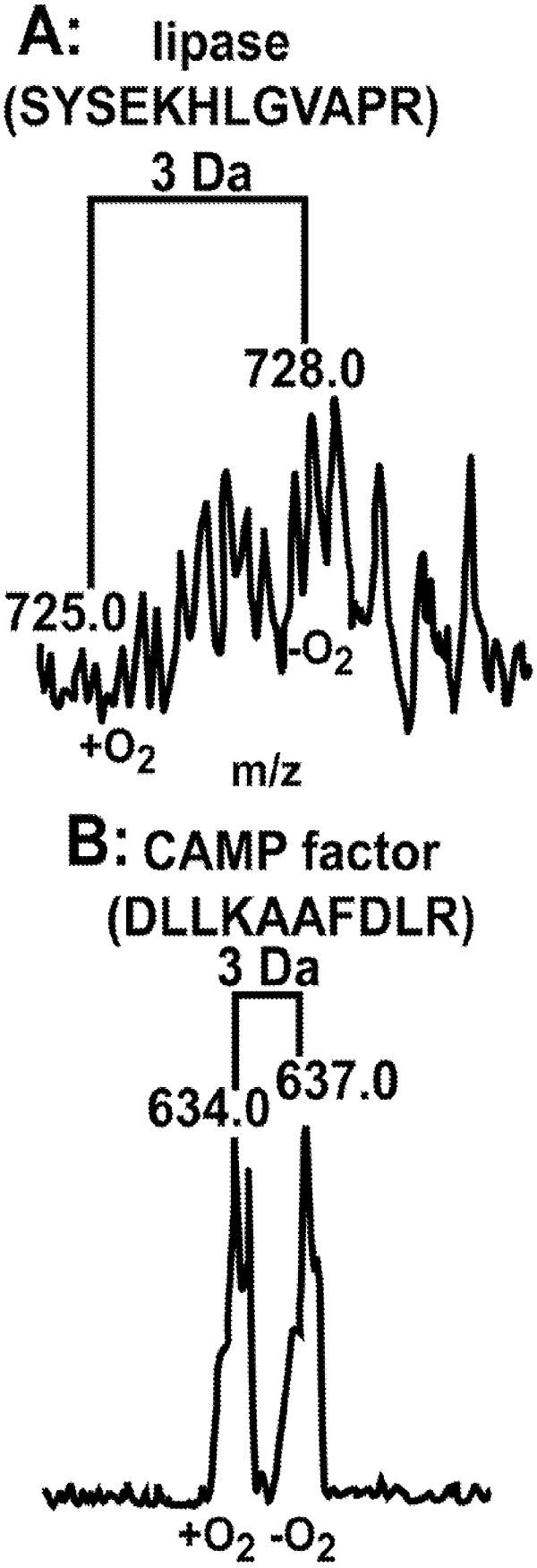
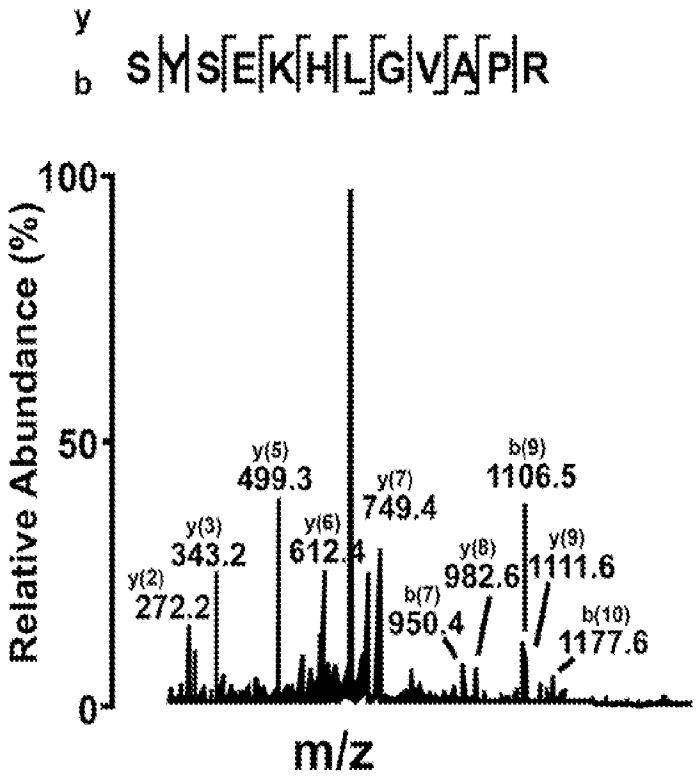

A: lipase
(SYSEKHLGVAPR)
3 Da
728.0
725.0
-O2
+O2
m/z
B: CAMP factor
(DLLKAAFDLR)
3 Da
634.0
637.0
+O2 -O2
m/z

FIGURE 5

C: lipase



D: CAMP factor

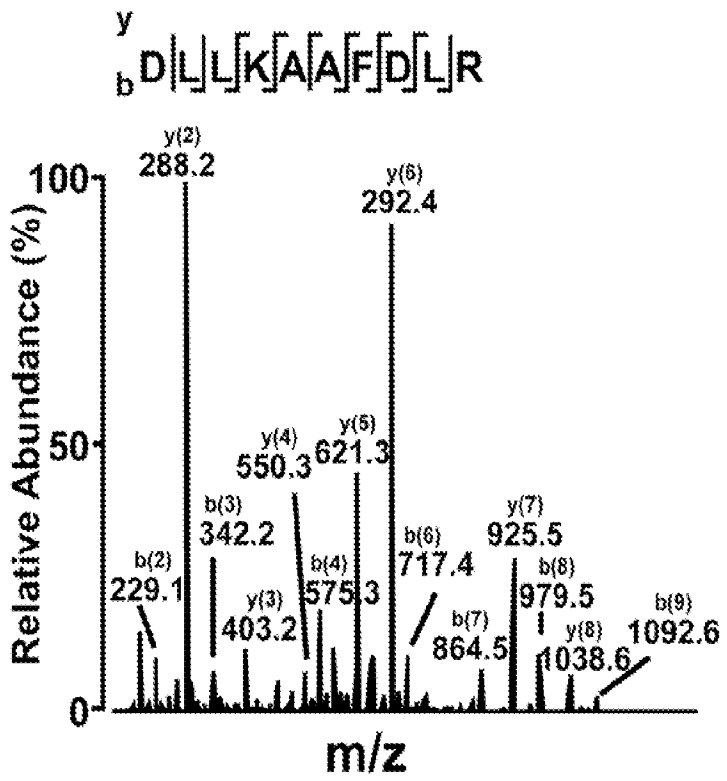


FIGURE 5 (cont'd)

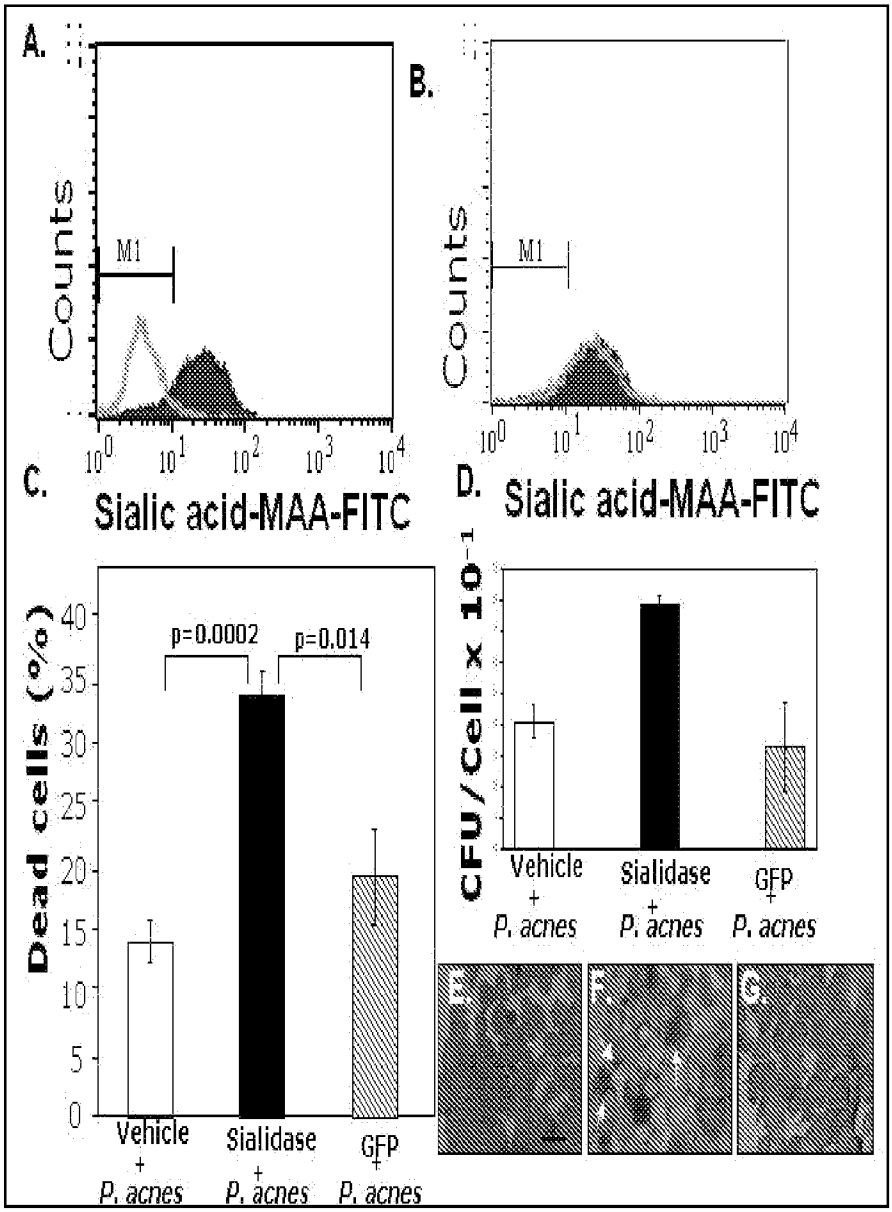

A.
Counts
M1
10^0 10^1 10^2 10^3 10^4
Sialic acid-MAA-FITC
B.
Counts
M1
10^0 10^1 10^2 10^3 10^4
Sialic acid-MAA-FITC
C.
Dead cells (%)
p=0.0002
p=0.014
0 5 10 15 20 25 30 35 40
Vehicle + P. acnes
Sialidase + P. acnes
GFP + P. acnes
D.
CFU/Cell x 10^{-1}
Vehicle + P. acnes
Sialidase + P. acnes
GFP + P. acnes
E.
F.
G.

FIGURE 6

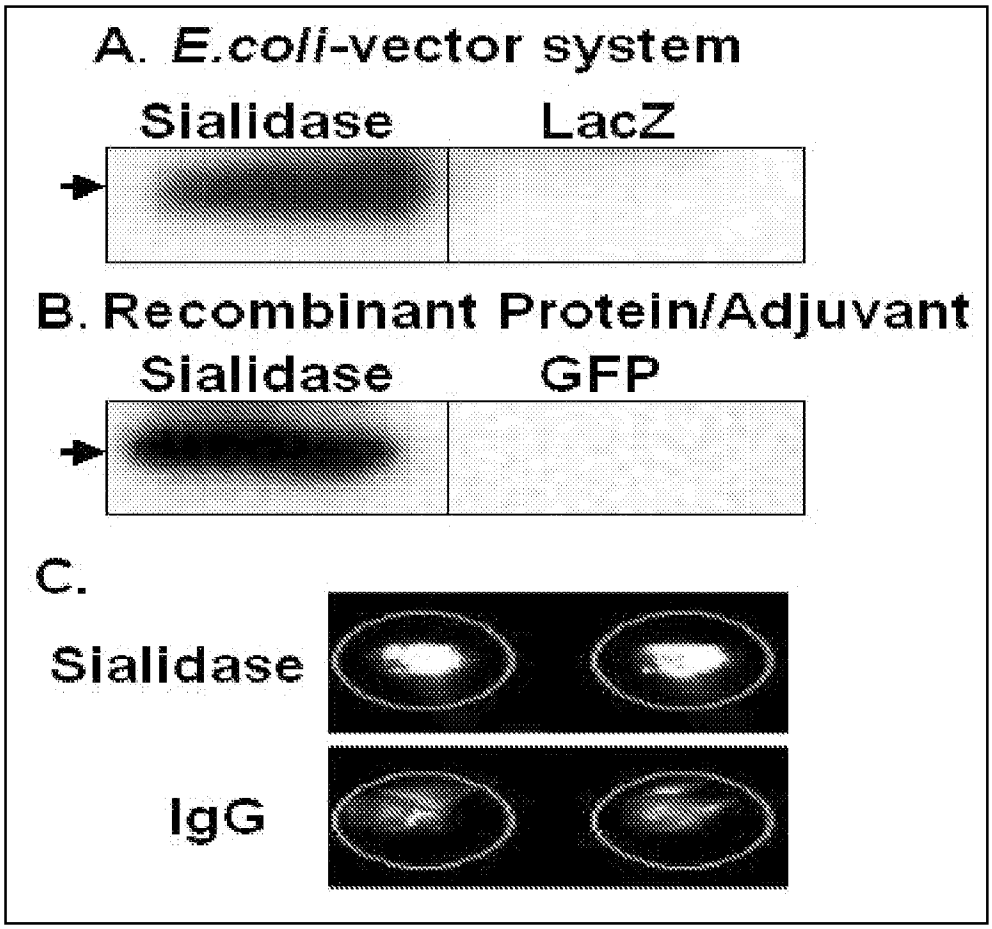


FIGURE 7

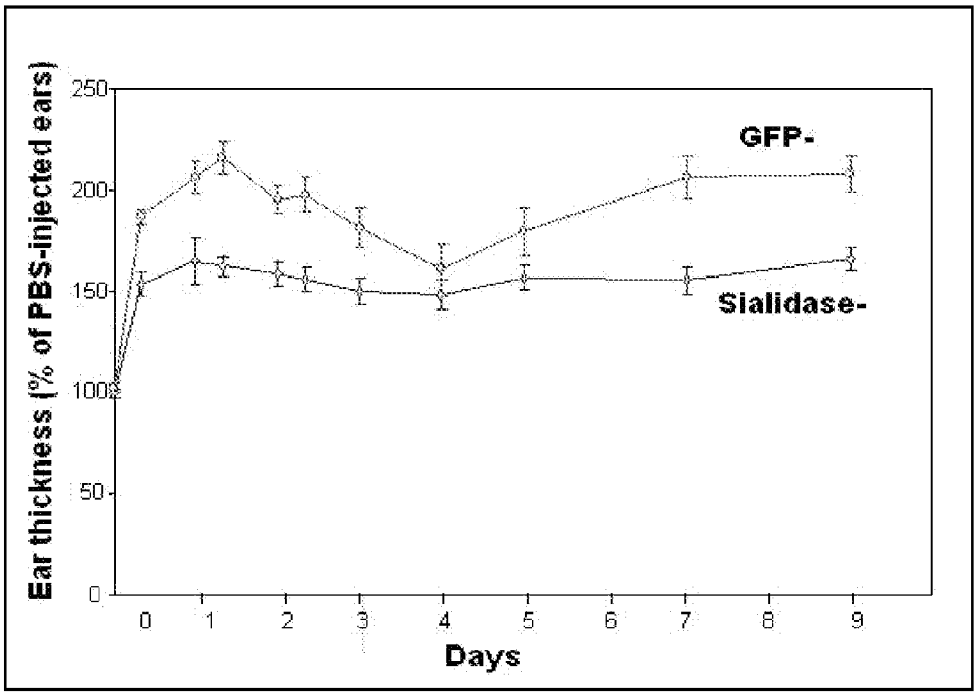


FIGURE 8

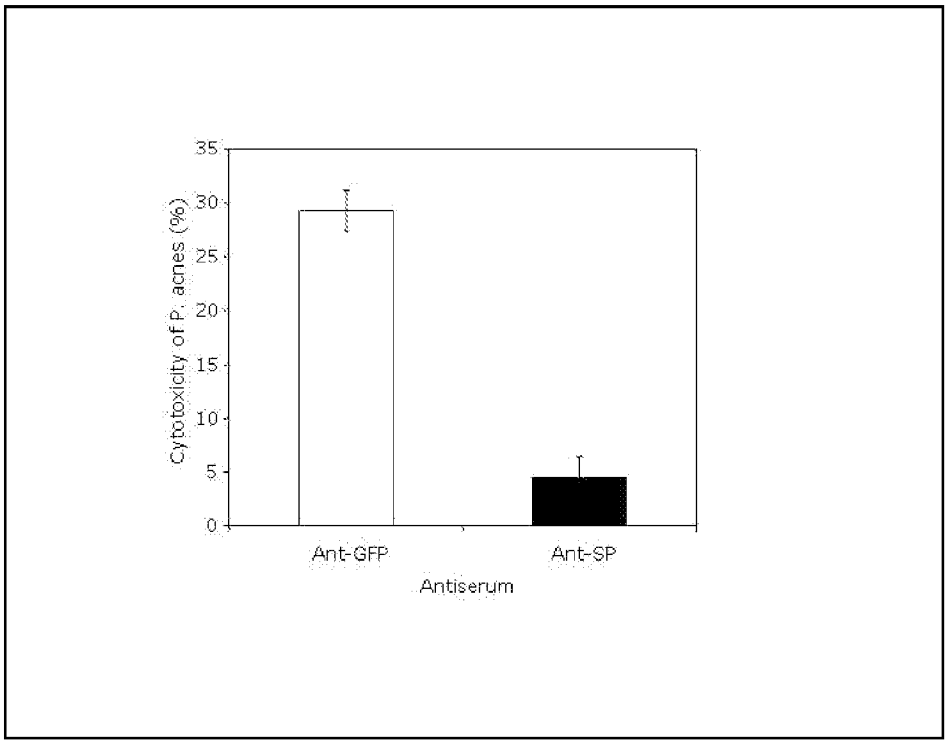

35
30
25
20
15
10
5
0
Cytotoxicity of P. acnes (%)
Ant-GFP
Ant-SP
Antiserum

FIGURE 9

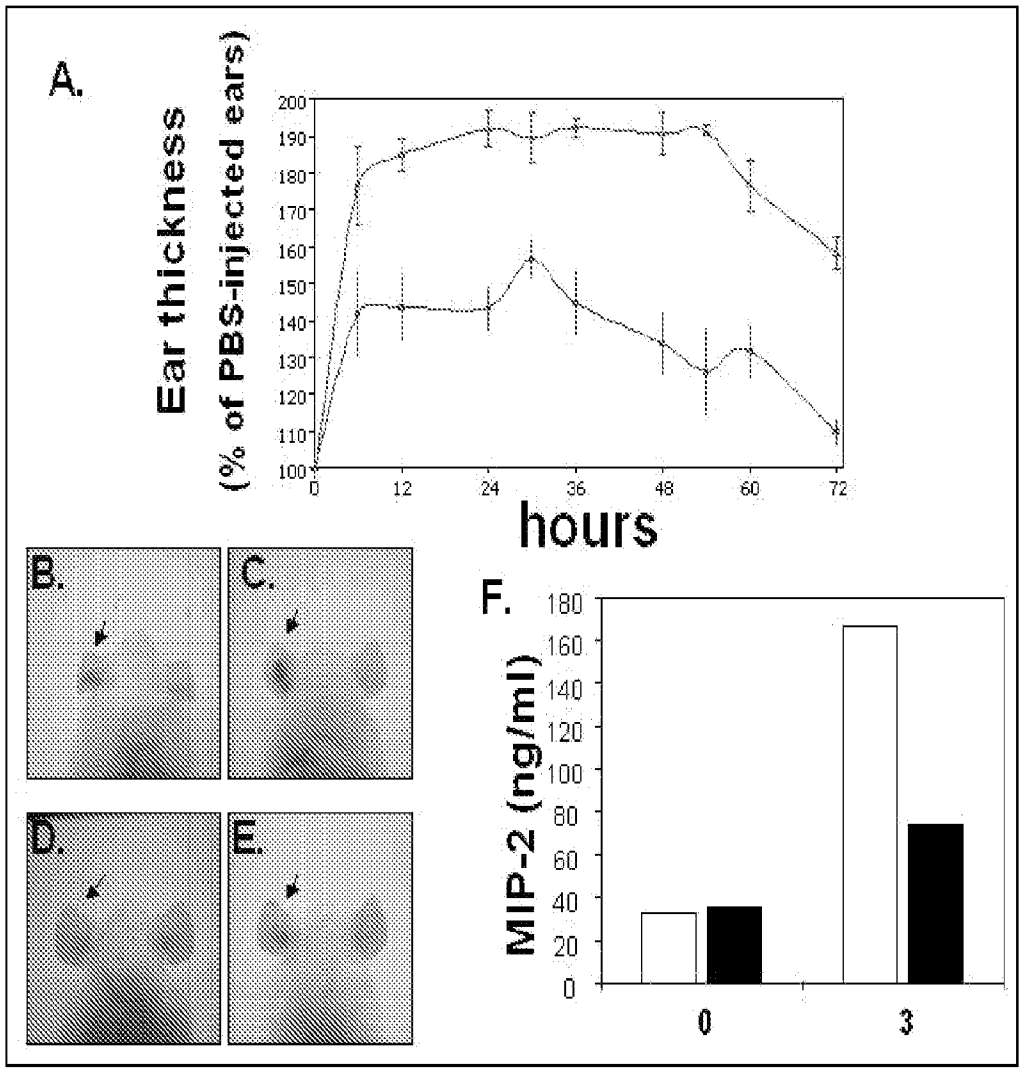

A.
Ear thickness
(% of PBS-injected ears)
200
190
180
170
160
150
140
130
120
110
100
0
12
24
36
48
60
72
hours
B.
C.
D.
E.
F.
MIP-2 (ng/ml)
180
160
140
120
100
80
60
40
20
0
0
3

FIGURE 10

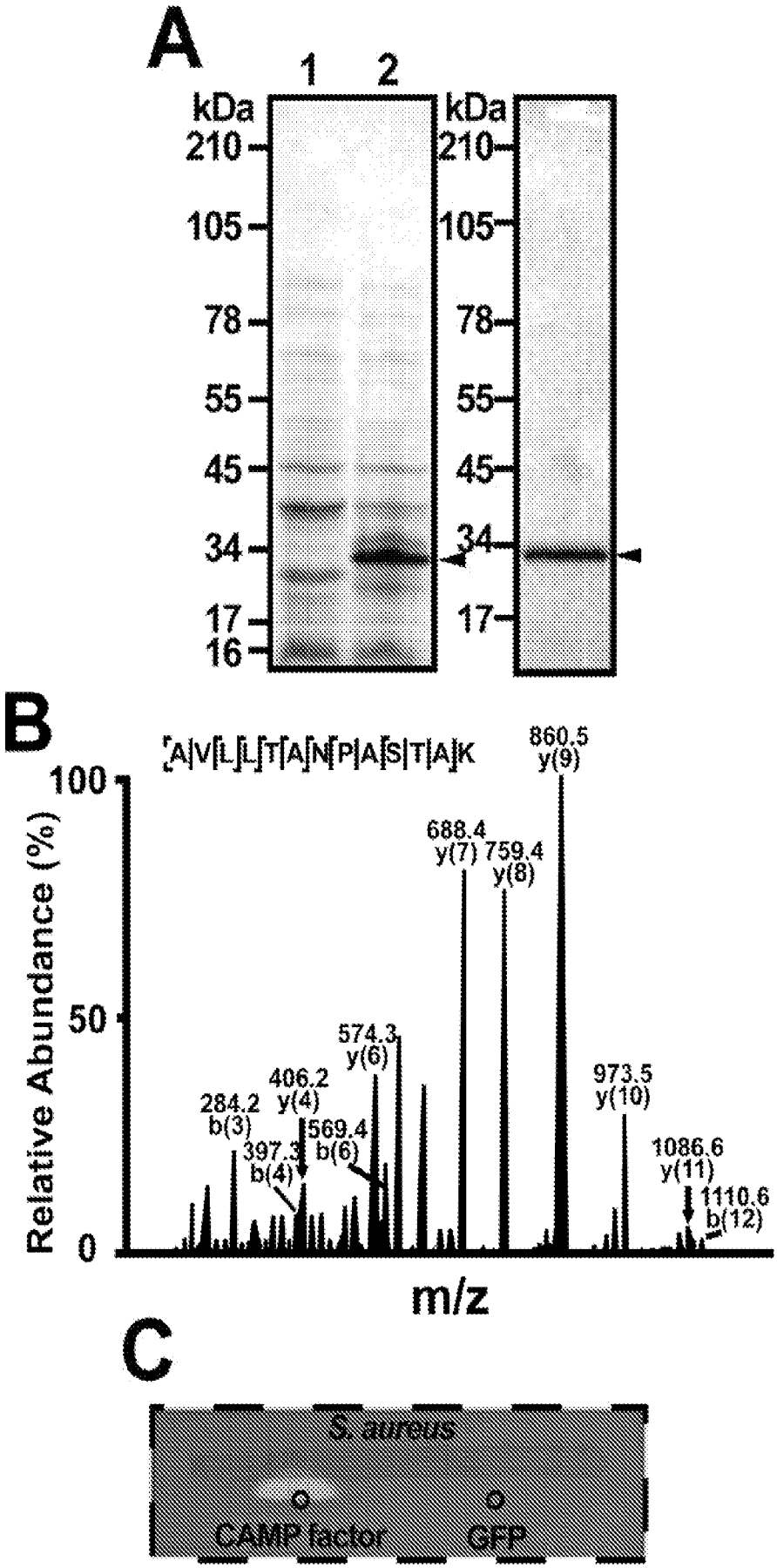

A
1
2
kDa
210
105
78
55
45
34
17
16
kDa
210
105
78
55
45
34
17
B
[A|V|L|L|T|A|N|P|A|S|T|A|K
860.5
y(9)
688.4
y(7)
759.4
y(8)
574.3
y(6)
406.2
y(4)
284.2
b(3)
397.3
b(4)
569.4
b(6)
973.5
y(10)
1086.6
y(11)
1110.6
b(12)
Relative Abundance (%)
100
50
0
m/z
C
S. aureus
CAMP factor
GFP

FIGURE 11

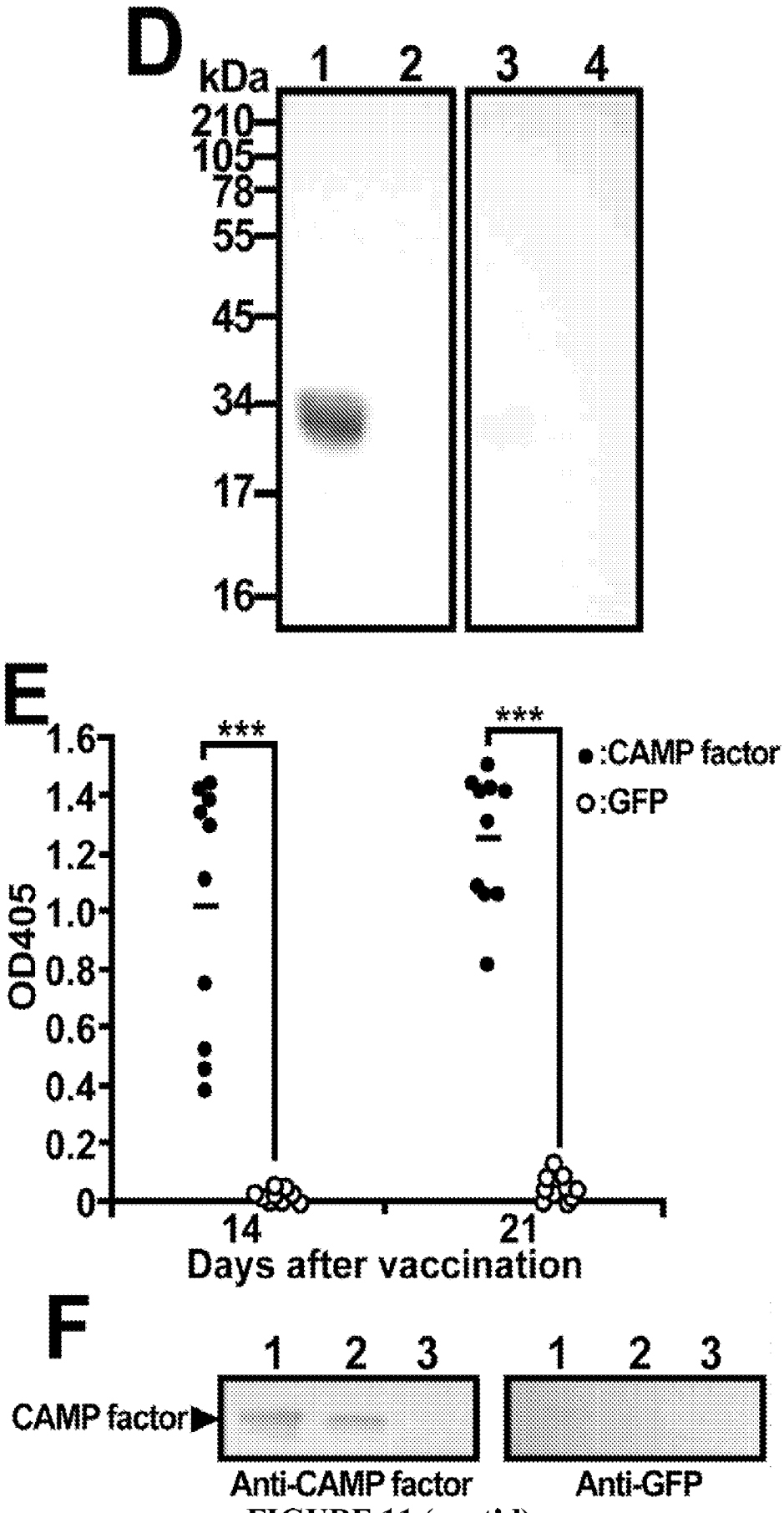


FIGURE 11 (cont'd)

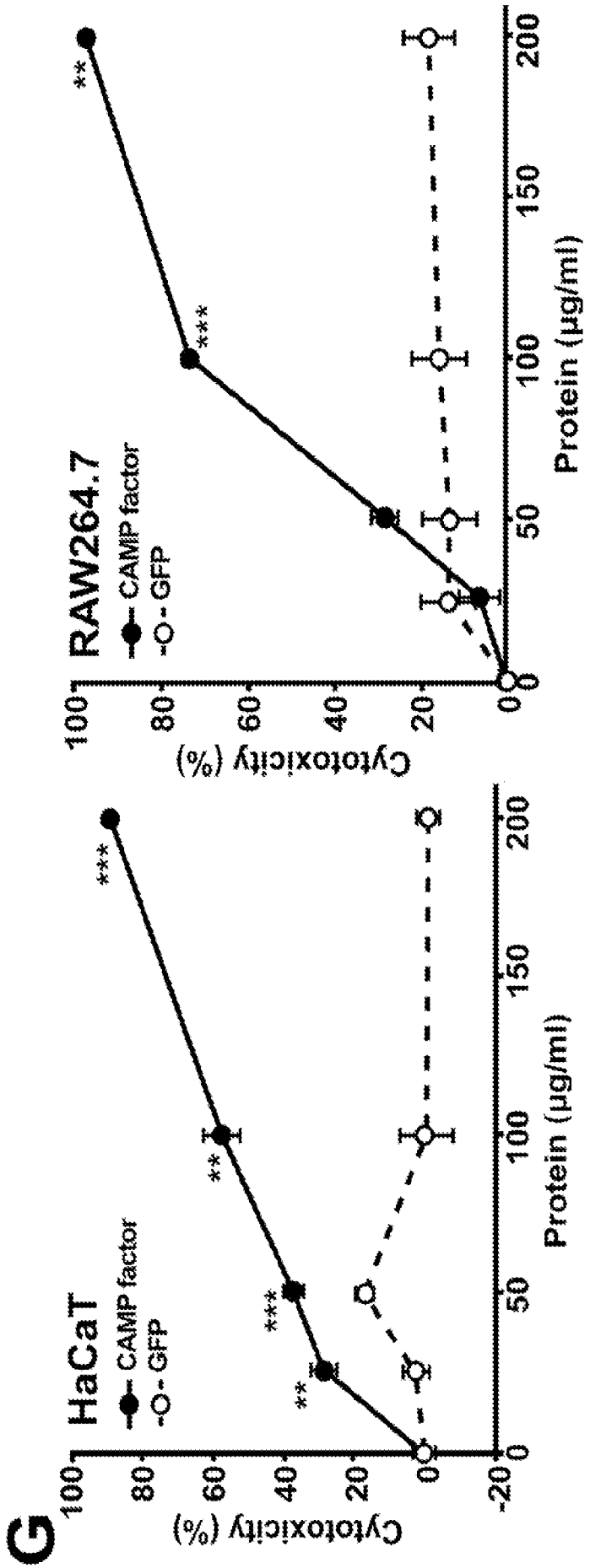


FIGURE 11 (cont'd)

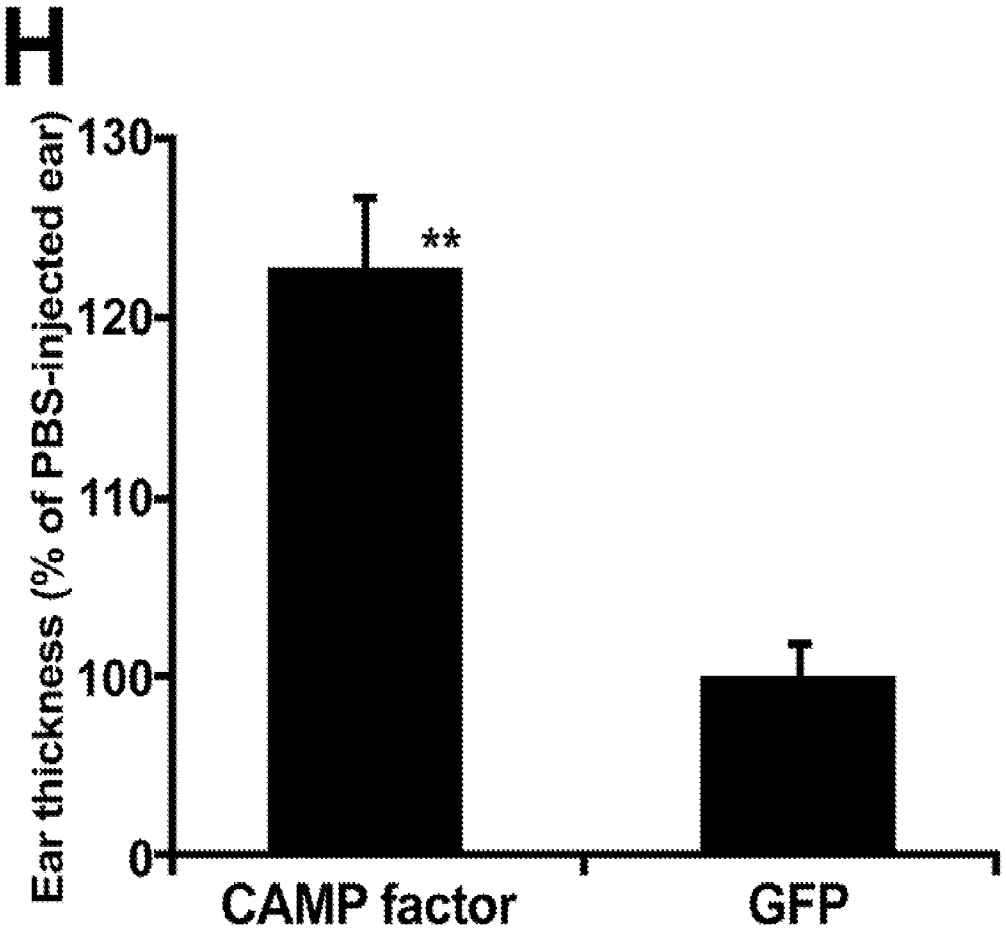


FIGURE 11 (cont'd)

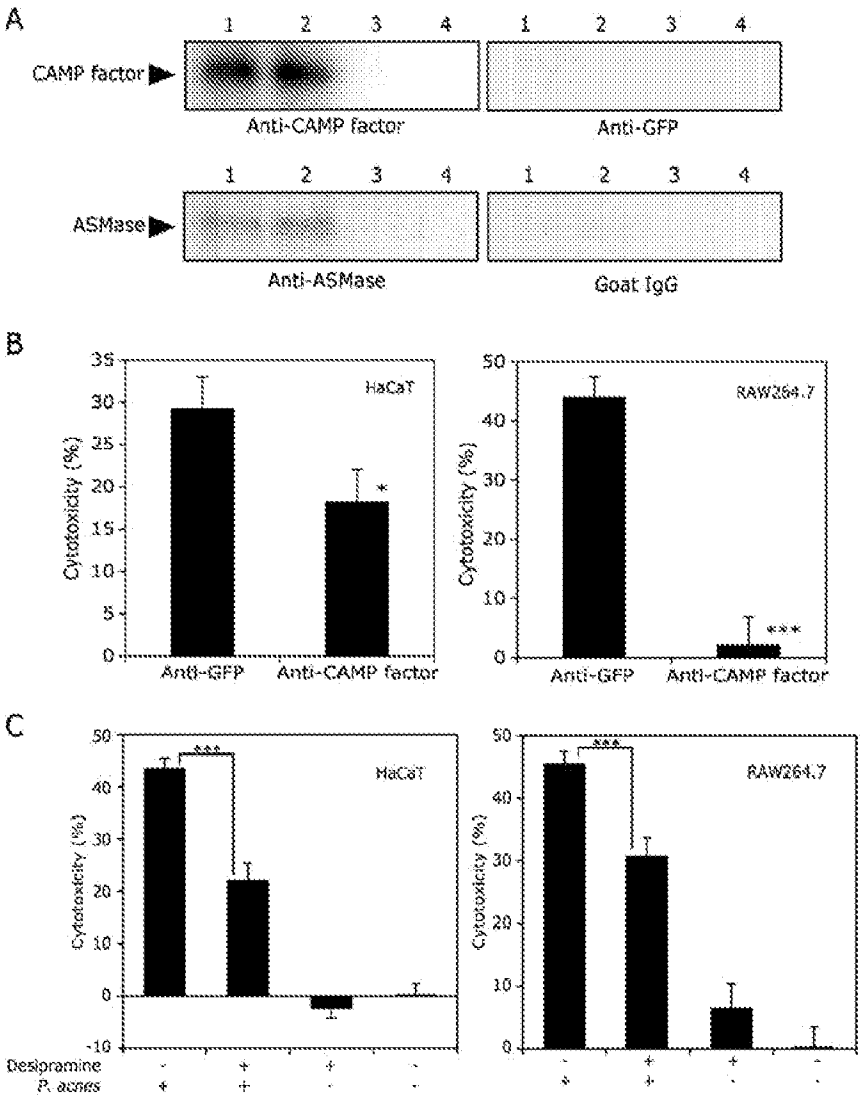

A
1 2 3 4 1 2 3 4
CAMP factor
Anti-CAMP factor
Anti-GFP
1 2 3 4 1 2 3 4
ASMase
Anti-ASMase
Goat IgG
B
HaCaT
Cytotoxicity (%)
Anti-GFP
Anti-CAMP factor
RAW264.7
Cytotoxicity (%)
Anti-GFP
Anti-CAMP factor
C
HaCaT
Cytotoxicity (%)
RAW264.7
Cytotoxicity (%)
Desipramine
P. acnes

FIGURE 12

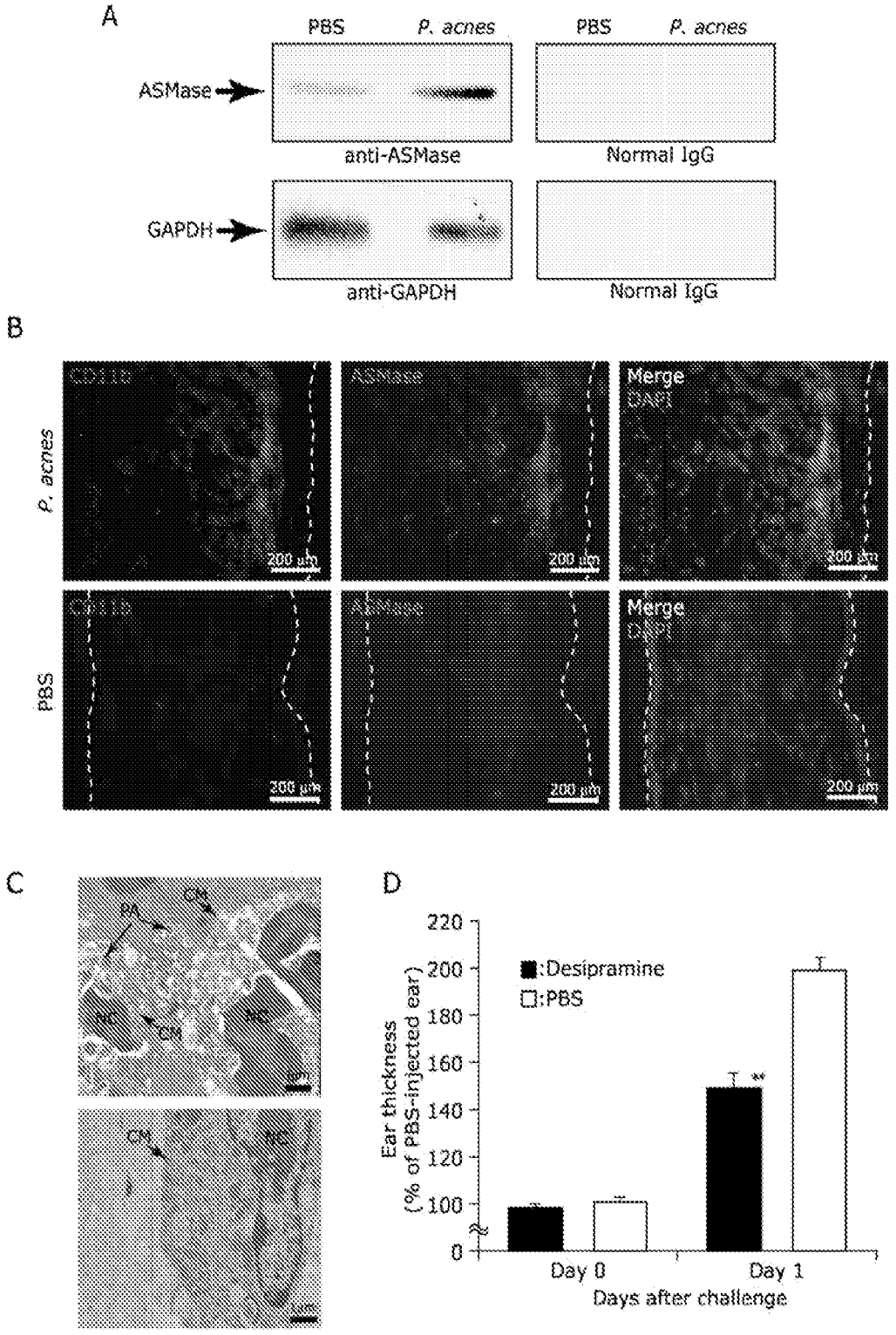

A
PBS
P. acnes
PBS
P. acnes
ASMase
anti-ASMase
Normal IgG
GAPDH
anti-GAPDH
Normal IgG
B
P. acnes
PBS
CD11b
ASMase
Merge
DAPI
200 μm
C
PA
CM
NC
D
:Desipramine
:PBS
Ear thickness
(% of PBS-injected ear)
220
200
180
160
140
120
100
0
Day 0
Day 1
Days after challenge

FIGURE 13

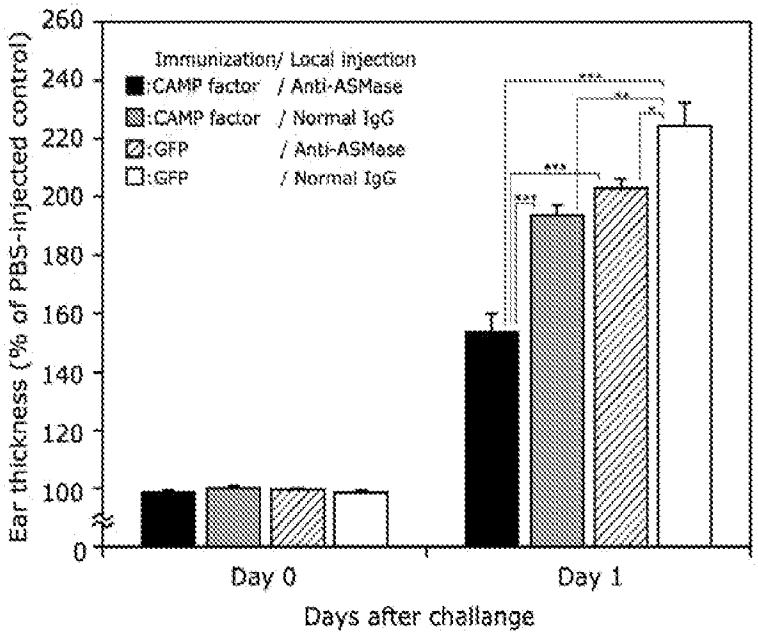


FIGURE 14

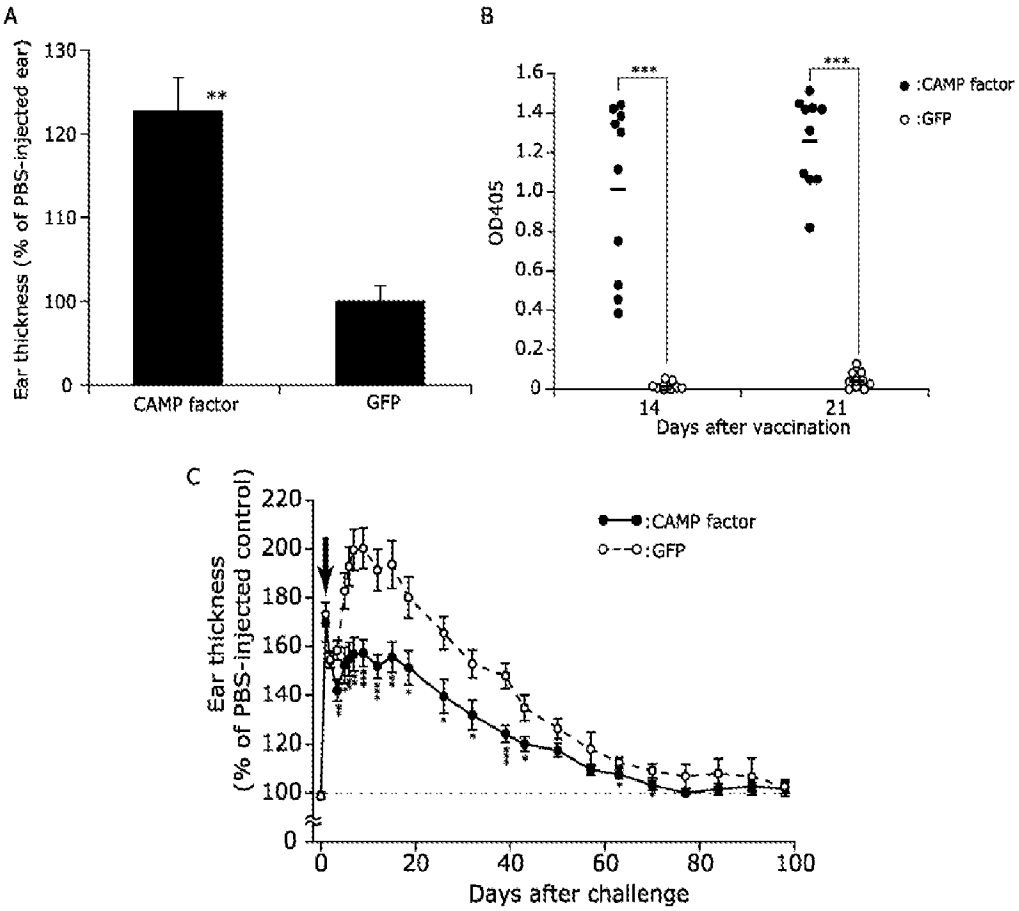


FIGURE 15

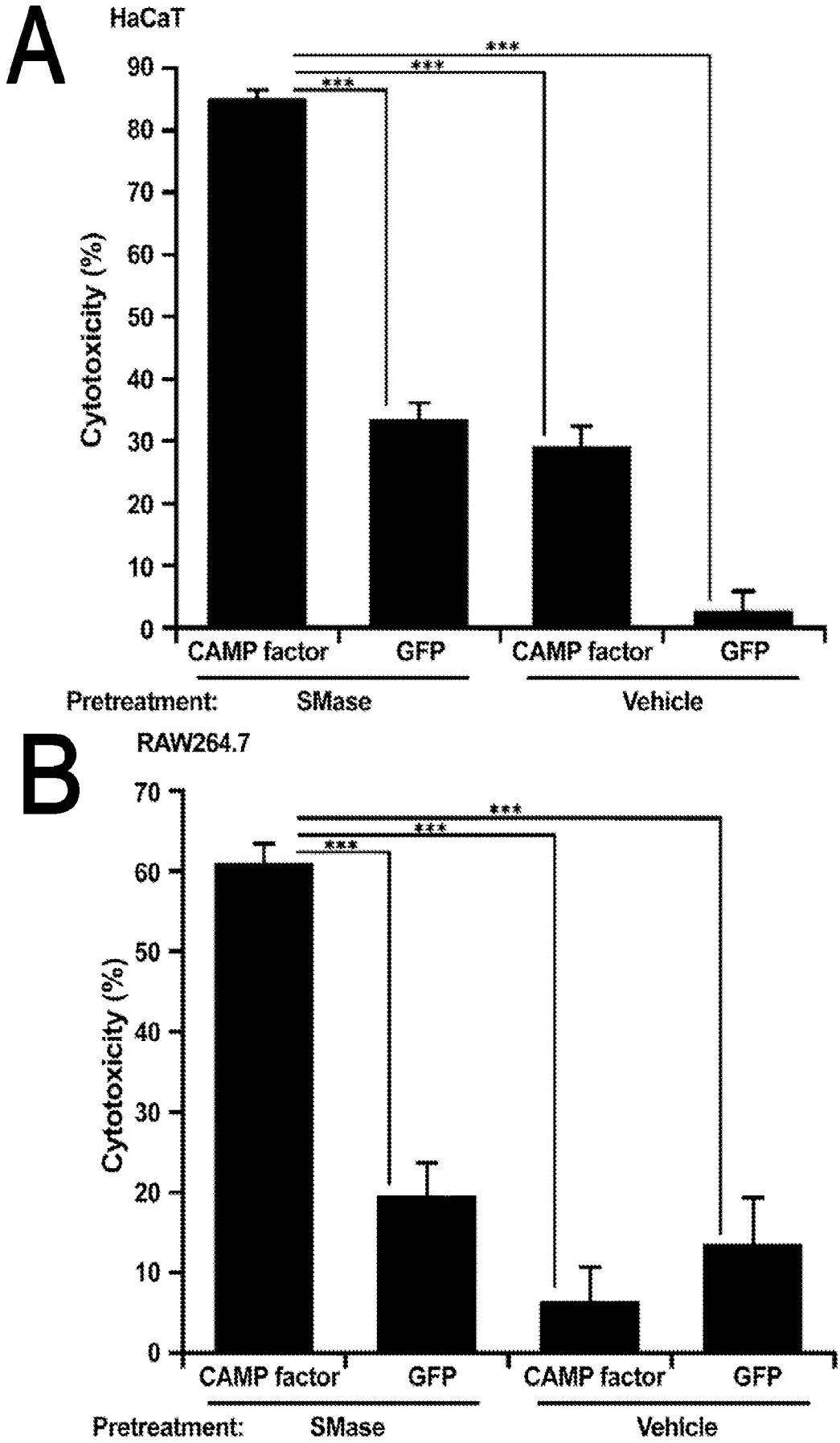

A
HaCaT
Cytotoxicity (%)
90
80
70
60
50
40
30
20
10
0
***
***
***
CAMP factor
GFP
CAMP factor
GFP
Pretreatment:
SMase
Vehicle
B
RAW264.7
Cytotoxicity (%)
70
60
50
40
30
20
10
0
***
***
***
CAMP factor
GFP
CAMP factor
GFP
Pretreatment:
SMase
Vehicle

FIGURE 16

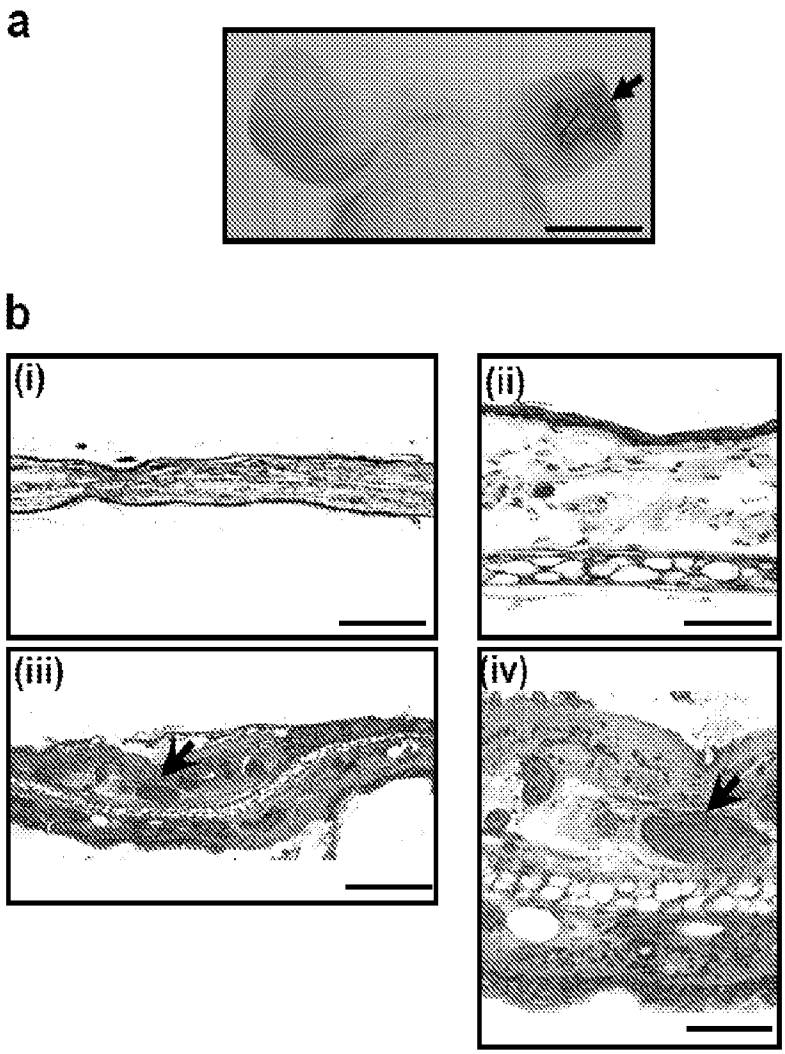


FIGURE 17

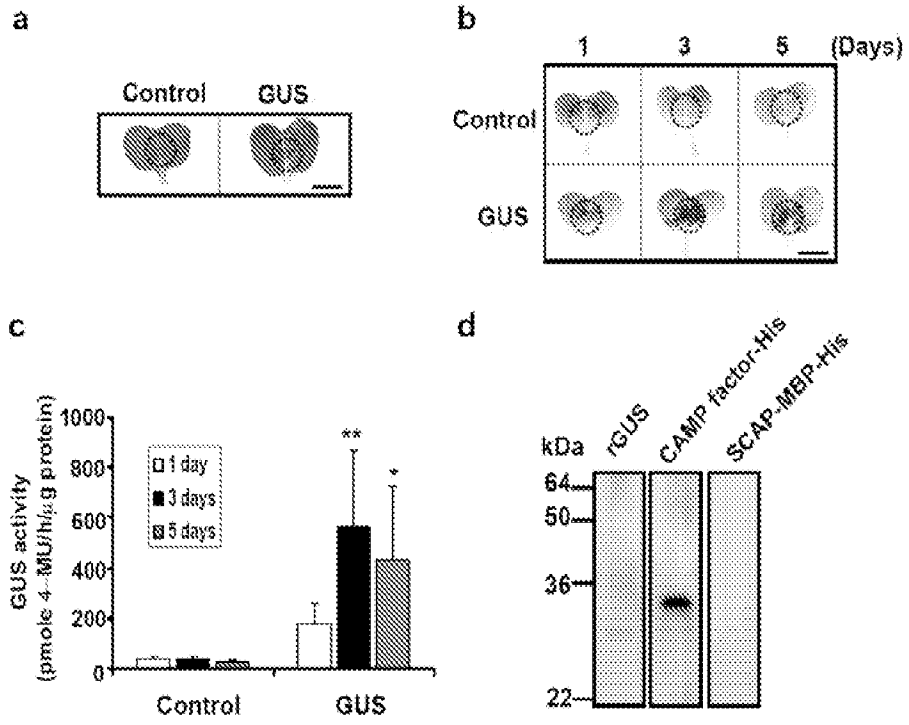


FIGURE 18

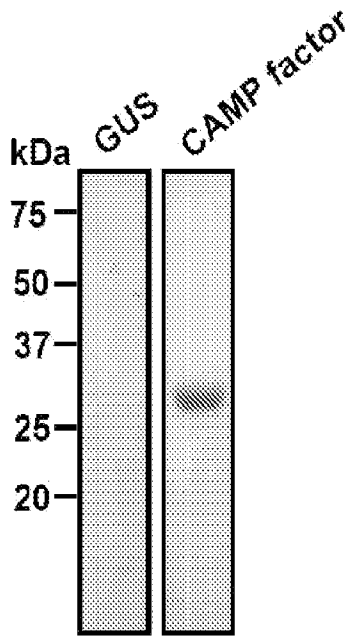


FIGURE 19

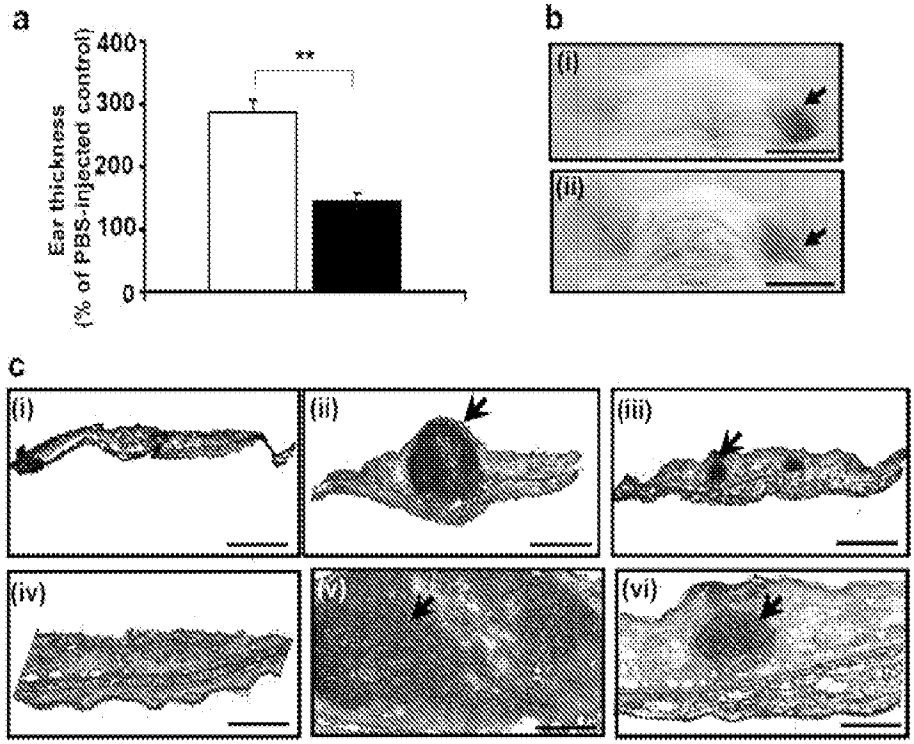


FIGURE 20

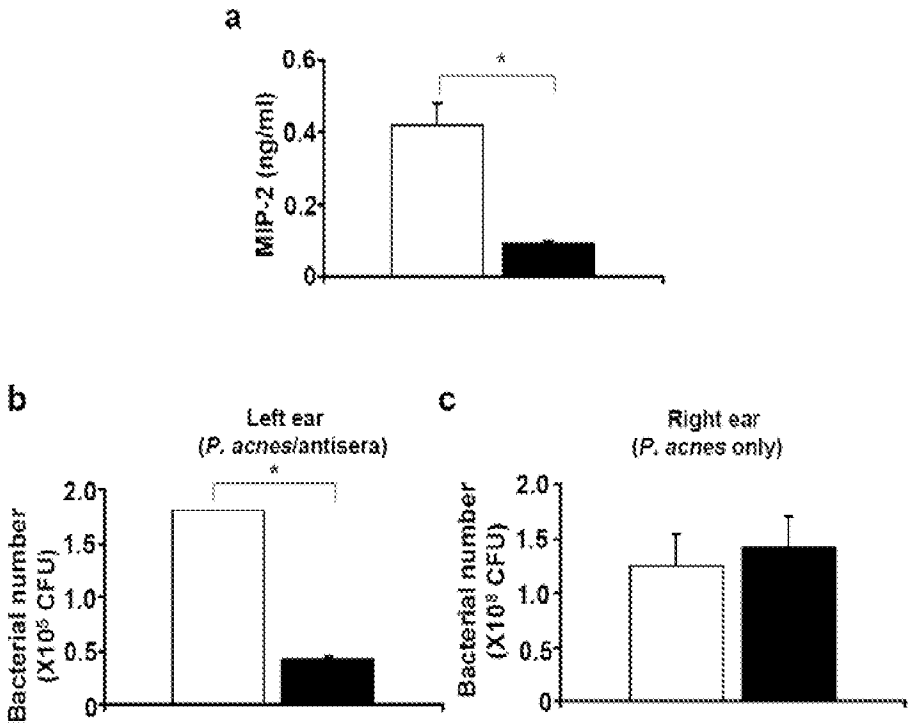


FIGURE 21

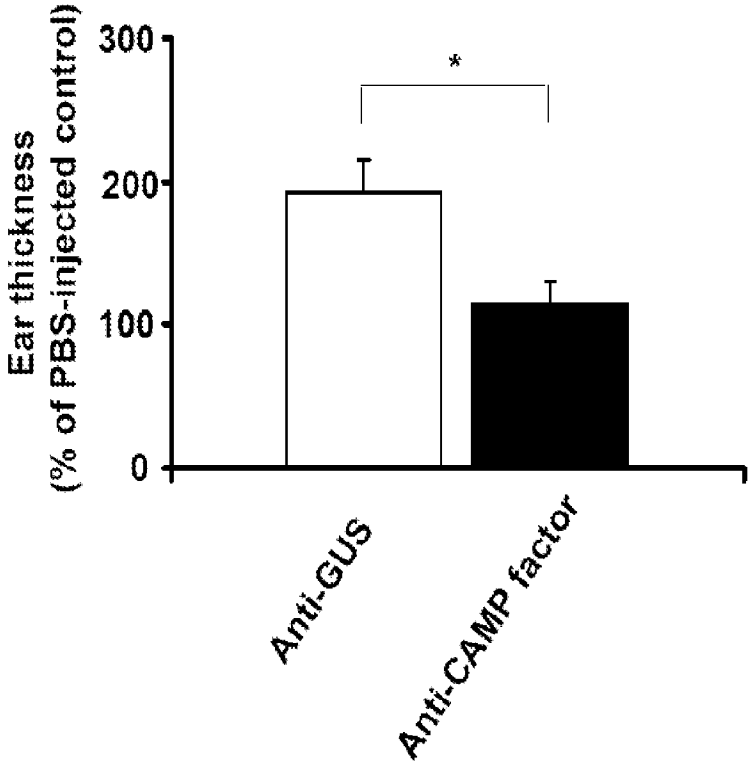

Ear thickness
(% of PBS-injected control)
300
200
100
0
*
Anti-GUS
Anti-CAMP factor

FIGURE 22