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Research Article

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Propionibacterium acnes-induced immunopathology correlates with health and disease association

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Genomic studies revealed the existence of health- and acne-associated *P. acnes* strains and suggested novel approaches for broadening understanding of acne vulgaris. However, clinical association of *P. acnes* with disease or health has yet to be corroborated experimentally. Current animal models of acne do not closely mimic human disease and have unclear translational value. We have developed a murine model of acne by combining *P. acnes* inoculation with topical application of a synthetic human sebum. We showed that human sebum promoted persistence of intradermally injected *P. acnes* with little loss of viability after 1 week and permitted use of more physiologic inoculums. Application of acne-associated *P. acnes* RT4/5 strains led to development of moderate to severe skin pathology compared with application of health-associated type II *P. acnes* strains (RT2/6). RT4/5 *P. acnes* strains uniformly induced higher levels of KC (IL-8), IL-1 α , IL-1 β , and IL-6 in vitro and in vivo compared with type II *P. acnes* strains. Overall, our data provide immunopathologic corroboration of health and disease association of clinical *P. acnes* strains and inform on a platform to query putative virulence factors uncovered by genomic studies.

Introduction

Acne vulgaris is an important disease affecting more than 80% of teenagers and young adults (1). It frequently results in permanent disfigurement, even with appropriate treatment. Common therapeutics include the use of antibiotics and antiinflammatory products, but a more targeted approach has eluded acne therapeutics. Although acne is a common disease, its etiology is still unclear. Acne is believed to be induced by multiple factors, including the abundant skin bacterium *Propionibacterium acnes*. Increased hormone levels and sebum production in puberty induce hyperkeratosis of the follicles and overgrowth of *P. acnes*, leading to skin inflammation.

Although *P. acnes* is thought to be important for the pathogenesis of acne vulgaris, it is found perplexingly to colonize both acne patients and healthy subjects. Recently, the enigma has been resolved through concerted effort by several groups: typing and genomic studies have identified various lineages of *P. acnes* with different degrees of health or acne association (2–4). Clade IA-2 strains (RT4 and RT5) have a particularly strong association with acne, while type II strains show a strong association with healthy human skin (3). Consistent with these clinical associations, genomic analyses revealed putative pathogenic factors encoded uniquely in acne-associated *P. acnes* strains and, therefore, suggest novel approaches to treat acne (3). In addition, a metagenomic study of the skin microbiome in acne suggests that the balance between health- and disease-associated microbes, rather than the presence of acne-associated strains, is an important determinant of acne (5). Altogether, the discovery of health- and acne-associated *P. acnes* strains suggests a novel approach to significantly broaden understanding of acne pathogenesis and further suggests that a targeted therapeutic approach should replace currently practiced nonspecific killing of *P. acnes*. However, transitioning from clinical association studies to pathogenesis and therapeutic studies requires first the corroboration of the clinical associations.

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To date, studies of human monocyte stimulation have shown induction of higher IL-17 and IFN- γ and lower IL-10 by acne-associated *P. acnes* strains compared with health-associated *P. acnes* strains (6, 7), thereby providing a link between host adaptive inflammatory mechanisms and acne. However, other studies that explored the relationship between *P. acnes* strains and innate proinflammatory responses have suggested enhanced host innate immune responses in association with health- rather than acne-associated strains (8). There is no report to date of in vivo studies comparing immune response induced by health- and acne-associated *P. acnes* strains. Although many animal models of acne exist (9), no current animal model of acne recapitulates all features of the disease in humans, and the clinical significance and translational value of existing models are unclear. Several models employ unconventional animals or animals with limited immunologic competence (rhino mice, Mexican hairless dogs, and nude mice transplanted with human skin) (10–12). Bacterial infection is not needed for induction of acne in many models, which clearly limits the relevance of those models to the human condition. A frequently used platform induces skin inflammation on the back or ear of rodents using high intradermal inoculums of *P. acnes* (10^7 – 10^9 live or dead *P. acnes*) (13, 14).

Here, we established an improved murine model of acne that combines application of *P. acnes* and human sebum and studied, in vitro and in vivo, the immunopathologic properties of *P. acnes* with strong health and disease association.

Results

An acne murine model that combines P. acnes and human sebum application. In initial experiments, we inoculated CD-1 mice in the back with 10^7 *P. acnes* based on a modified published protocol (13). As shown in Figure 1, intradermal inoculation of the mice with 10^7 acne-associated *P. acnes* RT5 strain HL043PA1 in PBS resulted in minimal lesions and was associated with rapid clearance of the bacteria from the site of inoculation, consistent with reports of difficulty inducing skin pathology with *P. acnes* in immunocompetent mice (13). Borrowing from other pathogen models of infection (15), we washed and inoculated *P. acnes* in media instead of PBS. Use of media improved *P. acnes* recovery from lesions after 3 and 7 days but induced lesions remain modest.

Hormonally stimulated production of sebum at the onset of puberty is an important contributor to acne. Sebum is particularly abundant at anatomic sites with high concentration of *P. acnes* (16), and the sebum component oleic acid has been reported to promote growth of *P. acnes* in culture (17). Mouse sebum is different from human sebum, and current models of acne do not add human sebum to *P. acnes* as part of the models. We therefore tested if the combined use of human sebum and *P. acnes* would lead to enhanced pathology. We synthesized human sebum based on a published protocol (18). We then inoculated CD-1 mice with *P. acnes* and additionally applied freshly made sebum daily. As shown in Figure 1, addition of human sebum dramatically enhanced survival of *P. acnes*, with only modest loss of viability after 7 days. Although sebum alone had no effect on skin pathology, sebum when applied with *P. acnes* promoted reproducible and significant skin pathology, characterized by abscess formation, erythema, induration, skin necrosis, and scaling (Figure 1B and Supplemental Figure 1B; supplemental material available online with this article; <https://doi.org/10.1172/jci.insight.124687DS1>). Accompanying the skin lesion, coapplication of human sebum and *P. acnes* induced higher levels of IL-6, KC, IL-1 α , and IL-1 β within the skin lesions compared with application of *P. acnes* alone. Sebum alone induced negligible levels of these cytokines (data not shown).

Up to 10^5 – 10^6 *P. acnes* could be routinely recovered from human body surfaces with high concentration of sebum (16). To determine the persistence of *P. acnes* with lower inoculums of *P. acnes*, we administered 10^5 – 10^7 CFU *P. acnes* intradermally with topical sebum. We showed that use of inoculums in the 10^5 – 10^6 range leads to more rapid clearance of *P. acnes* than use of 10^7 *P. acnes* (Supplemental Figure 1A).

Because the pathogenesis of acne vulgaris requires the presence of *P. acnes* in hair follicles, we also investigated if topical application of *P. acnes* along with topical sebum reproduced lesions that more closely mimic human disease. Although application of *P. acnes* with sebum, after tape stripping, induced skin erythema and plaques that were not observed with *P. acnes* or sebum application alone, individual lesions around hair follicles were not observed and bacteria were not always visualized within follicles. Therefore, the model was not perceived to be an improvement on the intradermal model (Supplemental Figure 2). As a consequence, we elected to investigate the pathogenic properties of health- and disease-associated *P. acnes* strains using the intradermal mouse model.

In vivo immunopathology induced by clinical P. acnes strains with disease and health association. MLST and microbiome studies have revealed that certain *P. acnes* strains are associated with acne, while others are associated with health (2–4). We have shown that clade IA-2 strains (RT4 and RT5) are strongly associated with acne, whereas type II strains (RT2/6) show strong association with healthy skin (3). We selected

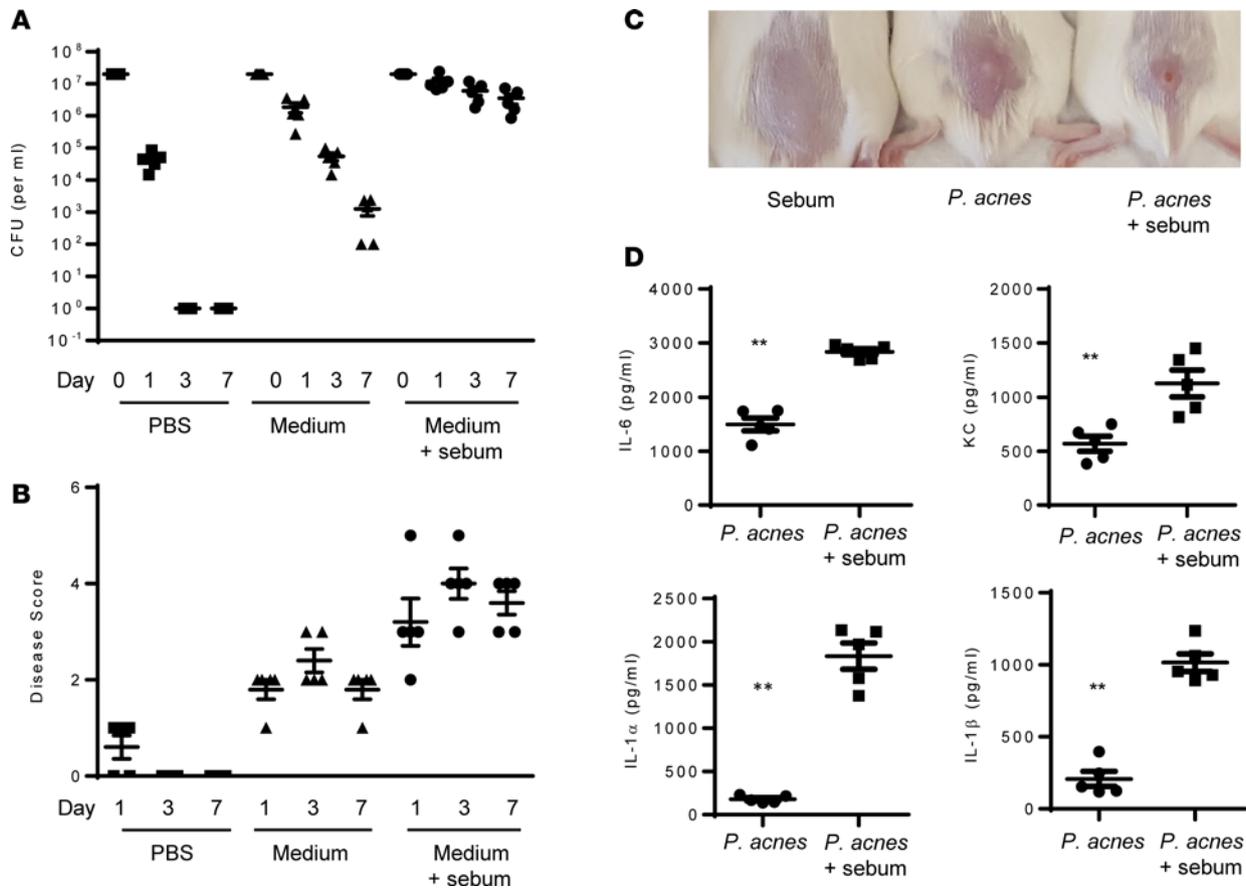


Figure 1. Sebum application enhances *P. acnes* survival in a murine model of acne immunopathology. *P. acnes* strain HL043PA1 (RT5, 10⁷ CFU) was washed and resuspended in PBS or intradermal injection, with or without daily topical application of sebum (20 μ l). (A) *P. acnes* recovery from skin lesions over 7 days. (B) Lesion sizes over 7 days and representative lesions on day 7. (C) Representative lesions induced with sebum alone, *P. acnes* alone, or both sebum and *P. acnes*. (D) Cytokines measured from skin homogenates, determined by ELISA. Data are shown as mean \pm SEM, $n = 5$ mice per group for A, B, and D. Data in A and B were analyzed by ANOVA and showed significance at $P < 0.01$ at day 1, 3, and 7 between groups. Data in D were analyzed using Mann-Whitney. ** $P < 0.001$.

several strains belonging to these clades for our study: 2 RT5 strains, 3 RT4 strains, 3 RT6 strains, and 1 RT2 strain (Supplemental Table 1). We applied these strains intradermally along with topical sebum and measured disease score based on skin induration, erythema, dermonecrosis, and scaling. Additionally, we measured histology and cytokines that are suggested to be important in acne disease. Although use of lower inoculums was possible, we inoculated 10⁷ *P. acnes* because that inoculum induces stable persistence of bacteria, which more closely mimics colonization.

As shown in Figure 2, RT4/5 strains uniformly induced higher disease scores compared with RT2/6 strains, without significant differences in bacteria clearance from the lesions on day 1 and 3. Consistent with skin immunopathology, higher cytokine levels (IL-6, IL-1 α , IL-1 β) and chemokine levels (KC) were also recovered from the lesions on day 3 (Figure 3).

Histology of skin lesions was studied and overall showed immunopathology that reflected the disease score, although there was more variation between samples compared with measured cytokines, chemokines, and disease scores (Figure 4 and Table 1). In all lesions, there was no apparent acute and chronic inflammation involving hair follicles and sebaceous glands. Minimal hyper- and/or parakeratosis was noted focally in rare cases. Most cases showed acute inflammation, located in the deep soft tissue underneath skeletal muscle layer, either in a localized mass-like (abscess) pattern or a diffuse band-like pattern, with mild spill over to the subcutaneous layer. Few cases demonstrated a mixed localized and diffuse pattern. Overall evidence of chronic inflammation was minimal or mild and was mostly in a diffuse band-like pattern, with rare cases showing vague granulomatous change. Gram stain of the slides showed approximate colocalization of the bacteria and immune cells, with many aggregates of Gram-positive rods within cytoplasm of neutrophils (Figure 4).

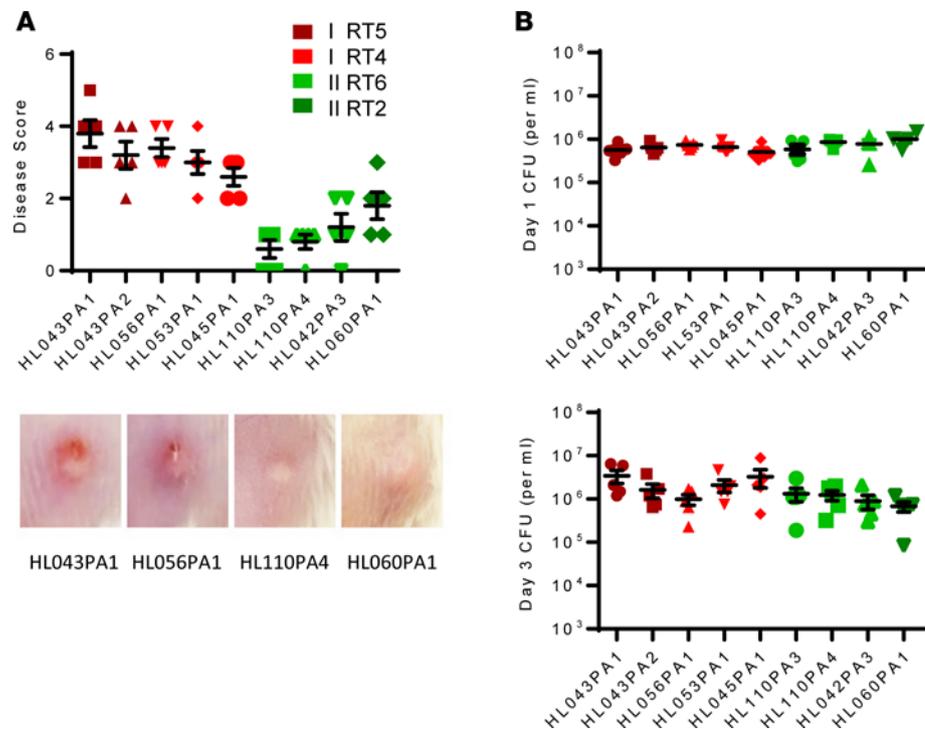


Figure 2. Induction of skin immunopathology by *P. acnes* strains associated with health and acne vulgaris. *P. acnes* strains (10^7 CFU) were injected intradermally, and sebum ($20\ \mu\text{l}$) was applied topically daily. (A) After 3 days, skin lesions were scored. Representative lesions induced by health- and disease-associated *P. acnes* are shown. (B) Skin was excised, and bacteria burden on day 1 and 3 was determined. Data are shown as mean \pm SEM, $n = 5$ mice per group. Data analysis compared cytokines induced by *P. acnes* RT4/5 (all 5 strains) versus RT2/6 (all 4 strains) using nonparametric Mann-Whitney *U* test. $P < 0.001$, comparing red and green strains.

In vitro host proinflammatory response to health- and disease-associated *P. acnes* strains. The robust correlation between *P. acnes* health and disease association and immunopathology prompted us to evaluate the proinflammatory properties of the strains *in vitro*. We focused on few cytokines and chemokines that have been shown to be important in acne pathogenesis, including IL-1 α , produced by keratinocytes and considered important for hyperkeratosis and comedo formation; IL-1 β and IL-6, produced by monocyte/macrophages particularly in response to TLR2; and IL-8 (or mouse homolog KC), an important chemokine linked to acne (19). We challenged the human keratinocyte HaCaT cell line and mouse primary macrophages with either killed *P. acnes* strains or their supernatant to assess proinflammatory activity of cell-associated and secreted *P. acnes* factors.

Consistent with the findings from the murine acne model, *P. acnes* RT4/5 strains uniformly induced significantly higher levels of IL-8 and IL-6 from keratinocytes and IL-1 α , IL-6, and KC from macrophages (Figures 5 and 6). Higher cytokine and chemokine levels were observed with killed bacteria and supernatants from *P. acnes* cultures, suggesting that both cell-associated and secreted factors from RT4/5 strains likely contribute to the severity of immunopathology. Minimal or no secreted IL-1 β or IL-1 α was detected in *P. acnes*-macrophage and *P. acnes*-keratinocyte coculture assays, respectively (data not shown). Combined with disease score and *in vivo* cytokines, these *in vitro* cytokine and chemokine data strongly corroborate the hypothesis that individual acne-associated *P. acnes* strains, through their background virulence genes, are sufficient to drive immunopathology associated with human acne disease.

Discussion

Although decades of studies suggest that *P. acnes* is important for pathogenesis of acne vulgaris, remarkably little is understood of how this commensal bacterium affects acne. Contributing to this poor understanding are the relative difficulty to manipulate *P. acnes* and uncertainty of the clinical relevance of the animal models (9). In this study, we applied a synthetic human sebum to old murine models of acne and showed that survival of *P. acnes* is significantly enhanced with sebum. It isn't clear how addition of human sebum improves survival of *P. acnes*, as both mice and human make sebum, albeit of different composition. The

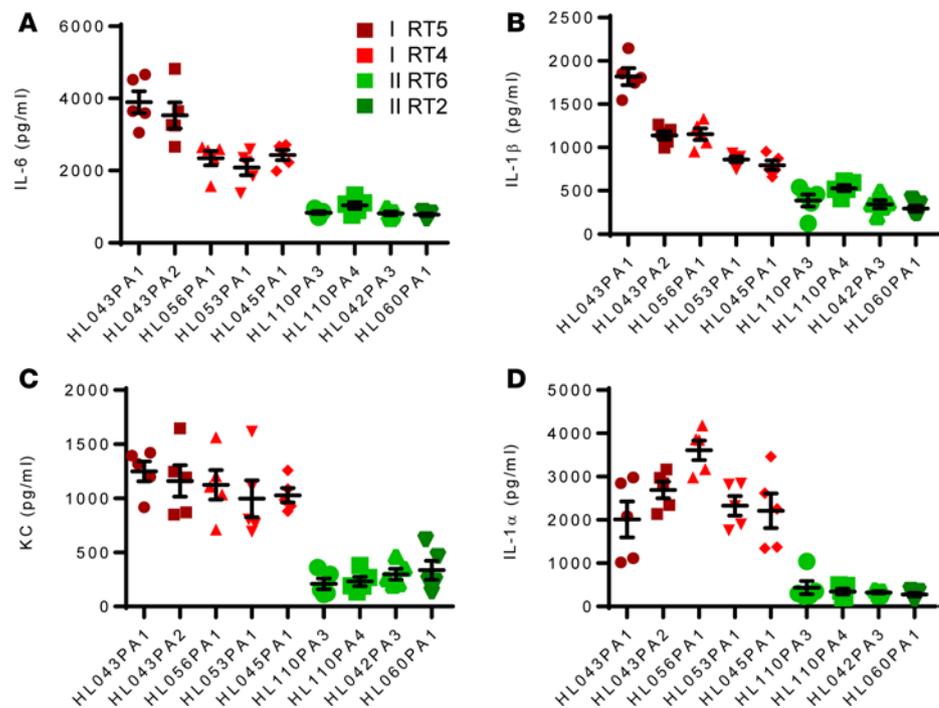


Figure 3. In vivo proinflammatory activity of health- and acne-associated *P. acnes* strains. Mice were injected with 1×10^7 *P. acnes*, and sebum (20 μ l) was applied topically daily. After 3 days the skin was excised and homogenized, and cytokines levels were determined by ELISA. Data are shown as mean \pm SEM ($n = 5$ mice per group). Data analysis compared cytokines induced by *P. acnes* RT4/5 (all 5 strains) versus RT2/6 (all 4 strains) using nonparametric Mann-Whitney *U* test. $P < 0.001$, comparing red and green strains.

applied synthetic sebum is made of triglycerides, oleic acid, wax esters, and squalene and is simplified from the much more complex human sebum, made of squalene, cholesterol, cholesteryl esters, fatty acids, triglycerides, diglyceride, and wax esters (20, 21). Mouse sebum, compared with human sebum, has significantly lower triglycerides and squalene and higher wax esters (21). In a study that assessed *P. acnes* growth on the skin of laboratory animals (mice, rats, rabbits, sheep, guinea pigs, and dogs), only the sebaceous (perianal gland) regions of guinea pigs harbored a significant burden of *P. acnes* (22). The authors attributed growth of *P. acnes* in the guinea pig sebaceous gland to the higher concentration of triglyceride found in the region. Consistent with this report, oleic acid, a breakdown product of triglyceride, promotes growth of *P. acnes* in vitro (17, 23), although these findings have been disputed by another group (24). Squalene, which is also abundantly found in human sebum but not in mouse sebum (21), is found in higher concentrations in sebum of subjects with acne compared with controls without acne (25), but its role in supporting *P. acnes* growth is unclear. Additionally, the sheer abundance of sebum could also account for improved *P. acnes* survival, as human studies have shown that sebum concentration is highest on the face and back and correlates with the number of *P. acnes* isolated from those sites (11).

Irrespective of the mechanism whereby human sebum enhances the mouse model, application of human sebum permits use of lower inoculums of *P. acnes* and leads to more stable bacteria burden and robust immunopathology. Our model has several caveats, including the intradermal administration of *P. acnes* and the relative acute nature of infection, and the model is not improved by topical application of *P. acnes* and sebum. Dysregulation of host skin environment and hypercornification of sebaceous gland have been proposed to be initial events facilitating *P. acnes* induction of inflammation (26). We have been unable to model these initial events; therefore, further studies are clearly needed to refine the murine model. Nonetheless, when rigorously selected health- and disease-associated strains are applied, the *P. acnes* strains produced disease scores and immunopathology that correlated strongly with their clinical association. These findings suggest that the virulence properties of *P. acnes* that are important for enhancing human acne inflammation are also important for immunopathology in the murine model. Altogether, we suggest that our validated model will be able to more reliably predict human disease compared with published models.

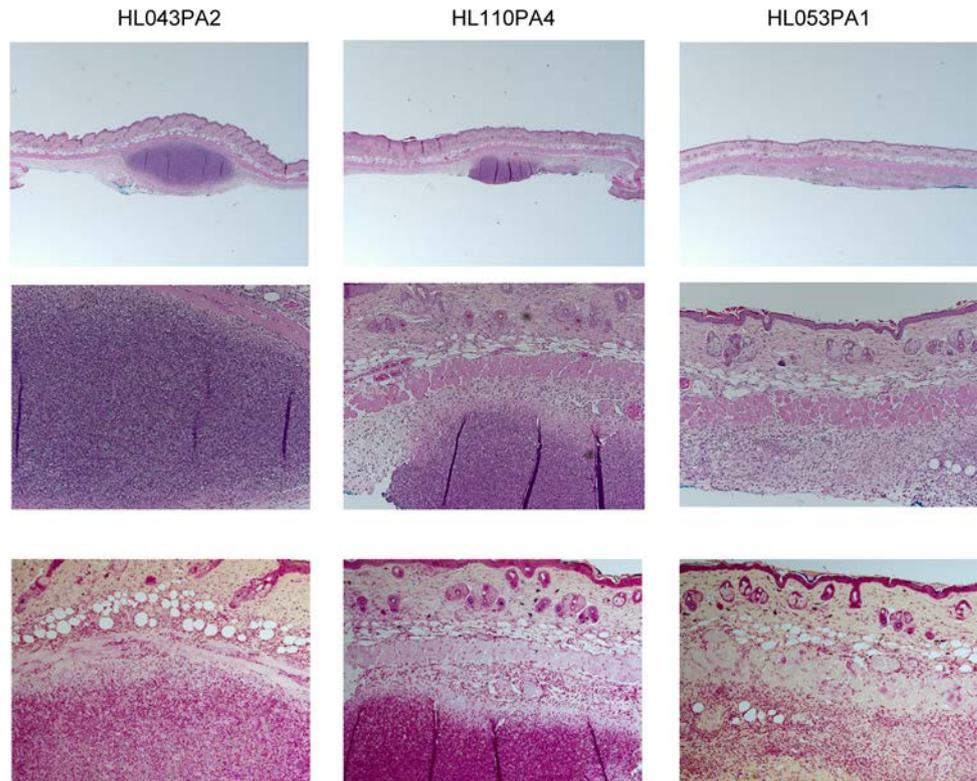


Figure 4. Histology of skin lesions induced by health- and acne-associated strains. Mice were injected intradermally with 1×10^7 *P. acnes*. Synthetic sebum (20 μ l) was applied daily, and skin was excised. H&E and Gram staining was performed after 7 days. Shown are slides representing 3 histologic patterns. Corresponding blinded scores are shown in Table 1. Original magnification, $\times 20$ (top, H&E); $\times 100$ (middle, H&E; bottom, Gram).

Acne vulgaris is a multifactorial disease involving *P. acnes* and host and environmental factors. The extent of bacterial contribution to the disease has been unclear, since acne patients and healthy individuals both harbor significant numbers of *P. acnes* on their skin. Genomic and metatranscriptomic studies indicate that acne-associated *P. acnes* strains (2–4, 27, 28) may contribute to the disease through virulence genes and transcriptional and metabolic activities and further suggest that putative virulence factors encoded by acne-associated strains could be exploited for therapeutics. Our study provides strong laboratory corroboration of genomic and clinical findings by demonstrating remarkable and uniform correlation between health/disease association of *P. acnes* and immunopathology induced by the strains. These findings suggest that the contribution of *P. acnes* genetic background to acne disease

Table 1. Histopathology of skin lesions induced by *P. acnes* strains

Strain name	Acne/health association	Acute inflammation score (\pm SD)
HL043PA1	Acne	2.0 \pm 1.73
HL043PA2	Acne	1.83 \pm 1.26
HL056PA1	Acne	2.16 \pm 1.4
HL045PA1	Acne	1.83 \pm 1.26
HL053PA1	Acne	0.66 \pm 0.57 ^A
HL110PA3	Health	1.0 \pm 1.4
HL110PA4	Health	1.25 \pm 1.1

Skin lesions were induced by intradermal injection of selective *P. acnes* strains and topical sebum, as described in the legend for Figure 2. Lesions ($n = 3$) were harvested on day 7, and H&E staining of the lesions was scored as described in Methods. ^AThis is the only strain that showed histologic evidence of chronic inflammation, with vague granuloma formation in 2 of 3 samples (see Figure 4).

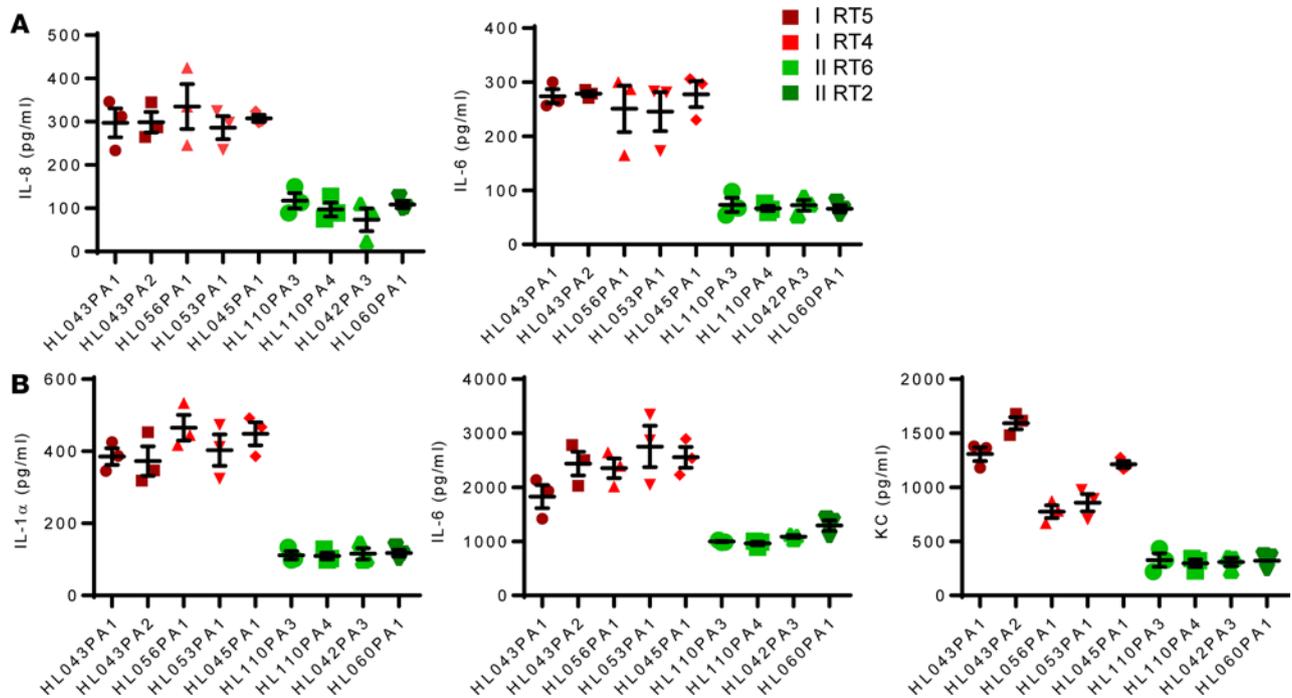


Figure 5. In vitro proinflammatory activity of heat-killed health- and acne-associated *P. acnes* strains. (A) HaCaT cells were stimulated for 12 hours with heat-killed *P. acnes* at an MOI of 10, and cytokines levels were determined by ELISA. (B) Bone marrow-derived macrophages were stimulated for 12 hours with heat-killed *P. acnes* at an MOI of 10, and cytokines were measured by ELISA. Data are shown as mean \pm SEM ($n = 3$) and are representative of 3 experiments. Data analysis compared cytokines induced by *P. acnes* RT4/5 (all 5 strains) versus RT2/6 (all 4 strains) using nonparametric Mann-Whitney *U* test. $P < 0.001$, comparing red and green strains.

must be substantial and that the investigation of putative virulence factors is likely to have translational and therapeutic value.

Currently, few *P. acnes* factors have been scrutinized for a potential role in acne pathogenesis. Huang and colleagues have shown that Christie-Atkins-Munch-Petersen (CAMP) factor contributes to inflammation and CAMP immunization ameliorates inflammation induced by *P. acnes* in the ear inflammation model (29, 30), although CAMP is expressed by both health- and acne-associated *P. acnes* strains/clades (6). We previously demonstrated that acne-associated *P. acnes* strains from type IA-2 RT4/5 strains produce higher levels of proinflammatory porphyrins compared with type II RT2/6 strains at baseline (27). In response to vitamin B12 supplementation, IA-2 RT4/5 strains further upregulate porphyrin production and promote inflammation, whereas type II RT6/2 strains are unresponsive to vitamin B12 supplementation (27, 28). Repressor gene *deoR*, which is present in type II strains but not in type IA-2 strains, is likely to be responsible for the modulation of porphyrin production (27, 28).

In addition to CAMP and porphyrin, comparative analysis of genomes from health- and acne-associated *P. acnes* strains has revealed other factors that could contribute to differences in immunopathology and induced inflammatory cytokines (6, 31–33). Specifically, IA-2 RT4/5 strains harbor two genomic islands and a linear plasmid with many unique genes, some of which are homologs to known virulence genes (3, 31). The linear plasmid encodes a tight adhesion (Tad) locus, which has demonstrated virulence functions in other microbes (34, 35). Within the genomic islands, a *Sag* gene cluster encodes proteins that have been shown to induce hemolysis in *S. pyogenes* and *S. iniae* with associated proinflammatory and pathogenic functions (36, 37). In addition, lipases and hyaluronidase are differentially expressed by acne- and health-associated strains and could also contribute to inflammation (31–33). At the proteome level, Kim and colleagues have shown that a cell wall hydrolase and several proteins of unknown function were expressed at significantly higher levels in acne-associated compared with health-associated strains (6). Characterization of these factors would significantly broaden our knowledge of acne pathogenesis and would likely be of clinical significance, given that *P. acnes* RT4/5 and RT2/6 strains have shown clear differences in pathology in our murine model.

Inflammation is an important driver of pathology in acne disease. Therefore, following the discovery of health- and acne-associated *P. acnes* strains and phylotypes, several groups have investigated the proinflam-

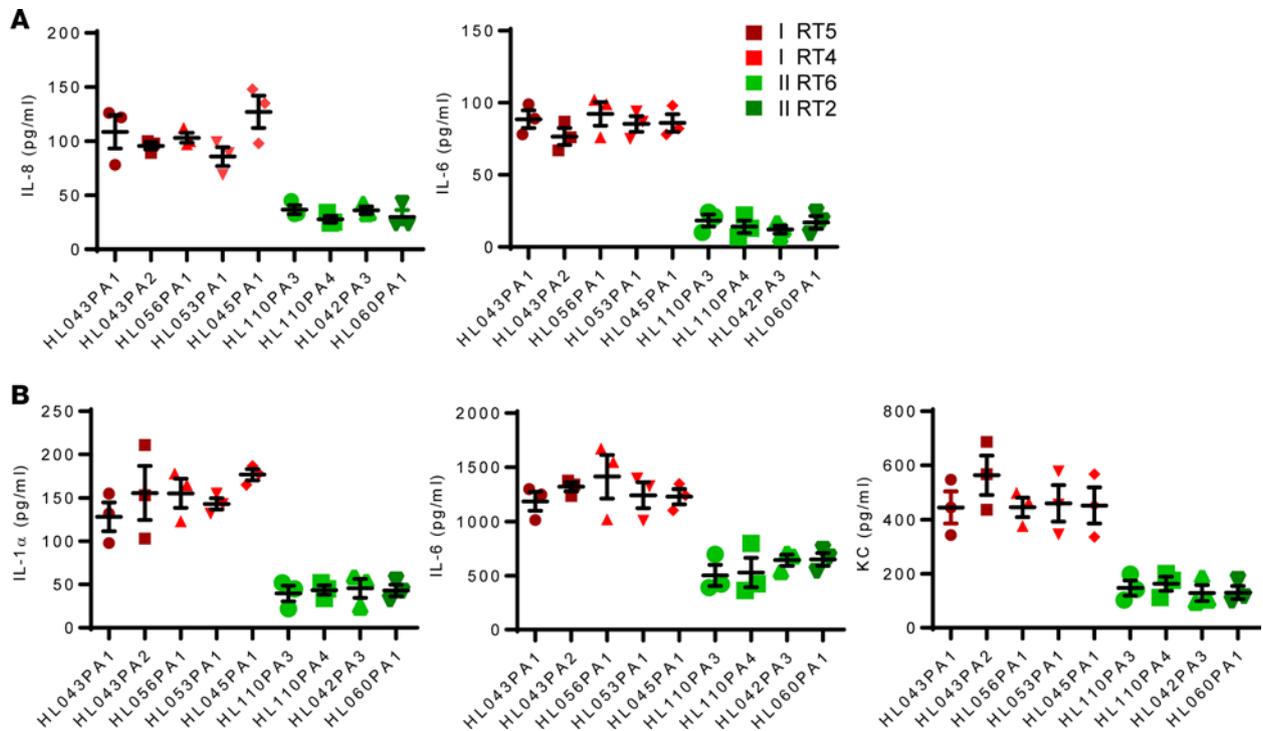


Figure 6. In vitro proinflammatory activity of supernatant from health- and acne-associated *P. acnes* strains. (A) HaCaT cells were stimulated for 12 hours with supernatant (10 μ l) from overnight *P. acnes* cultures, and cytokines levels were determined by ELISA. (B) Bone marrow-derived macrophages were stimulated for 12 hours with supernatant (10 μ l) from overnight *P. acnes* cultures, and cytokines were measured by ELISA. Data are shown as mean \pm SEM ($n = 3$) and are representative of 3 experiments. Data analysis compared cytokines induced by *P. acnes* RT4/5 (all 5 strains) versus RT2/6 (all 4 strains) using nonparametric Mann-Whitney *U* test. $P < 0.001$, comparing red and green strains.

matory activity of *P. acnes* strains from diverse phylotypes. Nagy and colleagues showed that strains from type IA₁ induced higher β -defensin from keratinocytes compared with a strain from type II (38). Conversely, the type II *P. acnes* induced higher levels of IL-8 (38). Jasson and colleagues challenged skin explant with lysates from *P. acnes* but showed higher expression of PAR-2, TIMP-2, MMP-13, and TNF- α by health- or non-acne-associated types (II and III) than by acne-associated strains (8). A caveat of these earlier studies is that the strains used were not well characterized for health and disease association and the studies looked mostly at single isolates from each phylotype. More recently, Kim and colleagues stimulated human monocytes with well-characterized health- and disease-associated *P. acnes* strains, including strains used in this study (6, 7). They concluded preferential induction of proinflammatory IL-17 by acne-associated *P. acnes* strains and enhanced induction of antiinflammatory IL-10 by health-associated *P. acnes* strains. Our in vitro studies further build on that study and demonstrate uniformly higher innate cytokine and chemokine induction by RT4/5 strains compared with type II strains. Increased IL-6 and IL-1 β induction, which promote Th17 development, is consistent with the reported association between acne and Th17. Overall, these data support the hypothesis that acne-associated *P. acnes* strains promote acute and chronic inflammation through activation of the innate and adaptive branch of the immune system. Because of the close association of select cytokine secretion with disease, proinflammatory cytokines deserve to be further studied as potential markers for health and acne association when screening *P. acnes* strains. Identification and manipulation of health- or disease-associated strains could represent a novel strategy to modify risk of severe acne.

In summary, our study has revealed an unexpectedly strong laboratory correlation between *P. acnes* immunopathology in animals and clinical disease and health association. The validated model system will facilitate investigation of putative virulence factors identified through the genomic studies.

Methods

Bacterial strains and growth conditions. *P. acnes* strains were isolated as stated previously (3). *P. acnes* strains were grown on blood agar plates and then isolated colonies were grown in BHI media at 37°C anaerobically until the stationary phase was reached. For supernatant samples, cultures were centrifuged at 3220 *g* for 5

minutes, and the supernatant was collected and filter sterilized. For heat-killed samples, overnight bacterial cultures were incubated at 65°C for 1 hour. For murine experiments, cultures were diluted 1:100 in fresh BHI and allowed to grow until an OD₆₀₀ of 0.1–0.3 was reached. Cultures were centrifuged at 3220 g for 5 minutes and washed in BHI 3 times, and the resulting pellets were resuspended in media.

Synthetic sebum. Synthetic sebum was prepared as previously described (18). Sebum was prepared on the day of experiment by mixing fatty acid (17% oleic acid, MilliporeSigma), triglyceride (45% triolein, Fisher), 25% wax monoester (25% jojoba oil), and squalene (13%, Fisher).

Murine model of acne immunopathology by intradermal injection of P. acnes. Eight-week-old CD-1 female mice (Jackson Lab) were shaved before application of Nair (Church & Dwight). The next day, the mice were injected intradermally with approximately 1×10^7 CFU of *P. acnes* in 50 μ l BHI media. Immediately following injection, 20 μ l freshly made synthetic sebum was applied to the skin and reapplied daily. Lesions were aseptically harvested at specified time points, and CFU was determined on agar plates incubated anaerobically. In addition, homogenized lesions were centrifuged at 9300 g for 10 minutes, and the supernatants were stored at –80°C for cytokine analysis by ELISA. For histology, skin was fixed in 10% formalin (Medical Chemical Corporation), embedded in paraffin, and submitted to the Department of Pathology at Cedars-Sinai Medical Center for H&E and Gram staining.

Disease scoring. Gross skin pathology was scored based on tabulation of the following: erythematous change (no = 0, mild = 1, and marked = 2); papule (flat = 0, small = 1, and large = 2); eschar (no = 0, mild = 1, and marked = 2); and xeroderma (no = 0, mild = 1, and marked = 2).

Histology slides were evaluated based on acute inflammatory changes in the intracorneal or subcutaneous/deep soft tissue (0 = normal, 1 = mild, 2 = moderate, 3 = severe).

Murine model of acne immunopathology by tape stripping and topical application of P. acnes. Eight-week-old CD-1 female mice were shaved before application of Nair (Church & Dwight). The following day, mice were anesthetized and tape stripped 10 times with masking tape. Immediately after, 1×10^7 CFU of *P. acnes* in 20 μ l was applied to the tape-stripped area with or without 20 μ l freshly made synthetic sebum and allowed to absorb into the skin. Sebum was applied daily and lesions were measured on day 3.

Cell culture. Bone marrow cells were isolated from the femurs and tibiae of 12-week-old C57BL/6 mice (Jackson Lab) and suspended in RPMI 1640 medium with 10% heat-inactivated fetal bovine serum. Ten percent supernatant from L929 cells (ATCC), containing M-CSF, was added to induce differentiation of bone marrow cells into macrophages (bone marrow-derived macrophages). The cells were cultured 5% CO₂ at 37°C for 7 days prior to use.

HaCaT cells (ATCC), a human keratinocyte cell line, were maintained in DMEM (Invitrogen) media with the addition of 10% heat-inactivated fetal bovine serum, 4.5 g/l glucose, and 2 mM L-glutamine. Keratinocytes were seeded at an appropriate density in tissue culture plates in 5% CO₂ at 37°C 1 day prior to stimulation.

ELISA. Mouse IL1- α - (catalog 433404, Biolegend), IL1- β - (catalog 432601, Biolegend), IL-6- (catalog 431301, Biolegend), and KC/CXCL1- (DY453-05, R&D Systems) and human IL-6- (catalog 430504, Biolegend) and IL-8-specific (catalog no. 431501, Biolegend) ELISAs were performed according to the manufacturer's instructions.

Statistics. Data are expressed as mean \pm SEM. Two-group analysis used unpaired *t* test (2 tailed) or non-parametric Mann-Whitney *U* test in the case of missing normality. Comparisons of multiple groups were performed using 1-way ANOVA and subsequent Bonferroni multiple comparisons. If normality or equal variance tests failed, then a Kruskal-Wallis test and subsequent Dunn's multiple comparisons were used. All in vitro studies were done with at least 3 sets of independent experiments. GraphPad Prism was used for all analyses. A *P* value of less than 0.05 was considered significant.

Study approval. All procedures were approved by the IACUC of Cedars-Sinai Medical Center and conducted in accordance with NIH guidelines for the care and use of laboratory animals.

Author contributions

SLK, GYL, JT, and HL conceived and designed the study. SLK and CMT performed the experiments. XF performed the pathological analyses. All authors contributed to data analyses. SLK and GYL wrote the manuscript. All authors contributed to manuscript editing.

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