Cutibacterium acnes infection induces type I interferon synthesis through the cGAS-STING pathway

1 **Katrin Fischer[1], Roland Tschismarov[2], Andreas Pilz[1], Susy Straubinger[3], Sebastian**
2 **Carotta[3], Andrew McDowell[4] and Thomas Decker[1*]**

3 [1]Max Perutz Labs, Department of Microbiology, Immunobiology and Genetics, University of
4 Vienna, Vienna Biocenter, Vienna, Austria
5 [2]Themis Bioscience GmbH, Vienna, Austria
6 [3]Boehringer Ingelheim RCV GmbH & Co KG, Vienna, Austria
7 [4]School of Biomedical Sciences, Ulster University, Coleraine, United Kingdom

8 *** Correspondence:**
9 Thomas Decker
10 Thomas.decker@univie.ac.at

11 **Keywords: Cutibacterium acnes, Interferon, Listeria monocytogenes, cGAS, STING,**
12 **TRIF, STAT, NFκB**

Abstract

14 *Cutibacterium* (previously *Propionibacterium*) *acnes* is an anaerobic, Gram-positive
15 commensal of the human body. The bacterium has been associated with a variety of diseases,
16 including acne vulgaris, prosthetic joint infections, prostate cancer and sarcoidosis. The
17 accumulation of *C. acnes* in diseases such as acne and prostate cancer has been shown to
18 correlate with enhanced inflammation. While the *C. acnes*-induced proinflammatory axis, via
19 NF-κB and MAPK signaling and inflammasome activation, has been investigated over the last
20 few decades, the potential role of *C. acnes* in triggering the type I interferon (IFN-I) pathway
21 has not been addressed. Our results show that *C. acnes* induces the IFN-I signaling axis in
22 human macrophages by triggering the cGAS-STING pathway. In addition, IFN-I signaling
23 induced by *C. acnes* strongly depends on the adapter protein TRIF in a non-canonical manner;
24 these signaling events occurred in the absence of any detectable intracellular replication of the
25 bacterium. Collectively, our results provide important insight into *C. acnes*-induced
26 intracellular signaling cascades in human macrophages and suggest IFN-I as a factor in the
27 etiology of *C. acnes*-induced diseases. This knowledge may be valuable for developing novel
28 therapies targeting *C. acnes* in diseases where the accumulation of the bacterium leads to an
29 inflammatory pathology.

Introduction

31 *Cutibacterium acnes* (previously *Propionibacterium acnes*) is an anaerobic, Gram-positive
32 bacterium that preferentially colonizes the human skin, as well as mucosal surfaces (1,2). While
33 *C. acnes* has traditionally been considered a non-pathogenic resident of the human microbiota,
34 current literature challenges this notion. In particular, *C. acnes* has been shown to play a
35 morbific role during the inflammatory skin disease acne vulgaris (3), as well as prostate disease
36 (2,4,5) and infections following implant surgeries (6–10). The bacterium produces a range of
37 pathogenic factors, including proteases, lipases and chemotactic factors for macrophages,
38 neutrophils and lymphocytes (11), and promotes innate immune cells such as macrophages to
39 secrete inflammatory cytokines, particularly tumor necrosis factor α (TNF-α), interleukin (IL)-
40 6, IL-8, IL-12 and IL-1 family members (12–15). Deciphering how *C. acnes* triggers the innate

41 immune response can be helpful for understanding diseases where the accumulation of *C. acnes*
42 plays a role, as well as for the development of host-directed novel therapies.

43 Type I Interferons (IFN-I) contribute to many aspects of immunity with a main focus on anti-
44 viral responses (16,17). IFN-I contribute to inflammatory responses by stimulating and
45 amplifying the secretion of chemokines and other immune mediators (18). Activation of the
46 IFN system in the defense of bacterial pathogens is not always beneficial, and may have
47 detrimental effects on the host during infection with certain pathogens (18). For instance,
48 enhanced IFN-I production during *Listeria monocytogenes* (*L.m.*) infections is associated with
49 decreased innate immunity and reduced host resistance (19). In general, there are several
50 possibilities for pathogens to stimulate the interferon pathway in host cells. For example,
51 conserved structures of bacterial cell surfaces can bind and activate membrane-bound Toll-like
52 receptors (TLR), with TLR4 as the main receptor for IFN-I induction. IFN stimulation via the
53 TLR4 axis requires the adapter molecule TIR-domain-containing adapter-inducing interferon-
54 β (TRIF) (20,21). As TLR4 recognizes lipopolysaccharides (LPS) derived from Gram-negative
55 bacteria, direct recognition of *C. acnes* by TLR4-TRIF appears unlikely. However, in some
56 macrophage populations, such as those residing in the mouse peritoneum, TRIF signaling
57 connects to TLR2, thus coupling the recognition of Gram-positive bacteria at the cell surface,
58 or in endosomes, to IFN-I synthesis (22). On the other hand, host cells can react to cytosolic
59 pathogenic intrusions by recognizing either foreign RNA (RIG-I-like receptors; RLR),
60 intracellular bacterial cell surface components (NOD-like-receptors; NLR) or cytoplasmic
61 DNA (cytosolic DNA-binding sensors). Cyclic GMP-AMP synthase (cGAS) is arguably the
62 most prominent DNA sensing receptor found in the cytoplasm with relevance during bacterial
63 infections (23,24). DNA recognition by the sensor protein cGAS causes the enzyme to produce
64 cyclic GMP-AMP (cGAMP), a cyclic di-nucleotide that associates with and activates stimulator
65 of interferon genes (STING), an essential scaffold for the activation of interferon regulatory
66 factors (IRF). As essential components of active IFN-I gene promoters, IRFs play a critical role
67 in the control of IFN-I production (25). Some intracellular bacteria, most prominently *L.m.,*
68 secrete cyclic-di-nucleotides that further stimulate the adapter STING in a cGAS-independent
69 manner (26,27). The potential role of *C. acnes* in triggering the interferon signaling axis, and
70 how it compares in this regard to intracellular *L.m.*, has not been considered yet and will be the
71 main focus of this study.

72 With our results we provide evidence that the Gram-positive bacterium *C. acnes* triggers the
73 interferon signaling axis in human macrophages. In accordance with published data
74 (12,21,28,29) we demonstrate a strong inflammatory cytokine response, as well as the
75 activation of the inflammasome. We further show that infection with *C. acnes* stimulates IFN-
76 I production via the cGAS-STING pathway with a strong dependency on the adapter molecule
77 TRIF. In contrast to *L.m.*, *C. acnes*-induced interferon production shows complete dependence
78 upon the cytoplasmic DNA sensor cGAS, suggesting DNA as the only trigger. While the pro-
79 inflammatory cytokine response shows little variation between *C. acnes* strains, not all strains
80 elicit a strong interferon response. However, for the tested strains the mechanism behind the
81 induction of interferon signaling in *C. acnes* infected macrophages, is the TRIF-dependent
82 activation of the cGAS-STING pathway.

Materials and Methods

Cell culture and differentiation

85 Human monocytic THP-1 cells (ATCC #TIB-202 and Invivogen #thp-isg) and U-937 cells
86 (ATCC #CRL-1593.2) were maintained in RPMI 1640 culture medium (Sigma-Aldrich)
87 supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Sigma-Aldrich) and
88 1% Penicillin/Streptomycin (both Sigma-Aldrich) (referred to as "complete medium").

89 For the differentiation into macrophage-like cells, human monocytic THP-1 or U-937 cells were
90 seeded in complete medium supplemented with 100nM phorbol 12-myristate 13-acetate (PMA)
91 (Sigma-Aldrich #P1585). Cells were differentiated for 2 days at 37°C and 5% CO_2 atmosphere.
92 On the third day, media was exchanged to RPMI 1640 supplemented with 10% (v/v) FBS and
93 incubation at 37°C and 5% CO_2 was continued for an additional 24 hours.

94 HaCaT cells obtained from the German Cancer Research Center (DKFZ, Heidelberg, Germany)
95 were maintained in DMEM culture medium (Sigma-Aldrich) plus 10% (v/v) FBS and 1%
96 Pen/Strep and kept at 37°C in a 5% CO_2 atmosphere. Cells were split twice a week by washing
97 once with PBS, incubating for 10 minutes with 0.05% EDTA in PBS at 37°C followed by a 10
98 minutes incubation with Trypsin-EDTA at 37°C.

Isolation of PBMCs and monocytes from human blood

100 Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation
101 with Lymphoprep™ (StemCell Technologies, #07851) from fresh buffy coats of three healthy
102 donors received from the Austrian Red Cross. Erythrocytes were removed using ACK lysing
103 buffer (Gibco, #A10492-01). After 3 washing steps, cell viability was measured (viability above
104 95%). Next, monocytes were isolated from PBMCs by negative selection using EasySep™
105 Human Monocyte Isolation Kit (StemCell Technologies, #19359) showing a viability above
106 91%. Monocytes were seeded on the same day in RPMI 1640 plus 10% (v/v) FBS and infected
107 the following day.

Differentiation of primary monocytes into monocyte-derived macrophages

109 Primary monocytes were seeded on the day of isolation in complete medium in 6-well plates.
110 For differentiation, cells were stimulated with 100ng/ml of recombinant human M-CSF (a kind
111 gift from L. Ziegler-Heitbrock, Helmholtz Center, Munich, Germany). At day 4, media were
112 refreshed (complete medium plus M-CSF). On day 6, media were exchanged to medium
113 containing only 10% (v/v) FBS. Infection experiments were performed with primary monocyte-
114 derived macrophages at day 7.

Bacterial preparation and infection

117 All *C. acnes* strains were cultured in brain-heart-infusion (BHI) media (BD Bioscience) at 37°C
118 under anaerobic conditions using BD GasPak™ (BD Bioscience). Bacteria were collected at
119 exponential growth phase (around 24 hours after inoculation), washed twice with 1x phosphate
120 buffered saline (PBS) and resuspended in RPMI 1640 supplemented with FBS. All
121 centrifugation steps were performed at 10,000xg for 5 minutes. The following strains were
122 used: NCTC737 (phylotype IA_1; ATCC6919); P. acn31 (phylotype IA_2); KPA171202
123 (phylotype IB; DSM16379); PV66 (phylotype IC); ATCC11828 (phylotype II); Asn12
124 (phylotype III) (see Table S1). Infection was performed with a multiplicity of infection (MOI)
125 of approximately 50. At different time-points, cells were washed twice with 1x PBS and lysed
126 for either RNA or protein isolation.

127 *L.m.* strain LO28 and the LLO-deficient strain LO28Δ*hly* were grown overnight in BHI media
128 at 37°C with continuously shaking. After reaching stationary phase, bacteria were washed twice
129 with 1x PBS and resuspended in RPMI 1640 supplemented with 10% (v/v) FBS. Centrifugation
130 was performed at 10,000xg for 5 minutes. Cells were infected with a MOI of approximately 40
131 for 1 hour at 37°C. Media was exchanged to RPMI 1640 supplemented with 10% (v/v) FBS
132 and 50µg/ml gentamycin (MP Biomedicals, Santa Ana, US). After one more hour, media was
133 replaced with RPMI 1640 supplemented with 10% (v/v) FBS and 10µg/ml gentamycin. After
134 different time-points, cells were washed twice with 1x PBS and lysed for RNA or protein
135 isolation.

136 LPS (Sigma-Aldrich #L2637) was used at a final concentration of 0.4µg/ml.

Colony-forming-unit (CFU) assay

138 THP-1 and U-937 cells were seeded in 96-well plates and differentiated using PMA (see above).
139 Cells were infected with *C. acnes* strain NCTC737, P. acn31, KPA171202, PV66, ATCC11828
140 and Asn12 or *L.m.* strain LO28 or *L.m.* mutant strain LO28Δ*hly*. After 1-hour post infection,
141 media of all wells was exchanged to RPMI 1640 supplemented with 10% (v/v) FBS and
142 50µg/ml gentamycin. After another hour, media was replaced by RPMI 1640 supplemented
143 with 10% (v/v) FBS and 10µg/ml gentamycin. After each timepoint, cells were washed twice
144 with 1x PBS. Subsequently, cells were burst open by adding sterile nuclease-free water.
145 Dilution series was made, and bacteria plated on either Brucella blood agar (*C. acnes*;
146 bioMérieux Austria GmbH) or BHI plates (*L.m.*). Brucella blood agar plates were incubated for
147 3 days at 37°C under anaerobic conditions. BHI plates with *L.m.* were kept for 1.5 days at 37°C.

RNA isolation, cDNA synthesis and RT-qPCR

149 Total RNA isolation was performed using the NucleoSpin RNA II kit (Macherey-Nagel,
150 Catalog #740955). For the synthesis of cDNA, Oligo (dT18) primer and RevertAid Reverse
151 Transcriptase (both Thermo-Fisher Scientific) were used. Real-time quantitative-PCR was run
152 on Eppendorf Mastercycler using SybrGreen (Promega Catalog # A6002). Primers for real-time
153 qPCR are listed in Table S2.

Western blot

155 THP-1 cells were lysed in Laemmli buffer (120mM Tris-HCl, pH 8, 2% SDS and 10% glycerol)
156 and protein concentrations measured using Pierce^{TM} BCA Protein Assay kit (Thermo-Fisher
157 Scientific). A total of 20µg of protein were resuspended with SDS-loading dye (50% β-
158 Mercaptoethanol, 0.02% Bromphenolblue), boiled and loaded on a 10% SDS polyacrylamide
159 gel. Proteins were transferred on a nitrocellulose or PVDF membrane for 16 hours at 200mA
160 and 2 hours at 400mA at 4°C using a carbonate transfer buffer (3mM Na_2CO_3, 10mM $NaHCO_3$
161 and 20% ethanol). Afterwards, membranes were blocked in 5% (w/v) milk powder in TBS-T
162 for 1-2 hours at room temperature. Membranes were incubated with the appropriate primary
163 antibody overnight at 4°C while shaking: α-Tubulin (Sigma Aldrich, Catalog # T9026, 1:5000);
164 GAPDH (Millipore, Catalog # ABS16, 1:3000); STAT1 (Santa Cruz, Catalog # SC346,
165 1:1000); Phospho-STAT1 (Tyr701; Cell signaling, Catalog # 9167, 1:1000); IRF9 (Santa Cruz,
166 Catalog # sc10793, 1:1000); IκB-α (Cell signaling, Catalog #9242, 1:1000); MyD88 (Cell
167 signaling, Catalog # 4283, 1:1000); TRIF (Cell signaling, Catalog # 4596, 1:1000); STING
168 (Cell signaling, Catalog # 13647, 1:1000); cGAS (Cell signaling, Catalog # 15102, 1:1000).
169 The membrane was then washed three times with TBS-T before incubating for 1-2 hours at
170 room temperature with the appropriate secondary antibody (Jackson ImmunoResearch Inc.,
171 Catalog # 111-035-003, 1:6000 and Catalog # 115-035-144, 1:6000). After three washing steps
172 with 1x TBS-T, the membrane was incubated with SuperSignal West Pico Chemiluminescent
173 substrate (Thermo-Fisher Scientific) and developed using the ChemiDoc^{TM} Imaging system
174 from Bio-Rad.

Luminex assay

176 ProcartaPlex Mix&Match human 8-plex from Affymetrix eBioscience was used for measuring
177 cytokine levels in the supernatant of *C. acnes* infected and uninfected cells. Protein levels were
178 measured at Affymetrix eBioscience, Campus Vienna Biocenter 2, 1030 Vienna. The kit was
179 used according to the manufacturer's specifications.

THP1 ISG reporter activity

181

182   Wildtype THP1-Blue[TM] ISG cells were differentiated in a 96-well plate (5×10^4 cells/well) with
183   PMA as described above. Cells were infected with either *L.m.* strain LO28 or *C. acnes* strains
184   NCTC737 and Asn12 or stimulated with 0.4µg/ml LPS for either 24, 48 or 72 hours. After each
185   time-point, 20µl of the supernatant was added to 180µl of QUANTI-Blue[TM] solution
186   (Invivogen, Cat. # rep-qbs) in a flat-bottom 96-well plate and incubated at 37°C. Absorbance
187   at 620nm was measured using BioTek Synergy H1 Plate reader.

189   **Genome-editing via CRISPR-Cas9 system**

190   The guide RNAs of human *MYD88*, *TICAM1* (gene encoding human TRIF) and *IRF9* were
191   designed   using   Broad   Institute   GPP   Web   Portal
192   (https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrnadesign). *LacZ* gRNA (non-
193   targeting   control   (TGCGAATACGCCCACGCGAT),   *MYD88*   gRNA
194   (TGTCTCTGTTCTTGAACGTG); *TICAM1* gRNA (GGAGAACCATGGCATGCAGG);
195   *IRF9* gRNA (ATACAGCTAAGACCATGTTC; published in Platanitis et al. (2019) (30)).
196   Oligos were ligated into the LentiCRISPRv2 plasmid. LentiCRISPRv2 was a gift from Feng
197   Zhang (Add gene plasmid # 52961; http://n2t.net/addgene:52961; RRID:Addgene_52961) (31).
198   THP-1 cells were transduced, and single cells selected.

199   Vector for the guide RNAs of human *TMEM173* (gene encoding human STING) and *CGAS*
200   were ordered from Sigma-Aldrich (U6gRNA-Cas9-2A-GFP-TMEM173 and U6gRNA-Cas9-
201   2A-GFP-MB21D1). *TMEM173* gRNA (GGGCCGACCGCATTTGGGAGGG); *CGAS* gRNA
202   (CGTCGGGCTGCTGAACACCGGG).

203   **Statistical information**

204   Data derived from RT-qPCR represent the mean values in a min/max box plot. Differences in
205   mRNA expression were compared using the two-tailed unpaired t-test of the log transformed
206   values. Differences in bacterial loads (CFU) were compared using the two-tailed unpaired t-
207   test. All statistical analysis was performed using GraphPad Prism. P-values: ns $P > 0.05$; $*P \leq$
208   0.05; $**P \leq 0.01$; $***P \leq 0.001$. Number of biological replicates is stated in the corresponding
209   figure legend.

210   # Results

211   ***C. acnes* intracellular degradation in human macrophages**

212   In order to analyze the ability of *C. acnes* to survive and/or replicate inside of its host cell, we
213   performed a CFU assay with *C. acnes*, as well as with the Gram-positive bacteria *L.m.* whose
214   intracellular fate is well studied (32–34). *L.m.* is an invasive bacterium that escapes the host
215   phagosome using the pore-forming toxin listeriolysin O (LLO). Recent literature suggests that
216   *C. acnes* may have developed strategies to neutralize phagosomes in order to survive or even
217   leave the phagosome without being targeted for degradation by bactericidal effector
218   mechanisms (35). For direct comparison, we included a mutant strain of *L.m.* (Δ*hly*, lacking
219   LLO) that is rapidly degraded by the host cell. Indeed, while the load of wildtype *L.m.* increased
220   immensely over a period of 24 hours (Figure 1A, Supplementary Figure 1A), survival and/or
221   propagation of the Δ*hly* strain was prevented by both THP-1 and U-937 cells in a similar time
222   period (Supplementary Figure 1B). *C. acnes* reference strain NCTC737 (phylotype IA$_1$) on the
223   other hand showed results comparable to the LLO mutant strain suggesting that THP-1 and U-
224   937 cells are indeed able to control *C. acnes* infections (Figure 1A, Supplementary Figure 1A).
225   A similar pattern could be observed after infection with strains representing each *C. acnes*
226   phylotype, suggesting a strain-independent host mechanism for *C. acnes* degradation in THP-1
227   cells (Figure 1B).

C. acnes stimulates an IFN-I response in human macrophages

We characterized the innate response to *C. acnes* infection by determining the production of typical innate cytokines by PMA-differentiated human THP-1 macrophages (Figure 2). *C. acnes* strain NCTC737 (phylotype IA$_1$) induced the expression of pro-inflammatory (TNF-α, IL-1β, IL-1α and IL-6) and anti-inflammatory (IL-10) cytokines (Figure 2A). Furthermore, *C. acnes*-infected THP-1 cells showed an increased secretion of IL-1β protein, confirming *C. acnes*-induced inflammasome activation as published by Qin et al. (2014) (Figure 2B) (28). The induction of inflammatory cytokines at the mRNA level correlated with the secreted protein level in the supernatant of infected cells. Interestingly, alongside a strong pro-inflammatory cytokine response, we observed an elevated expression of IFN-β as well as type I IFN-stimulated genes (ISGs), such as Interferon-induced GTP-binding protein Mx1 (MX1) and Interferon-induced protein with tetratricopeptide repeats 1 (IFIT1) in *C. acnes* infected THP-1 cells in the delayed phase of infection. This indicates that *C. acnes* not only activates an inflammatory response in human macrophages, but in addition triggers a type I IFN signature.

Comparing the cytokine response caused by infection with different strains of *C. acnes* (one strain of each phylotype) we did not observe any major differences in the amounts of inflammatory cytokine mRNAs. In contrast, the IFN-I-induced genes *MX1* and *IFIT1* differed vastly between strains (Figure 2C). Especially *C. acnes* strain Asn12 (phylotype III), which has been recently associated with the skin disease macular hypomelanosis (36), was remarkably potent in inducing ISG transcription. Thus, although *C. acnes* induced inflammatory cytokine response seems to be strain independent, the induction of ISGs differs when comparing various phylotypes.

Among the induced cytokines of figure 2B, IL-6 is controlled by both NFkB and IFN-I pathways (37), suggesting a potential contribution of IFN-I to cytokine secretion during *C. acnes* infection. To determine whether IFN-I activity alone suffices for the detection of secreted gene products we used THP1-Blue^{TM} ISG cells that monitor the interferon signaling pathway and its master transcription factor, the ISGF3 complex, through the stable integration of a secreted embryonic alkaline phosphatase (SEAP) reporter. Specifically, the reporter gene is under an ISG54 minimal promoter with five IFN-stimulated response elements (ISREs). Secreted SEAP can be detected by QUANTI-Blue^{TM} colorimetric enzyme assay. While stimulation with LPS and infection with *L.m.* showed high IFN-induced SEAP levels in the supernatant of cells infected for both 24 to 72 hours, absorbance increased over time with *C. acnes* strain Asn12 (Figure 2D). In line with the mRNA expression of ISGs and IFN-β, cells infected wth *C. acnes* strain NCTC737 released significantly smaller quantities of SEAP.

To corroborate our findings with data from primary cells, we analyzed the expression of pro-inflammatory cytokines and type-I IFN signaling in primary monocytes from three different donors. While mRNA expression of the pro-inflammatory cytokine TNF-α and IL-1β were greatly induced upon infection with either *L.m.* or *C. acnes* strains NCTC737 or Asn12 as well as upon stimulation with LPS, the induction of a type-I IFN response was only upregulated upon LPS treatment which could neither be seen with *L.m.* nor with any of the two *C. acnes* strains used (Supplementary Figure S2A). On the other hand, M-CSF-differentiated monocyte-derived macrophages (MDMs) from the same donors strongly induced the expression of IFN-β as well as MX1 in addition to a robust inflammatory cytokine response shown by elevated mRNA expression of TNF-α and IL-1β (Figure 3A). In addition, infection of primary MDMs with *L.m.* induced the phosphorylation of STAT1 on Tyr701at 6 and 24 hours post infection (Figure 3B). In line with the mRNA expression of IFN-β as well as MX1, we observed a strong phosphorylation of STAT1 at Tyr701 with *C. acnes* strain Asn12 and a much weaker signal with NCTC737.

276 As *C. acnes* is a commensal of the human skin, we were interested in the inflammatory cytokine
277 response as well as the IFN-I signature in human keratinocytes. Thus, we infected the human
278 keratinocyte cell line HaCaT with either *L.m.*, *C. acnes* strain Asn12 or stimulated them with
279 LPS for 48 hours. Compared to macrophages, HaCaT cells showed only a minor induction of
280 cytokine expression upon infection, even with our positive controls *L.m.* and LPS
281 (Supplementary Figure S2B). Although *L.m.* as well as Asn12 induced mRNA expression of
282 TNF-α, IL-6, MX1 and IFIT1, the levels were only slightly above the basal state. Taken
283 together, differentiated THP-1 cells as well as primary human macrophages induce an IFN-I
284 signature as well as a strong inflammatory response upon infection with *C. acnes*.

285 **Multiple intracellular pathways contribute to cytokine production by *C. acnes*-infected**
286 **cells**

287 Cytokine expression in infected cells is initiated by signals from cell surface or endosomal
288 TLRs as well as cytoplasmic nucleic acid receptors and inflammasomes (21). We sought to
289 determine the signals targeting inflammatory cytokines and IFN-I in *C. acnes*-infected cells by
290 studying cells deficient for either myeloid differentiation primary response 88 (MyD88) or
291 TRIF (Figure 4A-C, Supplementary Figure S3A), the eponymous adapters for the major
292 pathways downstream of TLR. Recent experimental evidence shows that *C. acnes* is recognized
293 by a TLR2/6 heterodimer in human keratinocytes (29), which signals via the adapter molecule
294 MyD88 to stimulate the expression of inflammatory cytokines. Consistent with this, a knockout
295 of MyD88 in THP-1 cells completely abolished the expression of the inflammatory cytokines
296 TNF-α and IL-1β upon infection with *C. acnes*, *L.m.*, or LPS (Figure 4A). In line with the
297 mRNA expression of inflammatory cytokines, cells lacking MyD88 were not able to degrade
298 IκB-α, the inhibitor of the transcription factor nuclear factor 'kappa-light-chain-enhancer' of
299 activated B-cells (NF-κB), upon infection with either *C. acnes* or *L.m.* (Figure 4B-C). Deletion
300 of TRIF slightly reduced TNF-α mRNA synthesis, but strongly increased that of IL-1β. This
301 suggests a negative feed-back of the TRIF pathway on IL-1β mRNA production through the
302 MyD88 pathway.

303 Unlike the inflammatory cytokines, IFN-I-dependent STAT1 phosphorylation and ISG
304 induction by either *C. acnes* or *L.m.* remained largely unaffected by the absence of MyD88. In
305 stark contrast, the knockout of adapter molecule TRIF strongly reduced ISG induction and
306 STAT1 Tyr701 phosphorylation upon infection with *C. acnes* or *L.m.* (Figures 4A-C). This
307 finding was surprising given the complete independence of *L.m.*-induced IFN-I production in
308 mouse bone marrow-derived macrophages (38). To examine whether the involvement of TRIF
309 reflected signaling through a non-canonical, endosomal TLR2-TRIF pathway as recently
310 observed in mouse peritoneal macrophages infected with *L.m.* (22), we infected THP-1 cells
311 with the escape deficient *L.m.* strain *Δhly*. LLO-deficient *L.m.* were still capable of inducing
312 the pro-inflammatory response via the TLR2 axis (TNF-α and IL-1β mRNA), however, they
313 were unable to stimulate IFN-I signaling as shown by the mRNA expression of MX1 and IFIT1
314 (Figure S3B). This result suggests that *L.m.*-induced ISG expression in THP-1 cells is
315 independent of TLR-TRIF signaling, however, dependent on the adapter TRIF in a different
316 signaling context.

317 IFN-I exert gene control via canonical and noncanonical mechanisms (39). The canonical mode
318 requires formation of the interferon-stimulated gene factor 3 (ISGF3) complex, a trimeric
319 protein complex consisting of STAT1, STAT2 and IRF9. We used IRF9 knockout THP-1 cells
320 to examine whether the expression of MX1 and IFIT1 was induced by the canonical IFN-I
321 signaling pathway in *C. acnes*-infected cells. Indeed, a knockout of IRF9 completely abolished
322 the expression of ISGs upon infection with *C. acnes* whereas it did not affect the
323 proinflammatory cytokine response (Figure 4D, Supplementary Figure S3C). Thus, *C. acnes*
324 induced IFN-I signaling occurs via the canonical type I IFN pathway.

C. acnes-induced type I IFN signaling requires a cGAS-STING pathway

To further interrogate the role of TRIF in IFN-I production, we investigated the role of STING and its upstream regulator cGAS. These studies were in accordance with a recent report demonstrating a TRIF requirement for STING signaling in cells infected with herpes simplex virus (40).

Unlike wildtype cells, ISG induction in response to infection with either *C. acnes* or *L.m.* was not detected in THP-1 cells lacking STING (Supplementary Figure S4A, Figure 5A). The complete lack of a response to IFN-I confirms the absence of TLR2-TRIF signaling, which is independent of STING. As expected, IFN signaling in LPS-stimulated cells was independent of STING signaling as we observed a slight increase in ISG expression. These results were corroborated by showing that the phosphorylation of STAT1 on tyrosine 701 is strongly reduced in both *C. acnes* and *L.m.*-infected cells lacking the adapter STING (Figure 5B-C). In contrast, the degradation of the NF-κB inhibitor IκB-α in wildtype and knockout cells was similar. These results demonstrate that *C. acnes* induced type I IFN signaling is dependent on the adapter STING, which is positively regulated by the adapter TRIF.

When infected with *C. acnes*, cGAS-deficient THP-1 cells revealed an essential function of the DNA sensor for the induction of ISG expression, very similar to the STING knockouts (Figure 6A). *L.m.* on the other hand was capable of ISG induction in absence of cGAS. This is in accordance with published data showing that *L.m.* is able to stimulate STING in a cGAS-independent manner (26,27). Interestingly, cGAS independence was much less pronounced when IFN-b synthesis was measured at earlier time points (41). This raises the possibility that cyclic di-nucleotides produced by *L.m.* accumulate to generate cGAS-independence of STING activation at delayed stages of infection.

In order to determine whether IFN signaling via the cGAS/STING pathway is generally employed by *C. acnes* to stimulate an IFN-I response independently of the phylotype, we performed infection experiments with *C. acnes* strain Asn12, the most potent IFN-I inducer (Figure 2C), in cells lacking either cGAS or STING. As depicted in Figure 6B, *C. acnes* strain Asn12 strongly induced ISG expression in wildtype cells. However, ISG expression was lost in cells lacking either cGAS or STING. Taken together our results suggest TRIF-dependent cGAS-STING signals in a default pathway of IFN synthesis in *C. acnes*-infected human macrophages (Figure 7).

C. acnes intracellular degradation is independent of inflammatory cytokine and type I IFN response

Cytokines shape the local inflammatory environment of a cell, but they can also influence the establishment of cell-autonomous immunity. To determine whether pro-inflammatory cytokine and/or interferon responses impact on effector mechanisms that influence bacterial growth we performed CFU assays with wildtype cells or cells lacking either MyD88, TRIF, STING or the ISGF3 subunit IRF9. As depicted in Figure 8A, *C. acnes* recovery from infected THP-1 cells did not significantly differ when comparing wildtype versus any of the knockout cells. Contrary to *C. acnes*, the intracellular growth of *L.m.* was curtailed by the MyD88-dependent proinflammatory response (Figure 8B). This differs from results obtained in mouse bone marrow-derived macrophages (42). Surprisingly, the knockout of TRIF reduced the growth of *L.m.* whereas such an effect was not observed when IFN-I synthesis or response was abrogated by the knockouts of STING or IRF9. This suggests that an IFN-independent activity of TRIF blunts the ability of THP-1 cells to restrict *L.m.* growth. Taken together, our data show that reduced proinflammatory cytokine or IFN-I synthesis fails to make macrophages permissive

371   for intracellular growth of *C. acnes*, and that the TLR adaptors MyD88 and TRIF impinge on
372   *L.m.* replication in opposing manners.

Discussion

374   Infection with *C. acnes* is associated with a variety of diseases ranging from inflammatory acne
375   lesions of the skin to prostate disease, soft-tissue infections and sarcoidosis. The latter disease,
376   characterized by bouts of inflammation in a variety of different organs, has been genetically
377   correlated with single-nucleotide polymorphism (SNPs) in the nucleotide binding domains of
378   the genes encoding the intracellular pattern recognition receptor NOD1 (43). The deposition
379   and survival of *C. acnes* in internal organs was proposed to result from its ability to survive in
380   circulating monocytes and epithelial cells [reviewed in (44)]. The pathogenesis of the human
381   skin disorder acne vulgaris is believed to occur from altered keratinization (45), as well as
382   elevated sebum production (46),leading to proliferation of *C. acnes* in the human hair follicle
383   (15,47). While the association of *C. acnes* with acne (3) and post-surgical implant infection (6–
384   10) is well-described, its intracellular fate and means of inducing innate immune signaling
385   pathways is still not fully understood.

386   It has previously been shown that *C. acnes* induces a strong inflammatory response *in vitro*
387   from THP-1, as well as U937 monocytes (12), which is consistent with data showing that
388   inflammatory acne lesions have an elevated expression of cytokines such as IL-1β, TNF-α and
389   IL-10 compared to uninvolved adjacent skin (48). We now extend these studies to primary
390   human monocytes as well as monocyte-derived macrophages. The data clearly show that the
391   ability to induce IFN-I synthesis is a property of differentiated macrophages much more than
392   of their monocyte progenitors. As *C. acnes* is a Gram-positive bacterium, several studies have
393   focused on the recognition of the *C. acnes*' peptidoglycan (PG) layer by intracellular NOD
394   receptors (43) and membrane-bound Toll-like receptors, particularly TLR2, 1 and 6 (29,49,50).
395   While there is a clear understanding that *C. acnes* triggers inflammatory cytokine expression
396   via heterodimers of TLR2 with TLR1 or 6, followed by the activation of the NF-κB as well as
397   mitogen-activated protein kinase (MAPK) signaling cascade (51,52), it has not been known if,
398   and indeed, how *C. acnes* may induce the interferon signaling pathway. In our studies, *C. acnes*
399   infection of THP-1 cells lacking the TLR adapter MyD88 clearly showed that production of
400   inflammatory cytokines, but not that of IFN-I, was driven by a MyD88 pathway. This is
401   perfectly in line with current literature suggesting a TLR2, 1 and 6 dependency.

402   To our surprise, we observed that the interferon response in a *C. acnes* infection strongly relied
403   on the adapter TRIF. This contradicts the notion that TRIF pathways are downstream of TLRs
404   3 and 4, but not those recognizing ligands of Gram-positive bacteria (53). Infection of
405   differentiated THP-1 cells with the Gram-positive bacterium *L.m.* showed similar dependency
406   on TRIF for IFN-I synthesis. The recognition of *L.m.* seems to strongly depend on the
407   macrophage population, as the IFN-I production in mouse bone marrow-derived macrophages
408   shows complete independence of TRIF (38) while macrophages residing the mouse peritoneum
409   show IFN-I synthesis via a TLR2-TRIF pathway upon *L.m.* infection (22). Therefore, one
410   possible interpretation of our results would be the wiring of a TLR2-TRIF pathway in human
411   macrophages. As for *L.m.* infections in THP-1 cells, a TLR2-TRIF pathway for IFN-I synthesis
412   can be ruled out due to the lack of ISG expression upon the infection with the escape-deficient
413   *hly* mutant strain which has been shown in this study. However, owing to our finding of a drastic
414   reduction of the response to IFN-I in either TRIF, cGAS and STING knockout cells, we posit
415   an association between the cGAS-STING pathway and TRIF as proposed by Wang et. al. (40).
416   These authors provide data showing that TRIF is needed for STING dimerization and is,
417   therefore, required for STING-mediated transcriptional activation of IRF3 homodimers. Thus,
418   our data would be the first to extend this signaling mechanism to a bacterial pathogen. Whereas

419 direct interaction between TRIF and STING was demonstrated, an involvement of TLR in this
420 interaction was not formally ruled out.

421 We noted an enhanced expression of pro-inflammatory cytokine mRNAs, especially those for
422 TNF-α, in knockouts of either STING or cGAS. This increase was not observed in cells
423 deficient for the ISGF3 subunit IRF9, suggesting it is not result of a transcription-based
424 cooperativity between IRF and STAT transcription factor families. A possible explanation is
425 provided by a report from Clark *et al.* (54) showing crosstalk between the canonical IκB kinase
426 (IKK) complex (IKKα-IKKβ) and the TANK-binding kinase 1 (TBK1)-IKKε kinases which
427 negatively regulates the canonical IKKs. Consequently, by blocking the TBK1-IKKε axis
428 downstream of STING activation one would lose the negative regulation of the canonical IKKs
429 and would, therefore, enhance the inflammatory response.

430 Our data showed that different *C. acnes* phylotypes differ significantly in their ability to
431 produce a response to IFN-I, however they show similar patterns in expression of pro-
432 inflammatory cytokines. This can be explained by the variations in morphological
433 characteristics such as cell wall composition, production of different virulence factors and other
434 immunologically relevant properties of the different *C. acnes* phylotypes (2). This raises the
435 possibility that inflammatory diseases associated with these phylotypes are more prone to the
436 proinflammatory effects, such as the synthesis of chemokines or the enhanced synthesis of some
437 inflammatory mediators such as nitric oxide (NO) (18).

438 We did not find any evidence of prolonged *C. acnes* survival in infected THP-1 or U937 cells
439 (Figure 1 and Supplementary Figure S1). In fact, the loss of viable bacteria corresponded well
440 to that of the escape-deficient *hly* mutant of *L.m*. This suggests that unlike wildtype *L.m*, *C.*
441 *acnes* is not endowed with a widely applicable mechanism for intracellular survival. Our data
442 do not rule out, however, that some primary cell types might present niches for intracellular
443 survival and organismic spread. In contrast to our data, Fischer *et al*. showed a persistence of
444 *C. acnes* in THP-1 cells over a period of 3 days (35). They suggested that *C. acnes* can either
445 neutralize the phagosomal acidic pH or escape the phagosome in order to prevent its
446 degradation, the latter being in line with our data showing *C. acnes*-induced cGAS-STING
447 signaling. However, while using the same host cell, Fischer *et al*. used a very low infection rate,
448 different bacterial strains, as well as different antibiotic treatments to kill extracellular bacteria,
449 which could explain the contrasting results. On the other hand, Nakamura *et al.* discovered that
450 *C. acnes* can be taken up by autophagic vacuoles, especially when using high infection rates
451 (56). While *C. acnes* could persist in those vacuoles in HeLa cells after 3 days of infection, the
452 loss of bacteria over time in either the murine macrophage cell line RAW264.7 or mouse
453 embryonic fibroblasts (MEFs) suggests a cell type specificity for *C. acnes* intracellular
454 degradation. Interestingly, type-I IFN signaling does not restrict *C. acnes* intracellular growth
455 and/or host-induced bacterial killing as seen in Figure 1B. Not even the most potent strain in
456 inducing an IFN-I axis, Asn12, showed differences in bacterial numbers counted by CFU.

457 Our data provide convincing evidence that *C. acnes* activates the cGAS-STING pathway and
458 that, unlike *L.m.,* does not secrete cyclic di-nucleotides capable of STING activation. An
459 important open question to be addressed in future research is how *C. acnes* DNA acquires
460 access to the intracellular sensor cGAS. This could occur either through phagosome leakage or
461 through a cytoplasmic escape mechanism that is not necessarily associated with intracellular
462 growth. Another open question concerns the consequences of an IFN response during *C. acnes*
463 infection, both for local and systemic inflammation. Taken together, studying the innate
464 immune signaling cascades activated downstream of *C. acnes* recognition is an important step
465 in order to fully understand pathogenic characteristics. This information can further be used for
466 pathogen-specific novel therapies against diseases where bacterial accumulation plays an
467 essential role.

Acknowledgement

Figure legend

Figure 1. Intracellular degradation of *C. acnes* by human macrophages. A CFU assay was performed using PMA-differentiated THP-1 cells. **(A)** THP-1 cells were either infected with *C. acnes* strain NCTC737 or *L.m.* for indicated timepoints. Graphs show the standard deviation and mean of five independent experiments. **(B)** Differentiated THP-1 cells were infected with one strain of each *C. acnes* phylotype: NCTC737 (phylotype IA$_1$), P. acn31 (phylotype IA$_2$), KPA171202 (phylotype IB), PV66 (phylotype IC), ATCC11828 (phylotype II) and Asn12 (phylotype III) for indicated timepoints. Graphs show the standard deviation and mean of three independent experiments. **(A-B)** Unpaired t-test of log transformed values compared to the 2-hour timepoint was calculated for each timepoint. P-values (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$).

Figure 2. Cytokine expression and secretion in response to *C. acnes*. **(A)** Differentiated THP-1 cells were infected with *C. acnes* strain NCTC737 for indicated timepoints. HPRT-normalized gene expression was measured using RT-qPCR and shown as log transformed fold-change to the uninfected sample. **(B)** Cytokine levels in the supernatant of **(A)** were measured using the Luminex assay. **(C)** Differentiated THP-1 cells were infected with one strain of each *C. acnes* phylotype: NCTC737 (phylotype IA$_1$), P. acn31 (phylotype IA$_2$), KPA171202 (phylotype IB), PV66 (phylotype IC), ATCC11828 (phylotype II) and Asn12 (phylotype III). HPRT-normalized gene expression was measured using RT-qPCR and shown as log transformed fold change to the uninfected sample. TNF-α, IL-1β, IL-1α, IL6 and IL-10 mRNA expression was measured after 6 hours post infection (pi). IFN-β, MX1 and IFIT1 mRNA expression was measured 24 hours pi. **(D)** THP1-Blue™ ISG cells (see text) were infected with *L.m., C. acnes* strain NCTC737 and Asn12 or stimulated with 0.4µg/ml LPS. After 24, 48 and 72 hours, supernatant was analyzed by measuring the absorbance at 620nm with QUANTI-Blue™ solution. **(A-D)** Data represent the mean values of three independent experiments. P. values were calculated using the unpaired t-test of log transformed values compared to the uninfected sample (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$).

Figure 3. Cytokine expression and type-I IFN signaling in primary monocyte-derived macrophages. Monocyte-derived macrophages were infected with either *C. acnes* strain NCTC737 and Asn12, *L.m.* or stimulated with LPS for either 6 or 24 hours. **(A)** HPRT-normalized gene expression of TNF-α, IL-1β, MX1 and IFN-β was measured using RT-qPCR and shown as log transformed fold change to the uninfected sample. Data represent the mean values of three independent experiments. P. values were calculated using the unpaired t-test of log transformed values (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$). **(B)** Protein expression of total STAT1, Phospho-Y701 STAT1 and GAPDH (housekeeping gene) was measured by Western blot.

Figure 4. Induction of innate immune signaling pathways in response to *C. acnes* infection in TRIF, MyD88 and IRF9 deficient human macrophages. **(A)** Differentiated wildtype,

MYD88-deficient and TRIF-deficient THP-1 cells were infected with either *C. acnes* strain NCTC737, *L.m.* or stimulated with lipopolysaccharide (LPS) for either 6 (TNF-α, IL-1β) or 24 hours (MX1, IFIT1). HPRT-normalized gene expression was measured using RT-qPCR and shown as log transformed fold change to the uninfected sample. (**B**) Differentiated wildtype, MYD88-deficient and TRIF THP-1 cells were infected with either *C. acnes* strain NCTC737 or *L.m.* for indicated timepoints. Protein expression of IκB-α, total STAT1, Phospho-Y701 STAT1 and tubulin (housekeeping gene) was measured by Western blot. (**C**) The representative blots in (**B**) were quantified using Image Lab. Relative intensities of the bands were normalized to their corresponding tubulin levels. Data represent relative intensities in percent to the corresponding uninfected control (equals 100%). (**D**) Differentiated wildtype and *IRF9⁻/⁻* THP-1 cells were infected with either *C. acnes* strain NCTC737, *L.m.* or stimulated with lipopolysaccharide (LPS) for either 6 (TNF-α, IL-1β) or 24 hours (MX1, IFIT1). HPRT-normalized gene expression was measured using RT-qPCR and shown as log transformed fold change to the uninfected sample. (**A, D**) Data represent the mean values of three independent experiments. P. values were calculated using the unpaired t-test of log transformed values (*P≤0.05; **P≤0.01; ***P≤0.001).

Figure 5. Induction of innate immune signaling pathways in response to *C. acnes* infection in STING-deficient human macrophages. (**A**) Differentiated wildtype and STING-deficient THP-1 cells were infected with either *C. acnes* strain NCTC737, *L.m.* or stimulated with lipopolysaccharide (LPS; 0.4µg/ml) for either 6 (TNF-α, IL-1β) or 24 hours (MX1, IFIT1). HPRT-normalized gene expression was measured using RT-qPCR and shown as log transformed fold change to the uninfected sample. Data represent the mean values of seven independent experiments. P values were calculated using the unpaired t-test of log transformed values (*P≤0.05; **P≤0.01; ***P≤0.001). (**B**) Differentiated wildtype and STING-deficient THP-1 cells were infected with either *C. acnes* strain NCTC737 or *L.m.* for indicated timepoints. Protein expression of IκB-α, total STAT1, Phospho-Y701 STAT1 and tubulin (housekeeping gene) was measured by western blot. (**C**) The representative blots in (**B**) were quantified using Image Lab. Relative intensities of the bands were normalized to their corresponding tubulin levels. Data represent relative intensities in percent to the corresponding uninfected control (equals 100%).

Figure 6. Induction of innate immune signaling pathways in response to *C. acnes* infection in cGAS-deficient human macrophages. (**A**) Differentiated wildtype, STING-deficient and cGAS-deficient THP-1 cells were infected with either *C. acnes* strain NCTC737, *L.m.* or stimulated with lipopolysaccharide (LPS; 0.4µg/ml) for either 6 (TNF-α, IL-1β) or 24 hours (MX1, IFIT1). (**B**) Differentiated wildtype, STING-deficient and cGAS-deficient THP-1 cells were infected with either *C. acnes* strain Asn12, *L.m.* or stimulated with lipopolysaccharide (LPS; 0.4µg/ml) for either 6 (TNF-α, IL-1β) or 24 hours (MX1, IFIT1). (**A-B**) HPRT-normalized gene expression was measured using RT-qPCR and shown as log transformed fold change to the uninfected sample. Data represent the mean values of four independent experiments. P values were calculated using the unpaired t-test of log transformed values (*P≤0.05; **P≤0.01; ***P≤0.001).

Figure 7. Model of the *C. acnes*-induced intracellular signaling pathways. *C. acnes* recognition in human macrophages is carried out by membrane bound toll-like receptors with its adapter MyD88. This further leads to the activation of the NF-κB pathway and the subsequent expression of inflammatory cytokines. The TLR adapter TRIF shows importance during *C. acnes*-induced IFN-signaling. TRIF can be activated by specific TLR activation on

the cell membrane or in the endosome and has also been shown to positively regulate the cGAS/STING pathway. Whether *C. acnes* is recognized by membrane-bound or endosomal TLR-TRIF or simply boosts the cGAS/STING pathway in a TRIF-dependent manner is not known.

Figure 8. Intracellular survival of *C. acnes* and *L.m.* in human macrophages deficient for either MyD88, TRIF, IRF9 or STING. Colony-forming-unit (CFU) assay was performed using PMA-differentiated THP-1 cells deficient for either MyD88, TRIF, IRF9 or STING. THP-1 cells were either infected with *C. acnes* strain NCTC737 (**A**) or *L.m.* (**B**) for indicated timepoints. Graphs show the standard deviation and mean of three independent experiments. Unpaired t-test of log transformed values was calculated. P-values (*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001).

Figure S1. (**A**) CFU assay was performed using PMA-differentiated U-937 cells. U-937 cells were infected with either *C. acnes* strain NCTC737 (left; grey) or wildtype *L.m.* (right; black) for indicated timepoints. (**B**) CFU assay was performed using PMA-differentiated THP-1 (black squares) and U-937 (grey triangles) cells. Cells were infected with the *L.m.* mutant strain Δ*hly* for indicated timepoints. (**A-B**) Representative of two independent experiments.

Figure S2. (**A**) Primary monocytes were infected with either *C. acnes* strain NCTC737 and Asn12, *L.m.* or stimulated with LPS for either 6 or 24 hours. HPRT-normalized gene expression of TNF-α, IL-1β, MX1 and IFIT1 was measured using RT-qPCR and shown as log transformed fold change to the uninfected sample. (**B**) HaCaT cells were infected with either *C. acnes* strain Asn12, *L.m.* or stimulated with LPS for 48 hours. HPRT-normalized gene expression of TNF-α, MX1, IFIT1 and IL-6 was measured using RT-qPCR and shown as log transformed fold change to the uninfected sample. (**A-B**) Data represent the mean values of three independent experiments. P. values were calculated using the unpaired t-test of log transformed values (*P≤0.05; **P≤0.01; ***P≤0.001).

Figure S3. (**A**) Confirmation of CRISPR/Cas9 generated MyD88 and TRIF knockout THP-1 cells. MyD88, TRIF and tubulin protein levels of wildtype, MyD88 and TRIF knockout THP-1 cells was analyzed by western blot. (**B**) Differentiated THP-1 cells were infected with either *C. acnes* strain NCTC737, wildtype *L.m.* or LLO-deficient *L.m.* strain Δ*hly* for either 6 (TNF-α, IL-1β) or 24 hours (MX1, IFIT1). HPRT-normalized gene expression was measured using RT-qPCR and shown as log transformed fold change to the uninfected sample. Data represent the mean values of three independent experiments. P. values were calculated using the unpaired t-test of log transformed values (*P≤0.05; **P≤0.01; P***≤0.001). (**C**) Confirmation of CRISPR/Cas9 generated IRF9 knockout THP-1 cells. IRF9 protein level of wildtype and IRF9 knockout THP-1 cells was analyzed by western blot.

Figure S5. (**A**) Confirmation of STING knockout THP-1 cells. STING and tubulin protein levels of wildtype and STING knockout THP-1 cells was analyzed by western blot. (**B**) Confirmation of cGAS knockout THP-1 cells. STING, cGAS and tubulin protein levels of wildtype, cGAS and STING knockout THP-1 cells was analyzed by western blot.

Table S1. *C. acnes* strains

Table S2. RT-qPCR primer

References

600   1. Grice EA, Segre JA. The skin microbiome. *Nat Rev Microbiol* (2011) **9**:244–53.
601   doi:10.1038/nrmicro2537

602   2. McDowell A, Nagy I. Molecular Medical Microbiology. *Part 7 Localized Infect*
603   (2015)837–858. doi:10.1016/b978-0-12-397169-2.00046-9

604   3. Taylor M, Gonzalez M, Porter R. Pathways to inflammation: acne pathophysiology. *Eur J*
605   *Dermatol* (2011) **21**:323–333. doi:10.1684/ejd.2011.1357

606   4. Davidsson S, Mölling P, Rider JR, Unemo M, Karlsson MG, Carlsson J, Andersson S-O,
607   Elgh F, Söderquis B, Andrén O. Frequency and typing of Propionibacterium acnes in prostate
608   tissue obtained from men with and without prostate cancer. *Infect Agents Cancer* (2016)
609   **11**:26. doi:10.1186/s13027-016-0074-9

610   5. Cohen RJ, Shannon BA, McNeal JE, Shannon T, Garrett KL. Propionibacterium acnes
611   associated with inflammation in radical prostatectomy specimens: A possible link to cancer
612   evlution? *J Urology* (2005) **173**:1969–1974. doi:10.1097/01.ju.0000158161.15277.78

613   6. Kadler BK, Mehta SS, Funk L. Propionibacterium acnes infection after shoulder surgery.
614   *Int J Shoulder Surg* (2015) **9**:139–44. doi:10.4103/0973-6042.167957

615   7. Pozo JLD, Tran NV, Petty PM, Johnson CH, Walsh MF, Bite U, Clay RP, Mandrekar JN,
616   Piper KE, Steckelberg JM, et al. Pilot study of association of bacteria on breast implants with
617   capsular contracture. *J Clin Microbiol* (2009) **47**:1333–7. doi:10.1128/jcm.00096-09

618   8. Rieger UM, Pierer G, Lüscher NJ, Trampuz A. Sonication of Removed Breast Implants for
619   Improved Detection of Subclinical Infection. *Aesthet Plast Surg* (2009) **33**:404–408.
620   doi:10.1007/s00266-009-9333-0

621   9. Piper KE, Jacobson MJ, Cofield RH, Sperling JW, Sanchez-Sotelo J, Osmon DR,
622   McDowell A, Patrick S, Steckelberg JM, Mandrekar JN, et al. Microbiologic diagnosis of
623   prosthetic shoulder infection by use of implant sonication. *J Clin Microbiol* (2009) **47**:1878–
624   84. doi:10.1128/jcm.01686-08

625   10. Portillo ME, Corvec S, Borens O, Trampuz A. Propionibacterium acnes: an
626   underestimated pathogen in implant-associated infections. *Biomed Res Int* (2013)
627   **2013**:804391. doi:10.1155/2013/804391

628   11. Holland C, Mak TN, Zimny-Arndt U, Schmid M, Meyer TF, Jungblut PR, Brüggemann
629   H. Proteomic identification of secreted proteins of Propionibacterium acnes. *Bmc Microbiol*
630   (2010) **10**:230. doi:10.1186/1471-2180-10-230

631   12. Vowels BR, Yang S, Leyden JJ. Induction of proinflammatory cytokines by a soluble
632   factor of Propionibacterium acnes: implications for chronic inflammatory acne. *Infect Immun*
633   (1995) **63**:3158–3165. doi:10.1128/iai.63.8.3158-3165.1995

634   13. Bojar RA, Holland KT. Acne and propionibacterium acnes. *Clin Dermatol* (2004)
635   **22**:375–379. doi:10.1016/j.clindermatol.2004.03.005

636 14. Kurokawa I, Danby FW, Ju Q, Wang X, Xiang LF, Xia L, Chen W, Nagy I, Picardo M,
637 Suh DH, et al. New developments in our understanding of acne pathogenesis and treatment.
638 *Exp Dermatol* (2009) **18**:821–832. doi:10.1111/j.1600-0625.2009.00890.x

639 15. Leeming JP, Holland KT, Cuncliffe WJ. The microbial colonization of inflamed acne
640 vulgaris lesions. *Brit J Dermatol* (1988) **118**:203–208. doi:10.1111/j.1365-
641 2133.1988.tb01775.x

642 16. McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious
643 disease. *Nat Rev Immunol* (2015) **15**:87–103. doi:10.1038/nri3787

644 17. Isaacs A, Lindenmann J. Virus interference. I. The interferon. *Proc Royal Soc Lond Ser B*
645 *Biological Sci* (1957) **147**:258–67. doi:10.1098/rspb.1957.0048

646 18. Rauch I, Müller M, Decker T. The regulation of inflammation by interferons and their
647 STATs. *Jak-stat* (2013) **2**:e23820. doi:10.4161/jkst.23820

648 19. Decker T, Müller M, Stockinger S. The Yin and Yang of type I interferon activity in
649 bacterial infection. *Nat Rev Immunol* (2005) **5**:675–687. doi:10.1038/nri1684

650 20. Abbas AK, Lichtman AH, Pillai S, by I, Baker DL. Cellular and Molecular Immunology.
651 *Sect Introd Immune Syst* (2010)19–46. doi:10.1016/b978-1-4160-3123-9.50007-3

652 21. Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune
653 defenses. *Clin Microbiol Rev* (2009) **22**:240–73. doi:10.1128/cmr.00046-08

654 22. Aubry C, Corr SC, Wienerroither S, Goulard C, Jones R, Jamieson AM, Decker T,
655 O'Neill LAJ, Dussurget O, Cossart P. Both TLR2 and TRIF contribute to interferon-β
656 production during Listeria infection. *Plos One* (2012) **7**:e33299.
657 doi:10.1371/journal.pone.0033299

658 23. Hansen K, Prabakaran T, Laustsen A, Jørgensen SE, Rahbæk SH, Jensen SB, Nielsen R,
659 Leber JH, Decker T, Horan KA, et al. Listeria monocytogenes induces IFNβ expression
660 through an IFI16-, cGAS- and STING-dependent pathway. *Embo J* (2014) **33**:1654–66.
661 doi:10.15252/embj.201488029

662 24. Ahn J, Barber GN. STING signaling and host defense against microbial infection. *Exp*
663 *Mol Medicine* (2019) **51**:1–10. doi:10.1038/s12276-019-0333-0

664 25. Chen Q, Sun L, Chen ZJ. Regulation and function of the cGAS–STING pathway of
665 cytosolic DNA sensing. *Nat Immunol* (2016) **17**:1142–1149. doi:10.1038/ni.3558

666 26. Woodward JJ, Iavarone AT, Portnoy DA. c-di-AMP secreted by intracellular Listeria
667 monocytogenes activates a host type I interferon response. *Sci New York N Y* (2010)
668 **328**:1703–5. doi:10.1126/science.1189801

669 27. Burdette DL, Monroe KM, Sotelo-Troha K, Iwig JS, Eckert B, Hyodo M, Hayakawa Y,
670 Vance RE. STING is a direct innate immune sensor of cyclic di-GMP. *Nature* (2011)
671 **478**:515–8. doi:10.1038/nature10429

672 28. Qin M, Pirouz A, Kim M-H, Krutzik SR, Garbán HJ, Kim J. Propionibacterium acnes
673 Induces IL-1β secretion via the NLRP3 inflammasome in human monocytes. *J Investigative*
674 *Dermatology* (2013) **134**:381–8. doi:10.1038/jid.2013.309

675 29. Su Q, Grabowski M, Weindl G. Recognition of Propionibacterium acnes by human TLR2
676 heterodimers. *Int J Med Microbiol* (2017) **307**:108–112. doi:10.1016/j.ijmm.2016.12.002

677 30. Platanitis E, Demiroz D, Schneller A, Fischer K, Capelle C, Hartl M, Gossenreiter T,
678 Müller M, Novatchkova M, Decker T. A molecular switch from STAT2-IRF9 to ISGF3
679 underlies interferon-induced gene transcription. *Nat Commun* (2019) **10**:2921.
680 doi:10.1038/s41467-019-10970-y

681 31. Sanjana NE, Shalem O, Zhang F. Improved vectors and genome-wide libraries for
682 CRISPR screening. *Nat Methods* (2014) **11**:783–4. doi:10.1038/nmeth.3047

683 32. Portnoy DA, Auerbuch V, Glomski IJ. The cell biology of Listeria monocytogenes
684 infection. *J Cell Biology* (2002) **158**:409–414. doi:10.1083/jcb.200205009

685 33. Portnoy DA, Chakraborty T, Goebel W, Cossart P. Molecular determinants of Listeria
686 monocytogenes pathogenesis. *Infect Immun* (1992) **60**:1263–1267. doi:10.1128/iai.60.4.1263-
687 1267.1992

688 34. Schnupf P, Portnoy DA. Listeriolysin O: a phagosome-specific lysin. *Microbes Infect*
689 (2007) **9**:1176–1187. doi:10.1016/j.micinf.2007.05.005

690 35. Fischer N, Mak TN, Shinohara DB, Sfanos KS, Meyer TF, Brüggemann H. Deciphering
691 the intracellular fate of Propionibacterium acnes in macrophages. *Biomed Res Int* (2013)
692 **2013**:603046. doi:10.1155/2013/603046

693 36. McDowell A, Nagy I, Magyari M, Barnard E, Patrick S. The opportunistic pathogen
694 Propionibacterium acnes: insights into typing, human disease, clonal diversification and
695 CAMP factor evolution. *Plos One* (2013) **8**:e70897. doi:10.1371/journal.pone.0070897

696 37. Wienerroither S, Shukla P, Farlik M, Majoros A, Stych B, Vogl C, Cheon H, Stark GR,
697 Strobl B, Müller M, et al. Cooperative Transcriptional Activation of Antimicrobial Genes by
698 STAT and NF-κB Pathways by Concerted Recruitment of the Mediator Complex. *Cell*
699 *Reports* (2015) **12**:300–312. doi:10.1016/j.celrep.2015.06.021

700 38. Stockinger S, Reutterer B, Schaljo B, Schellack C, Brunner S, Materna T, Yamamoto M,
701 Akira S, Taniguchi T, Murray PJ, et al. IFN Regulatory Factor 3-Dependent Induction of
702 Type I IFNs by Intracellular Bacteria Is Mediated by a TLR- and Nod2-Independent
703 Mechanism. *J Immunol* (2004) **173**:7416–7425. doi:10.4049/jimmunol.173.12.7416

704 39. Majoros A, Platanitis E, Kernbauer-Hölzl E, Rosebrock F, Müller M, Decker T. Canonical
705 and Non-Canonical Aspects of JAK-STAT Signaling: Lessons from Interferons for Cytokine
706 Responses. *Front Immunol* (2017) **8**:29. doi:10.3389/fimmu.2017.00029

707 40. Wang X, Majumdar T, Kessler P, Ozhegov E, Zhang Y, Chattopadhyay S, Barik S, Sen
708 GC. STING Requires the Adaptor TRIF to Trigger Innate Immune Responses to Microbial
709 Infection. *Cell Host Microbe* (2016) **20**:329–41. doi:10.1016/j.chom.2016.08.002

710 41. Hansen K, Prabakaran T, Laustsen A, Jørgensen SE, Rahbæk SH, Jensen SB, Nielsen R,
711 Leber JH, Decker T, Horan KA, et al. Listeria monocytogenes induces IFNβ expression
712 through an IFI16-, cGAS- and STING-dependent pathway. *Embo J* (2014) **33**:1654–1666.
713 doi:10.15252/embj.201488029

714 42. Edelson BT, Unanue ER. MyD88-Dependent but Toll-Like Receptor 2-Independent
715 Innate Immunity to Listeria : No Role for Either in Macrophage Listericidal Activity. *J*
716 *Immunol* (2002) **169**:3869–3875. doi:10.4049/jimmunol.169.7.3869

717 43. Tanabe T, Ishige I, Suzuki Y, Aita Y, Furukawa A, Ishige Y, Uchida K, Suzuki T,
718 Takemura T, Ikushima S, et al. Sarcoidosis and NOD1 variation with impaired recognition of
719 intracellular Propionibacterium acnes. *Biochimica Et Biophysica Acta Bba - Mol Basis Dis*
720 (2006) **1762**:794–801. doi:10.1016/j.bbadis.2006.07.006

721 44. Leheste JR, Ruvolo KE, Chrostowski JE, Rivera K, Husko C, Miceli A, Selig MK,
722 Brüggemann H, Torres G. P. acnes-Driven Disease Pathology: Current Knowledge and Future
723 Directions. *Front Cell Infect Mi* (2017) **7**:81. doi:10.3389/fcimb.2017.00081

724 45. Scott EJV, MacCardle RC. Keratinization of the Duct of the Sebaceous Gland and Growth
725 Cycle of the Hair Follicle in the Histogenesis of Acne in Human Skin 1. *J Invest Dermatol*
726 (1956) **27**:405–429. doi:10.1038/jid.1956.115

727 46. Grange PA, Weill B, Dupin N, Batteux F. Does inflammatory acne result from imbalance
728 in the keratinocyte innate immune response? *Microbes Infect Institut Pasteur* (2010) **12**:1085–
729 90. doi:10.1016/j.micinf.2010.07.015

730 47. Burkhart CG, Burkhart CN, Lehmann PF. Classic diseases revisited: Acne: a review of
731 immunologic and microbiologic factors. *Postgrad Med J* (1999) **75**:328–331.
732 doi:10.1136/pgmj.75.884.328

733 48. Kang S, Cho S, Chung JH, Hammerberg C, Fisher GJ, Voorhees JJ. Inflammation and
734 Extracellular Matrix Degradation Mediated by Activated Transcription Factors Nuclear
735 Factor-κB and Activator Protein-1 in Inflammatory Acne Lesions in Vivo. *Am J Pathology*
736 (2005) **166**:1691–1699. doi:10.1016/s0002-9440(10)62479-0

737 49. Kim J, Ochoa M-T, Krutzik SR, Takeuchi O, Uematsu S, Legaspi AJ, Brightbill HD,
738 Holland D, Cunliffe WJ, Akira S, et al. Activation of Toll-Like Receptor 2 in Acne Triggers
739 Inflammatory Cytokine Responses. *J Immunol* (2002) **169**:1535–1541.
740 doi:10.4049/jimmunol.169.3.1535

741 50. Zhang B, Choi YM, Lee J, An IS, Li L, He C, Dong Y, Bae S, Meng H. Toll-like receptor
742 2 plays a critical role in pathogenesis of acne vulgaris. *Biomed Dermatology* (2019) **3**:4.
743 doi:10.1186/s41702-019-0042-2

744 51. Chen Q, Koga T, Uchi H, Hara H, Terao H, Moroi Y, Urabe K, Furue M.
745 Propionibacterium acnes-induced IL-8 production may be mediated by NF-κB activation in
746 human monocytes. *J Dermatol Sci* (2002) **29**:97–103. doi:10.1016/s0923-1811(02)00013-0

747 52. Tsai H-H, Lee W-R, Wang P-H, Cheng K-T, Chen Y-C, Shen S-C. Propionibacterium
748 acnes-induced iNOS and COX-2 protein expression via ROS-dependent NF-κB and AP-1

749   activation in macrophages. *J Dermatol Sci* (2013) **69**:122–131.
750   doi:10.1016/j.jdermsci.2012.10.009

751   53. Kawasaki T, Kawai T. Toll-like receptor signaling pathways. *Front Immunol* (2014)
752   **5**:461. doi:10.3389/fimmu.2014.00461

753   54. Clark K, Takeuchi O, Akira S, Cohen P. The TRAF-associated protein TANK facilitates
754   cross-talk within the IkappaB kinase family during Toll-like receptor signaling. *P Natl Acad*
755   *Sci Usa* (2011) **108**:17093–8. doi:10.1073/pnas.1114194108

756   55. Guarda G, Braun M, Staehli F, Tardivel A, Mattmann C, Förster I, Farlik M, Decker T,
757   Pasquier RAD, Romero P, et al. Type I interferon inhibits interleukin-1 production and
758   inflammasome activation. *Immunity* (2011) **34**:213–23. doi:10.1016/j.immuni.2011.02.006

759   56. Nakamura T, Furukawa A, Uchida K, Ogawa T, Tamura T, Sakonishi D, Wada Y, Suzuki
760   Y, Ishige Y, Minami J, et al. Autophagy Induced by Intracellular Infection of
761   Propionibacterium acnes. *Plos One* (2016) **11**:e0156298. doi:10.1371/journal.pone.0156298