Extracellular traps released by antimicrobial TH17 cells contribute to host defense

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Short title: Antimicrobial TH17 cells contribute to host defense through the release of extracellular traps.

One Sentence Summary: Our study makes a significant conceptual advance in TH17 biology and T cell-microbe interaction via the identification of a subpopulation of antimicrobial TH17 cells that have an ability to form T cell extracellular traps and secrete a combination of antimicrobial molecules to kill both Gram-positive and Gram-negative bacteria.
Abbreviations: AMT\textsubscript{H17}, Antimicrobial T\textsubscript{H17} cells; nAMT\textsubscript{H17}, Non-antimicrobial T\textsubscript{H17} cells; C\textsubscript{A}, C. acnes strains associated with acne; C\textsubscript{H}, C. acnes strains associated with healthy skin; CFU, Colony forming units; TETs, T cell extracellular traps

Key words: C. acnes; histones H2B; Extracellular traps; Interleukin 17.

ABSTRACT

T\textsubscript{H17} cell subpopulations have been defined that contribute to inflammation and homeostasis, yet the characteristics of T\textsubscript{H17} cells that contribute to host defense against infection are not clear. To elucidate the antimicrobial machinery of the T\textsubscript{H17} subset, we studied the response to Cutibacterium acnes, a skin commensal that is resistant to IL-26, the only known T\textsubscript{H17} secreted protein with direct antimicrobial activity. We generated C. acnes-specific antimicrobial T\textsubscript{H17} clones (AMT\textsubscript{H17}) with varying antimicrobial activity against C. acnes, which we correlated by RNA-seq to the expression of transcripts encoding proteins that contribute to antimicrobial activity. Additionally, we validated that AMT\textsubscript{H17}-mediated killing of C. acnes as well as bacterial pathogens, was dependent on the secretion of granulysin, granzyme B, perforin and histone H2B. We found that AMT\textsubscript{H17}s can release fibrous structures composed of DNA decorated with the histone H2B that entangle C. acnes that we call T cell extracellular traps (TETs). Within acne lesions, H2B and IL-17 colocalized in CD4\textsuperscript{+} T cells, in proximity to TETs in the extracellular space composed of DNA decorated with H2B. This study identifies a functionally distinct subpopulation of T\textsubscript{H17} cells with an ability to form TETs containing secreted antimicrobial proteins that capture and kill bacteria.
INTRODUCTION

T cell responses represent an important component of the adaptive immune response and contribute to host defense against microbial pathogens by secreting cytokines that activate antimicrobial effector pathways and proteins that directly lyse infected targets (1). Classically, CD4<sup>+</sup> T cell subsets with diverse immunological functions have been distinguished based on unique cytokine secretion patterns and transcription factor profiles (2-7). T<sub>Η17</sub> cells express the transcriptional factor RORγt and secrete IL-17, IL-22, and IL-26 among others. Cytokines such as TGF-β, IL-1-β, and IL-6 are involved in T<sub>Η17</sub> cell differentiation (8-10), and signals from these cytokines result in the activation of the transcription factor STAT3, which directly regulates downstream genes involved in T<sub>Η17</sub> differentiation (11, 12). Defective T<sub>Η17</sub> cell responses in STAT3 deficient patients have been associated with increased susceptibility to bacterial infections, indicating that the T<sub>Η17</sub> subset has a major role in host defense (13-16).

The ability of T cells to lyse infected targets cells can be accompanied by the release of antimicrobial effector molecules that kill both intracellular and extracellular bacteria. Two major mechanisms are responsible for T cell-mediated cytolytic activity. The first involves the secretion of lytic granules containing perforin and granzymes by T cells upon contact with a target, and the second involves the interaction of membrane-bound Fas ligand on T cells with Fas molecule on the target cell (17, 18). In addition to CD8<sup>+</sup> T cells, several in vivo studies have demonstrated that cytolytic CD4<sup>+</sup> T cells can play a protective role in viral clearance, antimicrobial activity against intracellular bacteria (19) and elimination of tumors (20-25).

The identification of CD4<sup>+</sup> T mediated killing of target cells has been described within the entire heterogeneous CD4<sup>+</sup> T cell population and little is known about the extent to which CD4<sup>+</sup> T subsets are involved in CD4<sup>+</sup> T cell-mediated antimicrobial activity. In the case of the T<sub>Η17</sub> cells, most of the work has been done in defining their role in pathologic inflammation and disease (26,
It is unclear what distinguishes inflammatory Th17 cells elicited by pathogens from tissue resident Th17 cells induced by commensals. Two functionally distinct populations of Th17 cells can simultaneously reside within the gut during pathogen-induced inflammation; notably, Th17 induced by segmented filamentous bacteria (SFB) was shown to induce non-inflammatory homeostatic Th17 cells, whereas *Citrobacter rodentium*-induced Th17 cells exhibited a high inflammatory cytokine profile reflecting an inflammatory effector potential (28). Similarly, we and others have demonstrated that *Cutibacterium acnes* is a potent inducer of IL-17 and IFN-γ in CD4+ T cells, and that IL-17+ cells are present in perifollicular infiltrates of acne lesions, indicating that Th17 cells contribute to the pathogenesis of the disease (8, 29). Moreover, acne-associated (CA) and healthy-associated (CH) strains of *C. acnes* differentially modulate the CD4+ T cell responses to induce an IL-17/IFN-γ or IL-17/IL-10+ secreting Th17 cells respectively. However, little is known about how Th17 cells contribute to the killing of *C. acnes* as Th17-mediated release of the antimicrobial protein IL-26 did not reduce bacterial viability (30).

Here, we used RNA sequencing (RNA-seq) to determine the mechanism(s) involved in antimicrobial Th17 cell-mediated killing of bacteria, initially studying the immune response to *C. acnes*. We generated *C. acnes*-specific antimicrobial Th17 clones (AMTh17) with varying antimicrobial activity against *C. acnes*. We show that *C. acnes*-induced AMTh17 cells represent a subset of CD4+ TEM and TEMRA cells. RNA-seq analysis indicate that cytotoxic gene expression in AMTh17 clones correlate with both protein secretion and antimicrobial activity against *C. acnes* and is dominated by a number of known antimicrobial proteins. We found that AMTh17 cells release histone-rich T cell extracellular traps (TETs) in conjunction with antimicrobial proteins that can entangle and kill bacteria. This suggests that AMTh17-mediated killing of bacteria may be a general mechanism that contributes to homeostatic regulation of bacterial colonization.
RESULTS

*C. acnes*-specific AMT$_{H17}$ are highly enriched in cytotoxic genes

Besides the CD8$^+$ cytolytic T lymphocytes (CTLs), human CD4$^+$ T cells with cytolytic functions have been reported in response to viral infections (31-34). The CD4$^+$ T cells are able to function as CTLs *ex vivo* and can be detected following vaccinations, including against poliovirus, smallpox and in response to vaccines against HIV infection (35, 36) and likely to play a role in host defense (37). Since during the propagation of long-term CD4$^+$ T cell lines in the absence of cloning, cells expressing cytotoxic and antimicrobial activity are lost after several weeks of culture (17), we developed a cloning strategy that involves the use of whole *C. acnes* bacteria to stimulate immune cells, and used sterile cell sorting to select for *C. acnes*-specific T$_{H17}$ cells (Fig. S1). We generated and maintained short-term cultures of stable *C. acnes* strain-specific T$_{H17}$ clones, which enabled us to recapitulate the spectrum of the biology, present in *ex vivo* T$_{H17}$ cells (28). The quick expansion also permitted the analysis of transcripts associated with T$_{H17}$ cells. We first compared the antimicrobial activity of supernatants derived from these clones against *C. acnes* and several bacterial strains. We identified antimicrobial T$_{H17}$ cells, hereafter termed AMT$_{H17}$ that had antimicrobial activity against *C. acnes* and other Gram-positive and Gram-negative bacteria (Fig. 1, A and B). We also identified non-antimicrobial T$_{H17}$ clones, hereafter termed, n-AMT$_{H17}$, that lacked antimicrobial potency (Fig. 1A). Both the AMT$_{H17}$ and n-AMT$_{H17}$ clones were able to secrete IL-17 upon stimulation with α-CD3/CD28 antibodies (Fig. 1C). In further comparisons of the cytokine secretion patterns of the AMT$_{H17}$ and n-AMT$_{H17}$ clones, we observed that the secretion of IL-17, IL-22, IL-26 and IFN-$\gamma$ ($p<0.001$) was higher in the n-AMT$_{H17}$ than in the AMT$_{H17}$ clones (Fig. 1D). On the other hand, IL-10 levels were elevated within the AMT$_{H17}$ compared to the n-AMT$_{H17}$ clones (Fig. 1E) suggesting that the AMT$_{H17}$ subset likely produce IL-10 in addition to other cytokines as an important regulatory molecule to dampen excessive inflammation.
We next investigated the phenotype of the \( \text{TH}_{17} \) clones via flow cytometry. We analyzed 15 AM\( \text{TH}_{17} \) clones and discovered that a mean of 64% of the AM\( \text{TH}_{17} \) were enriched in the CD4-\( \text{TEM} \) (T effector memory) and 34% within the \( \text{TE}_{\text{MRA}} \) subsets defined as (CD4+CD45RA-CCR7- and CD4+CD45RA+CCR7-) cells respectively (Fig. 2, A and B). On the other hand, of the 5 n-AM\( \text{TH}_{17} \) clones that we analyzed, 82% were highly enriched within the CD4-\( \text{TEM} \) and 15% CD4-\( \text{CM} \) (CD4+CD45RA-CCR7-) (Fig. 2, A and C). In addition, these clones expressed transcripts associated with tissue resident memory T cells such as CXCR6, ITGAE (CD103), KFL2 and SIPRI (Fig. S2) (38, 39). These data suggest that the AM\( \text{TH}_{17} \) cells are at an advanced stage of differentiation, and may have the ability to exert antimicrobial activity as they home to peripheral non-lymphoid tissues such as the skin.

**AM\( \text{TH}_{17} \) cells exhibit antimicrobial activity as early as six hours**

T cells are generally thought to contribute to antimicrobial activity either by releasing cytokines, which recruit and activate other cells, or by major histocompatibility complex (MHC)-restricted lysis of infected host cells (40). The fact that only supernatants derived from activated AM\( \text{TH}_{17} \) clones had an ability to kill *C. acnes* in *vitro* CFU assays, suggested that these T cells were producing soluble bactericidal product(s). To further understand the mechanism(s) of \( \text{TH}_{17} \) cell mediated killing, we used RNA-seq to determine differential antimicrobial gene expression in AM\( \text{TH}_{17} \) and n-AM\( \text{TH}_{17} \) clones. To this end, we took advantage of the finding that AM\( \text{TH}_{17} \) clones had varying levels of antimicrobial activity, which we termed Low, Medium and High based on the results of *C. acnes* CFU assays. Against *C. acnes* strain HL005PA2 (Fig. 2D), reductions greater than 5-log, 3-log and 1-log in CFU were observed using undiluted supernatants derived from activated High, Medium and Low AM\( \text{TH}_{17} \) clones respectively. In contrast, supernatants from activated n-AM\( \text{TH}_{17} \) clones did not exhibit antimicrobial activity against the three *C. acnes* strains that we tested (HL005PA2, HL096PA1, and HL110PA1). We next determined the killing kinetics of AM\( \text{TH}_{17} \) supernatants against *C. acnes*. As shown in (Fig. 2E), we established that
antimicrobial activity was detectable after 6h, reaching a 2-log reduction after 12h of incubation. In contrast, supernatants derived from activated n-AM\textsubscript{T\textsubscript{H}17} clones lacked antimicrobial activity against \textit{C. acnes} even after 24h incubation. Thus, in subsequent bulk RNA-seq experiments, 15 \textit{AM}\textsubscript{T\textsubscript{H}17} clones with varying antimicrobial activity were stimulated with α-CD3/CD28 for 6h and 12h, and as a control, we used 5 n-AM\textsubscript{T\textsubscript{H}17} clones (Fig. 2D).

**Identification of antimicrobial proteins of AM\textsubscript{T\textsubscript{H}17} by RNA sequencing**

Using the transcriptome sequencing data, we next correlated the genes that had a greater than twofold expression within AM\textsubscript{T\textsubscript{H}17} over the n-AM\textsubscript{T\textsubscript{H}17} with antimicrobial activity as determined by \textit{in vitro} \textit{C. acnes} CFU activity. There were 431 and 983 genes identified in the AM\textsubscript{T\textsubscript{H}17} specific signatures for the 6 and 12h time point, respectively (Fig. 3, A and B). Subsequent, overlap of these genes with an antimicrobial gene list from the Gene Cards database revealed 50 and 98 common genes with significantly higher expression in AM\textsubscript{T\textsubscript{H}17} compared to the n-AM\textsubscript{T\textsubscript{H}17} clones at 6 and 12h time points, respectively (Fig. 3, A and B). These common genes included cytotoxic granule and antimicrobial protein genes encoding GNLY, GZMB, GZMA, PRF1, and histones H2B and H4, and were also highly enriched in the High killer AM\textsubscript{T\textsubscript{H}17} as compared to the n-AM\textsubscript{T\textsubscript{H}17} clones (Fig. 3, C and D, Table S1 and S2). Transcripts encoding transcription factors and receptors related to T\textsubscript{H}17 cells such as \textit{RORc}, \textit{IL17RE} were also expressed at high levels in the AM\textsubscript{T\textsubscript{H}17} clones (Fig. S3). The \textit{GNLY} transcript had the highest mean expression in AM\textsubscript{T\textsubscript{H}17} compared to the n-AM\textsubscript{T\textsubscript{H}17} clones at both the 6h and 12h time-points (Fig. S3, A and B). \textit{GNLY} is linked to the cytotoxic function of natural killer and CD8\textsuperscript{+} T cells, and has a wide range of antimicrobial activity against bacteria and fungi (18, 19).

**Cytotoxic gene expression in AM\textsubscript{T\textsubscript{H}17} clones is highly correlated with protein secretion and antimicrobial activity**
To assess the functional capacity of AMT\textsubscript{H17}, we confirmed the expression of some of the cytotoxicity-related transcripts (\textit{GNLY}, \textit{GZMB}, and \textit{PRF1}) at the protein level following 6 and 12h \textit{in vitro} stimulation with \(\alpha\)-CD3/CD28 antibodies. We found that gene expression of \textit{GNLY}, \textit{GZMB}, \textit{PRF1} as determined by RNA-seq had a high positive correlation with the protein secretion data (\(r = 0.85, 0.79\) and 0.58 at 6h), and (\(r = 0.87, 0.64\) and 0.39 at 12h), respectively (Fig. 4B and D). We then performed CFU experiments using the same supernatants that were measured using ELISA. We observed a negative correlation in \textit{GNLY}, \textit{GZMB} and \textit{PRF1} gene expression and antimicrobial activity in AMT\textsubscript{H17} (\(r = -0.89, -0.75\) and -0.71 at 6h), and (\(r = -0.94, -0.81\) and -0.63 at 12h), respectively (Fig. 4A and 4C), suggesting that the products of these genes may play an important role in AMT\textsubscript{H17}-mediated antimicrobial activity against a wide variety of pathogens. The correlation between \textit{PRF1} gene correlation and perforin protein expression decreased from 6 to 12 hours. In looking at a dynamic process in which the transcripts and protein are induced and degraded with different kinetics, the correlation may vary with time (41). We further validated that granulysin, granzyme B and perforin are highly enriched within the AMT\textsubscript{H17} and not the n-AMT\textsubscript{H17} clones (Fig. S4, A and B). Therefore, our combined transcriptomics, protein analysis and antimicrobial CFU data suggest that AMT\textsubscript{H17}-mediated killing is a general mechanism, and just like CD8\textsuperscript{+} cytolytic T lymphocytes, AMT\textsubscript{H17} can secrete granulysin, granzymes, perforin and other molecules as part of their antimicrobial arsenal, and these molecules can act synergistically to target \textit{C. acnes} and a multitude of other cutaneous pathogens.

\textbf{Histones H2B contributes to AMT\textsubscript{H17}-mediated antimicrobial activity}

RNA-seq data revealed that \textit{GNLY} was the top gene expressed in activated AMT\textsubscript{H17} compared to n-AMT\textsubscript{H17}. The high values of \textit{GNLY} expression are consistent with the role of granulysin as a protein with broad-spectrum antimicrobial activity against microbial pathogens (1). Neutralizing the effect of granulysin using a monoclonal antibody led to a 2-log reduction but not a complete abrogation in bacteria CFU (Fig. 5A). We therefore reasoned that the AMT\textsubscript{H17}-mediated killing
can involve a complex of other molecules and further mined the RNA-seq data to gain a global view of additional genes highly expressed in AMT_H17 in comparison to n-AMT_H17 clones. We discovered that histones (HIST2H2BE, HIST4H4 and HIST1H2BG) were among the top genes that were highly expressed after stimulation of AMT_H17. Specifically, we observed a negative correlation in HIST2H2BE gene expression and antimicrobial activity in AMT_H17 (r = -0.67, at 6h), and (r = -0.77, at 12h), respectively (Fig. 5, B and D). We also found that gene expression of HIST1H2BE as determined by RNA-seq had a high positive correlation with the protein secretion data (r = 0.79, at 6h) and (r = 0.88, at 12h) respectively (Fig. 5, C and E). These data therefore suggested that histone H2B contributes to the AMT_H17-mediated antimicrobial activity.

Histone proteins share essential traits of cationic antimicrobial peptides (CAMPS), and are a major antimicrobial component of neutrophil extracellular traps (NETs) (42). To confirm that histone H2B can negatively affect C. acnes growth, we used recombinant histone H2B and H4 and performed CFU assays. Indeed a 1 and 2.5-log reduction in bacterial CFU was observed when C. acnes was incubated with recombinant H2B and H4 respectively (Fig. 5F). Treatment of AMT_H17 supernatants with neutralizing antibodies to histone H2B and H4 led to a 1 to -2-log reduction in bacteria CFU (Fig. S5, A). A pronounced decrease in CFU was observed against E. coli, and S. aureus (Fig. 5, G and H) treated with recombinant histone H4. Histones have been reported in the mitochondria, cytosolic granules and cell surface (43), and on this basis, we reasoned that the AMT_H17 may have an ability to secrete histones upon exposure to bacteria and that these extranuclear histones can play an important role in host defense. We therefore stained the AMT_H17 clones with α-H2B histone antibodies and DAPI and found that histone H2B could localize to the cell surface of AMT_H17 clones (Fig. S5, B-D). To address whether AMT_H17 secrete histone H2B, we stimulated AMT_H17 and n-AMT_H17 clones, harvested the supernatants and lysates and performed ELISA and western blots. Indeed, the AMT_H17 and not the n-AMT_H17 clones were able to secrete histones (Fig. S6). Together these data support our notion that histones can be
secreted by AM T\textsubscript{H}17 and that they are antimicrobial against both Gram-positive and Gram-negative bacteria.

Previous studies detected DNA in supernatants of peripheral blood mononuclear cells stimulated with phytohemagglutinin (44, 45). Although both human and mouse CD4\textsuperscript{+} T cells could release DNA and histones, it was not determined which T cell subset was involved (46). We therefore next examined the ability of T\textsubscript{H}1 and T\textsubscript{H}2 cells to secrete histones. We demonstrate that both T\textsubscript{H}1 and T\textsubscript{H}2 cell lines release the signature cytokines IFN-\(\gamma\) and IL4, respectively upon stimulation with PMA (Fig. S6, D and E). However, both cell lines lacked the ability to secrete histones and subsequently kill \textit{C. acnes in vitro} (Fig. S6, F). In addition, histone/DNA complex formation by these cells were undetectable by confocal microscopy (Fig. S7, A and B). These data indicate that the ability of AM T\textsubscript{H}17 cells to secrete histone-coated ETs as part of an antimicrobial response is specific to this T cell subpopulation.

\textit{AM T\textsubscript{H}17 cells release T cell extracellular traps (TETs) that entangle \textit{C. acnes}}

Based on the fact that the \textit{AM T\textsubscript{H}17} were viable after histone secretion, we hypothesized that the mechanism of histone secretion involves an early non-lytic extracellular trap formation that can be induced by the recognition of bacterial stimuli/products. As shown previously, the formation of extracellular traps by immune cells is an important mechanism in the innate immune response (42, 47). Extracellular traps are composed of chromatin coated with histones, proteases and cytosolic proteins that not only ensnare bacteria fungi and protozoans, but also provide a high concentration of antimicrobial molecules that help trap and kill bacteria and fungi (42, 48-51). To study the mechanism of T\textsubscript{H}17 extracellular trap formation, we stimulated \textit{AM T\textsubscript{H}17} and \textit{n-AM T\textsubscript{H}17} clones with phorbol 12-myristate 13-acetate (PMA), \(\alpha\)-CD3/CD28 antibodies or \textit{C. acnes}, either in the presence or in the absence of deoxyribonuclease (DNase). Confocal staining shows histone H2B accumulated in the cytoplasm and cell surface of \textit{AM T\textsubscript{H}17} suggesting that traps can mediate T cell antimicrobial activity (Fig. 6 and S5, C and D). Furthermore, confocal microscopy
demonstrate that activated $\text{AM}_{\text{T}_{17}}$ form TETs, that are fibrous structures composed of DNA prominently decorated with histone H2B (Fig. 6, S7 and S8). To closely visualize the TETs, we used scanning electron microscopy and revealed that $\text{AM}_{\text{T}_{17}}$ are able to externalize a meshwork of extracellular traps into the extracellular space that entangle $C. \text{acnes}$ (Fig. 7 and S9). We next tested the TET forming characteristics of $\text{AM}_{\text{T}_{17}}$ and $\text{n-AM}_{\text{T}_{17}}$ clones activated only by contact with $C. \text{acnes}$. We observed that $C. \text{acnes}$ were able to induce TETs in $\text{AM}_{\text{T}_{17}}$ and not the $\text{n-AM}_{\text{T}_{17}}$ (Fig. 7 E and F), and that these structures could trap bacteria (Fig. 7, F). Because extracellular traps are degraded by treatment with DNase (42) this enzyme was added to PMA-activated $\text{AM}_{\text{T}_{17}}$ followed by addition of $C. \text{acnes}$. Treatment of $\text{AM}_{\text{T}_{17}}$ with DNase led to a reduction in TET formation (Fig. S9, G and H).

To explore the disease relevance of $\text{T}_{17}$ TET formation in vitro, we investigated whether such extracellular structures could be detected in vivo in biopsy specimens from acne patients. We detected H2B and IL-17 in the inflammatory infiltrate in acne lesions but not in normal skin (Fig. S10). We further investigated the presence of extracellular traps in acne lesions using confocal microscopy labelling CD4, IL-17 and H2B as well as DAPI. We identified CD4$^+$ T cells expressing IL-17 in acne lesions (Fig. S11). The area containing CD4$^+$ IL-17$^+$ cells was selected and H2B visualized (Fig. 8). IL-17 and H2B colocalized with DNA in fibrous structures in the extracellular space proximal to the CD4$^+$ T cells indicative of extracellular trap formation. Identical structures were detected in a second acne biopsy sample (Fig. S12). The isotype controls for both samples was negative (Fig. S13). In summary, our data demonstrate that, as in several innate immune cells (42, 47, 52-54), $\text{AM}_{\text{T}_{17}}$s can release traps composed of DNA decorated with lysine-rich histones such as H2B, providing a mechanism by which the adaptive T cell response can monitor and regulate commensals such as $C. \text{acnes}$ and invading pathogens including $S. \text{aureus}$. 

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DISCUSSION

Most of our understanding about mechanisms of host defense against infectious disease has come from exploration of the response to pathogenic microbes. However, the vast majority of microbial encounters are those resulting from commensal and/or symbiotic relationship with the microbiota.

In the case of acne vulgaris, while most humans harbor C. acnes on their skin, the loss of the skin microbial diversity together with the action of the innate immune response, in particular, is thought to drive the chronic inflammatory condition (55). The ability of C. acnes strains to induce differential activation of both the innate and adaptive arms of the immune response are most likely due to differences in lineage specific genetic elements among the strains (56). In this study, we identify AM T\textsubscript{H}17 cells as a population within the CD4\textsuperscript{+} T\textsubscript{H}17 subset, discovered through RNA-seq and functional analysis that utilizes a combination of antimicrobial molecules to kill C. acnes and other microbial pathogens. Importantly, AM T\textsubscript{H}17 cells have the ability to form T cell extracellular traps (TETs) \textit{in vitro} which are also detected \textit{in vivo} in acne lesions.

Previous studies have reported the presence of extracellular traps in neutrophils, mast cells, macrophages and basophils (42, 48, 57-59). The ETs entrap not only Gram-positive and Gram-negative bacteria, such as \textit{Staphylococcus aureus}, \textit{Salmonella typhimurium}, \textit{Streptococcus pneumoniae} and Group A streptococci, but also pathogenic fungi, such as \textit{Candida albicans} (42, 49, 60-62). However, it is not known whether T cells form ETs and trap bacteria such as \textit{C. acnes}. Notably, we observed that \textit{C. acnes} can activate AM T\textsubscript{H}17 leading to the formation of T cell extracellular traps, fibrous structures composed of DNA that are prominently decorated with histone H2B, and that the TETs upon release form a meshwork in the extracellular space that have the capacity to entangle \textit{C. acnes}. After entrapment, we visualized through scanning EM that most of the \textit{C. acnes} were killed. However, some bacteria have developed strategies to reduce trapping and killing by repelling cationic antimicrobial peptides (CAMPs) in ETs (63) or by degrading the DNA backbone with a deoxyribonuclease (DNase) (60, 62). Treatment of TETs
with commercial DNase rendered the TETs ineffective suggesting that DNA is required for the TET structure and function. As part of the pathogenesis of acne, disruption of the pilosebaceous unit results in the entry of *C. acnes* into the dermis, which contributes to the induction of an inflammatory response. We have previously determined that IL-17+ cells are present in the perifollicular infiltrate of inflamed acne lesions (64). Herein, we visualized TETs *in vivo* in biopsy specimens from acne lesions, observing the colocalization of fibrous structures composed of DNA and H2B in proximity to CD4+ T cells expressing IL-17. We demonstrate that these TETs can contribute to an antimicrobial response against *C. acnes*, but may also contribute to inflammation.

We further characterized the full repertoire of antimicrobial molecules expressed by AMT_H17 in our RNA-seq dataset, and identified histones as a component of AMT_H17-mediated immunity. Histones have been reported to coat ETs of neutrophils and other innate immune cells (42, 47). Four core histones (H2A, H2B, H3, and H4) form an octamer, around which DNA is wrapped in nucleosomes. These histones can display biological activities different from nucleosome structures and form an important part of skin defense (65). Histones are hydrophobic, cationic, and can form amphipathic α-helical structures and therefore share essential traits of CAMPS. Lysine-rich histones H2A and H2B are present on the epithelial surface of the placenta, providing the placenta and fetus protection against microbial infection (43). In addition, histone H2A and H2B both possess the capacity to neutralize endotoxin (66) and in our study, we demonstrate antimicrobial activity against *E. coli*, *S. aureus* and *C. acnes*. Therefore, the observation that histone H2B gene expression highly correlated with granulysin activity in CFU assays is consistent with its antimicrobial action (67), but how this increased antimicrobial response is activated *in vivo* is unknown. We also observed high expression of the arginine-rich histone H4 in AMT_H17. Histone H4 is known to mediate antimicrobial activity through the destruction of the cell membrane, and human sebocytes can release H4, which displays bactericidal activity against *S.
*aureus* and *C. acnes*. The antibacterial activity of H4 is enhanced by the presence of fatty acids on the skin (67).

**T**H17 cells are well known for their host protective role against fungal infections in barrier tissues, in particular, those caused by *Candida albicans* and in protection against extracellular bacteria (68-71). Our findings highlight the relevance of **T**H17 immunity to the skin commensal *C. acnes* and other bacterial strains. Direct comparison of **AM**T**H**17 and **n-AM**T**H**17 clones confirmed that **AM**T**H**17 displayed antimicrobial activity against both Gram-positive and Gram-negative bacteria. We show that the antimicrobial activity of **AM**T**H**17 is associated with a rapid expression and induction of antimicrobial transcripts, the impact of which is underscored by the finding that the top most abundantly secreted antimicrobial molecules of **AM**T**H**17 (granulysin, histones H2B) alone accounted for nearly 50% of the antimicrobial killing. We further identified multiple antimicrobial transcripts/molecules that are functionally important to immune defense. These results suggest that the antimicrobial molecules including granulysin, granzyme B, and perforin can act synergistically as part of the antimicrobial arsenal of **AM**T**H**17. It therefore seems more likely that the **AM**T**H**17 is a functionally distinct population that serves a protective role during infection. We suggest a model where, in the case of extracellular bacteria, **AM**T**H**17 cells can secrete granulysin that is then attracted to the bacterial cell wall by ionic interactions mediated by positively charged arginine residues. These residues interact with the negatively charged phospholipids on the surface of the pathogen, and granulysin can then alter membrane permeability, leading to osmotic lysis by itself. We also envisage a scenario where granulysin can colocalize with the pore forming molecule perforin and act synergistically with granzyme B, histone H2B and H4 leading to osmotic lysis. Both mechanisms can allow granulysin to access the intracellular compartments in which the pathogens reside leading to bacteria killing. Additionally, the impact of other antimicrobial cytokines such as IL-26 cannot be definitively
excluded in the bacterial killing even though we did not see significant difference in IL-26 expression between the AM\textsubscript{TH17} and AM\textsubscript{TH17} clones.

ETs have been observed in diseases such as human appendicitis (42) sinusoids of the liver and lungs during sepsis (72). The evidence presented in the paper shows that those T\textsubscript{H17} cells that express histones make TETs and secrete a combination of molecules that have antimicrobial activity against extracellular bacteria. The TETs were detected in acne lesions, linking them to the site of disease where they could contribute to the antimicrobial response in the extracellular environment, such as in the extracellular matrix for example. Human genetic studies indicate that alterations in T\textsubscript{H17} cell differentiation due to STAT3 mutations, or deletion of IL-17 receptors predisposes to multiple infections, such that these mechanisms are necessary for host defense (13, 69). Patients with Hyper – immunoglobulin E syndrome (HIES), caused by mutations in STAT3 have few detectable T\textsubscript{H17} cells in peripheral blood (69); and a failure of T\textsubscript{H17} CD4 cell differentiation \textit{in vitro} (13, 15, 73-75). Therefore, it is not possible to study the role of TETs in STAT3 deficient T\textsubscript{H17} cells in humans. In addition murine and human genetic approaches point strongly to the model that IL-17RA/RC signaling in non-myeloid cells as a necessary \textit{in vivo} effector mechanism of T\textsubscript{H17} cells (76, 77). However, these studies do not indicate whether T\textsubscript{H17} production of IL-17 is sufficient for host defense. It will be difficult to assess whether TETs are also necessary for host defense, as inherited mutations in HIST2H2BE have not been reported. Nevertheless, it is possible that both IL-17 and TETs contribute to host defense against extracellular bacteria. It is likely that there is redundancy in the immune response such that several antimicrobial mechanisms work additively or \textit{in synergy} \textit{in vivo} to destroy extracellular bacteria.

Although our data indicates that TETs are involved in antimicrobial responses, as are other ETs, we cannot exclude the possibility that TETs contribute to pathology. In psoriasis, neutrophil ETs
may contribute to T\(_{H17}\) induction as part of the disease pathogenesis (78). In addition, a
correlation between the presence of neutrophil ET-associated DNA and pathology has also been
implicated in other diseases (42, 70), and whether this is true for \textit{C. acnes}-induced TETs remains
to be explored. A charge-mediated mechanism whereby cationic antimicrobial molecules and
histones such as H2B and H4 in TETs trap negatively charged commensals such as \textit{C. acnes}
seems plausible. The fact that T\(_{H17}\) cells can release traps implies that these cells can act as an
important link between the innate and adaptive responses targeting efficient clearance of invading
pathogens. Taken together, our data identifies a functionally distinct subpopulation of T\(_{H17}\) cells
with an ability to secrete antimicrobial proteins and T cell extracellular traps to capture and kill
extracellular bacteria.
MATERIALS AND METHODS

Bacterial strains

C. acnes strains used in this study were obtained from Biodefense and Emerging Infections Research Resources Repository (BEI Resources) and cultured as previously described (30). Staphylococcus aureus SA113, Pseudomonas aeruginosa PAO1 and Escherichia coli DH5α were grown in Luria broth (LB) overnight at 37°C with agitation. Overnight bacterial cultures were sub cultured and incubated until midlog was reached, which was determined to be OD$_{600} = 0.4$. Cultures were washed in sterile PBS and renormalized to OD$_{600} = 0.4$ in culture media.

PBMC isolation, stimulation and cytokine ELISAs

Peripheral blood mononuclear cells (PBMCs) were obtained from healthy donors with written informed patient consent, as approved by the University of California, Los Angeles Institutional Review Board. PBMCs were then isolated using Ficoll–Paque gradients (GE Healthcare) as previously described (30). Briefly, cells were cultured in T cell media (RPMI 1640, 10% heat inactivated human serum (Gemini), 2mM L-glutamine, 10U/ml penicillin and 100 µg/ml streptomycin) and stimulated with different strains of C. acnes at 1 multiplicity of infection (1 MOI). Levels of cytokines accumulated in culture supernatants were measured by ELISA. As a positive control for NET formation, neutrophil isolation was done using the Neutrophil Isolation Kit (Miltenyi Biotec, Auburn, CA) following manufacturer’s protocol and assessed for spontaneous NET formation (incubated with RPMI medium with 2% fetal calf serum for 130 min) and for NET formation after stimulation with 20nM phorbol 12-myristate 13-acetate (PMA) for 80, 100, and 130 min as previously described (42).

Sterile Cell sorting, T$_H$17 cloning and neutrophil isolation

We developed a cloning system that uses C. acnes microbes and autologous monocytes as APCs. This cloning approach provides a large number of antigens and a variety of stimuli to innate
receptors to elicit polarizing cytokines for Th17 differentiation. Briefly, PBMCs were stimulated for 16 hours with *C. acnes* strains, and cytokine secretion determined using IL-17 cytokine secretion capture assay following the manufacturer’s protocol (Miltenyi). After IL-17 staining, the cells were further stained with α-CD4 antibodies (BD, clone RPA-T4) and the CD4⁺ IL-17⁺ cells sorted under sterile conditions using Beckton Dickinson FACS Vantage (San Jose, CA). Dead cells were excluded by DAPI staining. Sorted cells were cloned in Terasaki plates (Nunc Microwell, Sigma-Aldrich) as previously described (30) and maintained in T cell media supplemented with 100 U/ml IL-2 and 2ng/ml IL-23. To avoid the effect of long-term culture, Th17 cell clones were expanded for a maximum of 13 days aliquoted and frozen, and/or used immediately for RNA-seq and subsequent functional experiments. Samples were acquired on BD Biosciences FacsScan, and analyzed using FlowJo software (V7.6). In additional experiments, human Th1 and Th2 cells were isolated using CD4⁺ T cell isolation kits (Miltenyi) and cultured in the presence of IL-2 and AB serum as previously described (79). Levels of IFN-γ and IL-4 were determined by ELISA (R&D).

**Bacterial CFU assay**

*C. acnes* strains were grown under anaerobic conditions in Reinforced Clostridial Medium (Oxoid, Basingstroke, England) for 2 days and collected in mid-log phase. The bacteria were washed three times with the assay buffer (10 mM Tris pH 7.4, supplemented with 0.03% volume trypticase soy broth, Tris-TSB), and enumerated by applying a conversion factor of 7.5 x 10⁷ bacteria per mL=1 OD₆₀₀. Th17 culture supernatants were diluted in Tris-TSB and the CFU assays performed as previously described (80, 81). For the *S. aureus, E. coli* and *P. aeruginosa*, CFU assays; bacteria were grown as described above and resuspended in RPMI 1640. Depletion of granulysin was performed by incubating supernatants with 10µg/ml of neutralizing α-granulysin mAb (Biolegend, clone DH10) or an isotype mAb for 12 h at 4°C. 100µl reactions
(bacteria + T\(_{H17}\) supernatants or rhIL-26 or \(\alpha\)-granulysin or \(\alpha\)-H2B, or \(\alpha\)-H4, Abcam) were added to 1.5-ml tubes and incubated at 37\(^{\circ}\)C with shaking for 1, 3, or 24 h after the specified incubation periods, 10-fold serial dilutions were plated on LB plates to quantify surviving CFU.

**Bulk RNA-seq Library and Sequencing**

Fifteen \(_{AM}T_{H17}\) and five \(_{n-AM}T_{H17}\) clones (control) generated from six healthy donors were stimulated with \(\alpha\)-CD3/CD28 (BD) in T cell media. Total RNA was isolated at two time points (6 and 12h) after treatment using RLT buffer supplemented with 1% \(\beta\)-mecaptoethanol (QIAGEN). RNA extraction was performed on a total of forty samples according to manufacturer’s instructions using RNAeasy Micro Kit (QIAGEN), including the on-column DNase treatment step. Extracted RNA was quantified with Quant-iT RiboGreen RNA Assay Kit (Invitrogen) and RNA quality was assessed using the Agilent 2200 Tapestation (RNA Assay). mRNA libraries were prepared using the Illumina TruSeq mRNA Library Prep kit following manufacturer’s protocol. Briefly, total RNA was subjected to poly-A-selection to purify messenger RNA, then fragmented and converted into double stranded cDNA. Double stranded cDNA was then end-repaired, ligated to adapters and amplified. Final libraries were quantified using PicoGreen (Invitrogen) and the quality was assessed using the Agilent 2200 Tapestation (D1000 Assay). Libraries were pooled (4 per lane) at equimolar quantities (10uM each library) and sequenced on a HiSeq 2000 sequencer (Illumina) with 50bp single-end protocol. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (82).

**Bioinformatics methods**

The alignment of the samples was performed using STAR 2.5.3 (83) using the human genome (GRCh38.90). We explored the data to check for outliers and one sample (S31) was removed from downstream analyses. For each experiment, the 19 samples were divided into groups (Low, Medium, High, and \(_{n-AM}T_{H17}\)) based on in vitro \(C.\ acnes\) CFU killing assay. We filtered reads for
low counts and remaining were normalized using TMM (trimmed mean of M-values) in the
dgeR package (84) in R. Reads were then processed by voomwithqualityweight in Limma to
vert into log2 counts per million (logCPM) with associated precision weights (Law et. al
2014; Ritchie et al 2015), followed by contrast comparisons. 11,995 genes and 12,040 genes were
kept for contrast comparisons in the six hours and 12 hours stimulation experiments respectively.
For CFU, correlation analysis, AMTH17 and n-AMTH17 (controls) clones were stimulated with α-
CD3/CD28 (BD), total RNA was isolated (6 and 12h), and processed for RNA-seq. Specific
AMTH17 gene signatures with a twofold or more expression in comparison to the n-AMTH17 clones
were used in a correlation analysis with % antimicrobial activity determined by in vitro C. acnes
activity. Genes with a coefficient of correlation (r) >0.5 were overlapped with a list of
antimicrobial related molecules obtained from the Gene Cards database
(https://www.genecards.org/). The RNAseq data have been deposited in NCBI's Gene Expression
Omnibus and are accessible through GEO Series accession number GSE144852

Scanning Electron Microscopy

TH17 clones were adhered on silicon wafers (Ted Pella Inc.) treated with 0.01% Poly-L-lysine
(Sigma). C. acnes added at a 1:1 ratio were incubated for 20, 40, 60 and 90 minutes at room
temperature. Samples were rinsed with warm fixative (2.5% glutaraldehyde in 0.1M sodium
cacodylate buffer, pH 7.4) then incubated with fresh fixative for 1h on ice. Next, samples were
rinsed 5 times (2min each) with 0.1M sodium cacodylate and then post-fixed with 2% osmium
tetroxide in 0.1M sodium cacodylate for 30min on ice. Following the incubation with osmium,
the samples were rinsed five times (2min each) with diH2O and then dehydrated by incubating
with an ascending series of ethanol concentrations (30, 50, 70, 85, 95% 2min each). Dehydration
was completed by washing the samples in 3 changes (2min each) of 100% anhydrous ethanol.
Next, samples were loaded into a Tousimis Autosamdr810 critical point dryer and dried at the
critical point of CO\textsubscript{2} before mounting the silicon wafers onto aluminum SEM stubs with double-sided carbon tape and transferring them to an ion-beam sputter coater and coating with approximately 5nm of iridium. Finally, secondary electron images were acquired with a Zeiss Supra 40VP scanning electron microscope set to 3.5kV accelerating voltage. All reagents were purchased from Electron Microscopy Sciences (Hatfield, PA).

**Cell culture, immunoperoxidase and immunofluorescence labeling**

*C. acnes* were labeled with PKH26 (Sigma) following manufactures protocol. T\textsubscript{H}17 clones were then treated with PMA, PKH26-labeled *C. acnes* or left untreated in T cell medium for 3h. Following stimulation, both T\textsubscript{H}17 clones and PKH-labeled *C. acnes* and were adhered to Poly-L-lysine-coated transwells for one hour. Cells were then washed and fixed for 30 minutes with BD Cytofix/Cytoperm (BD Biosciences) before being washed again. Next cells were blocked with normal Goat Serum for 20 minutes, and immunolabeled with primary antibodies for Histone H2B (Abcam) for one hour. Following washing, cells were stained with secondary antibodies for one hour, washed and mounted with DAPI. Immunofluorescence of cell cultures was examined using a Leica-TCS-SP8 MP inverted single confocal laser-scanning microscope (Leica) at the Advanced Microscopy/Spectroscopy Laboratory Macro-Scale Imaging Laboratory (California NanoSystems Institute, UCLA). For immunoperoxidase labeling, de-identified normal skin and acne lesion specimens were obtained from the UCLA Translational Pathology Core Laboratory after signed written informed consent. Staining for histone H2B and IL-17 (Abcam) was performed using the standard streptavidin–biotin technique, using the commercial kit HRP-AEC system following manufacturer’s recommendations (R&D Systems). For confocal imaging of acne tissues, immunofluorescence labeling was performed by serially incubating cryostat tissue sections with anti-human mAbs for 2 hours and washed 3 times with 1× PBS, followed by incubation with specific, fluorochrome-labeled (A488, A568, A647) goat anti–mouse immunoglobulin antibodies (Molecular Probes) for 90 minutes. Controls included staining with
isotype-matched antibodies. Nuclei were stained with DAPI (Invitrogen, Life Technologies, Thermo Fisher Scientific). Immunofluorescence of skin sections was examined using Leica-TCS-SP8 MP as described above.

**Histone H2B Western blot analysis**

Western blot assays were performed using supernatants and whole cell lysates from T\textsubscript{h}17 clones. Protein concentrations were estimated by Bradford method (Thermo fisher). Briefly, lysates prepared from cells in a lysis buffer containing protease inhibitor cocktail (Roche) were separated by SDS-PAGE, transferred to PVDF membranes, and subjected to immunoblotting. Immunoblots were performed with lysates and supernatants using anti-H2B antibody (1:1000, Abcam) and b-actin (1:5000, Abcam, ab8227) as an internal control overnight.

**Statistical analysis**

For statistical analysis, data obtained from at least three independent experiments were performed using GraphPad Prism software version 8. If datasets were not normally distributed, a non-parametric test was used to determine significance. If more than two datasets were compared, One-way analysis of variance was used to compare variances within groups. *Post hoc* two-tailed Student’s *t*-test was used for comparison between two groups. For comparisons among 3 or more groups, we used repeated measures one-way ANOVA with Greenhouse-Geisser correction, along with Tukeys’s multiple comparison test, with individual variances computed for each comparison. Significant differences were considered for those probabilities $\leq 5\% (P\leq 0.05)$.

**Study approval.** This study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by UCLA IRB (#118-00193). All donors and acne patients provided written informed consent for the collection of peripheral blood and subsequent analysis.
Author contributions

GWA conceived, designed the experiments, analyzed the data, and wrote the manuscript. GWA performed most of the experiments. RT and TW participated in confocal and scanning EM experiments. AM, PA and MM helped with RNA-seq and bioinformatics analyses. RLM and MP supervised the study, provided critical suggestions and discussions throughout the study, and revised the manuscript.

Conflict of interest

The authors state no conflict of interest

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FIGURE LEGENDS

Fig. 1. AM\textsubscript{T\textsubscript{H}17} secrete T\textsubscript{H}17-associated cytokines and are antimicrobial against \textit{C. acnes} and other bacterial strains. (A) Observed CFU activity against \textit{C. acnes} strain HL005PA1 after 4 h incubation with \textsubscript{AM}\textsubscript{T\textsubscript{H}17} clone S26 and \textsubscript{n-AM}\textsubscript{T\textsubscript{H}17} clone S35 supernatants. (B) Observed CFU activity against several bacterial strains after 24 h incubation with \textsubscript{AM}\textsubscript{T\textsubscript{H}17} clone S26 and \textsubscript{n-AM}\textsubscript{T\textsubscript{H}17} clone S35 supernatants. Data represents the mean ± SEM. n>3. ***p<0.0001 by repeated measures 1-way ANOVA for treatment groups compared to \textsubscript{n-AM}\textsubscript{T\textsubscript{H}17} supernatants in panel D and \textit{C. acnes} in panel E. (C) \textsubscript{AM}\textsubscript{T\textsubscript{H}17} and \textsubscript{n-AM}\textsubscript{T\textsubscript{H}17} clones were stimulated with \(\alpha\)-CD3/CD28 for 5h and IL-17 and IFN-\(\gamma\) expression determined by flow cytometry. n>3. (D-E) Cytokine levels in \textsubscript{AM}\textsubscript{T\textsubscript{H}17} clones (S26, S27, S28) and \textsubscript{n-AM}\textsubscript{T\textsubscript{H}17} clones (S35, S38, S44) as determined by ELISA. Data are shown as mean ± SEM. n>3 (D and E). *p<0.05, **p<0.01, ***p<0.001 by 2-tailed Students’s t test.

Fig. 2. AM\textsubscript{T\textsubscript{H}17} are CD4\textsuperscript{+} T\textsubscript{EM} and T\textsubscript{EMRA} cells and demonstrate antimicrobial activity as early as six hours. (A) \textsubscript{AM}\textsubscript{T\textsubscript{H}17} and \textsubscript{n-AM}\textsubscript{T\textsubscript{H}17} clones were stimulated with \(\alpha\)-CD3/CD28 and stained with antibodies to CD4, CD45RA and CCR7. The \textsubscript{AM}\textsubscript{T\textsubscript{H}17} clones consisted of primarily CD4\textsuperscript{+}CD45RA\textsuperscript{−}CCR7\textsuperscript{RA} (T\textsubscript{EM}) and CD4\textsuperscript{+}CD45RA CCR7\textsuperscript{−} (T\textsubscript{EMRA}) whereas the \textsubscript{n-AM}\textsubscript{T\textsubscript{H}17} clones consisted mainly of T\textsubscript{EM} and ‘CD45RA CCR7\textsuperscript{+} (T\textsubscript{CM}). Data is representative of four independent experiments using clones derived from four different donors. (B and C) Analysis of memory markers in \textsubscript{AM}\textsubscript{T\textsubscript{H}17} clones (S5, S16, S26, S28) and \textsubscript{n-AM}\textsubscript{T\textsubscript{H}17} clones (S10, S13, S35, S38) by flow cytometry (n=4). ****p<0.0001 by repeated measures 1-way ANOVA for T\textsubscript{EM} compared to T\textsubscript{CM}, T\textsubscript{EMRA} and T\textsubscript{N}. (D) Several \textsubscript{AM}\textsubscript{T\textsubscript{H}17} and \textsubscript{n-AM}\textsubscript{T\textsubscript{H}17} clones were stimulated with \(\alpha\)-CD3/CD28 and supernatants used for CFU assays against \textit{C. acnes} strain HL096PA1. The \textsubscript{AM}\textsubscript{T\textsubscript{H}17} clones were subsequently stratified into High, Medium, and Low based on the results of the CFU assays. ****p<0.001 by repeated measures 1-way ANOVA, Low, Medium and High killer \textsubscript{AM}\textsubscript{T\textsubscript{H}17} compared to \textsubscript{n-AM}\textsubscript{T\textsubscript{H}17}. (E) Observed antimicrobial kinetics of supernatants derived from
activated AMTH17 clones against several C. acnes strains (HL110PA1, HLA110PA3, HL043PA1, HL096PA1 HL005PA2, and ATCC6919) in CFU assays. Data are shown as mean ± SEM. n>3.

****p<0.0001 by repeated measures 1-way ANOVA for treatment groups compared to C. acnes control.

**Fig. 3. Antimicrobial transcripts are highly expressed in AMTH17.** (A and B) AMTH17 genes with a log2Fold-change (FC) >2 and positively correlated with % antimicrobial activity (r>0.5) were overlapped with an antimicrobial gene list from the Gene Cards database. (C-D) Heatmap of the top 20 highest correlated genes with % antimicrobial activity found in the AMTH17 clones with Low (sky blue), Medium (yellow) and High (purple) antimicrobial activity against C. acnes at 6h (C) and 12h (D). Annotation for % antimicrobial activity and correlation coefficient values for each sample and gene are displayed on top (dark blue) and on the left (green). Gene expression values are displayed as Z-scores of log10 normalized counts.

**Fig. 4. Antimicrobial gene expression in AMTH17 clones highly correlate with both protein secretion and antimicrobial CFU activity.** (A-D) Correlation plots of GNLY, PRF1 and GZMB expression in stimulated AMTH17 as determined by RNA-seq. Specific AMTH17 gene signatures with a twofold or more expression in comparison to the n-AMTH17 clones and that highly correlated with C. acnes CFU activity (A and C) and ELISA protein secretion (B and D) are shown for the 6h and 12h time points. p value by Student’s t test (n=15).

**Fig. 5. Histones H2B is a component of AMTH17 antimicrobial activity.** (A) Supernatants derived from activated AMTH17 clone S26 were incubated with α-granulysin neutralizing antibody or control IgG for 1h prior and used in CFU assay against C. acnes strain HL005PA1. Data are shown as mean ± SEM. n>3. ****p<0.0001 by repeated measures 1-way ANOVA for treatment
groups compared to C. acnes control. (B-E) Correlation plots of HIST2H2BE gene expression in AMTh17 as determined in RNA-seq against CFU assays and ELISA protein secretion after 6h (B and C), and 12h (D and E). \(p\) value by Students t test (\(n=20\)). (F) Observed CFU activity against C. acnes strain HLA110PA3 after 4 h incubation with recombinant histones H2B, H4 and heat inactivated controls. Data are representative of 4 independent experiments. \(* * * p<0.0001\) by repeated measures 1-way ANOVA for treatment groups compared to C. acnes control.

(G) Supernatants derived from activated AMTh17 clone S26 were incubated with \(\alpha\)-H2B neutralizing antibody or control IgG for 1h prior and used in CFU assay against E. coli. Data are representative of 3 independent experiments. \(* * * p<0.0001\) by repeated measures 1-way ANOVA for treatment groups compared to E.coli control. (H) S. aureus after 24h incubation with recombinant histone H2B and H4. Data shows average CFU from three independent experiments \(* * * p<0.0001\) by repeated measures 1-way ANOVA for treatment groups compared to S. aureus control.

**Fig. 6. AMTh17 extracellular structures are prominently coated with Histone H2B.** (A-B) n-AMTh17 clones S13 (A) and AMTh17 clone S16 (B) were stimulated with PMA for 2 hours as previously described (42) and incubated with PKH-labeled C. acnes (red) (1:1). Cells were fixed, and stained with DAPI (blue) and \(\alpha\)-histone H2B (green). Confocal staining images are shown. White arrows indicate T cell extracellular traps and ensnared C. acnes. Magnification 63X.

**Fig. 7. Antimicrobial Th17 release extracellular traps that entangle C. acnes.** Scanning electron microscopy of the interaction of AMTh17 and C. acnes at different time points. (A) AMTh17 clones were stimulated with PMA for 30 minutes. (B and C) PMA and C. acnes for 30 minutes. (D) \(\alpha\)-CD3/CD28 for 30 minutes. (E and F) C. acnes 30 minutes. (G) PMA and C. acnes for 40 minutes, and (H) PMA, C. acnes and DNase for 40 minutes (42). Extended and released TETs can be seen attached to bacteria.
Fig. 8 Expression of T cell extracellular traps in acne lesions. (A). Confocal images of IL-17 (red), histone H2B (green), and nuclei (DAPI, blue) in acne lesions. Dashed-line boxes identify the area further studied at higher power. (B). Higher power magnification of the delineated regions marked in (A) showing H2B (green) and DAPI (red) only. White arrows indicate T cell extracellular traps in proximity to CD4⁺IL-17⁺H2B⁺ triple-positive cells within acne lesions. TETs are visualized as fibrous structures containing DNA (DAPI, blue) decorated with histone H2B (green) in the extracellular space. The images are projections of confocal z stacks generated from sections of 10µm thickness. Magnification, (A) 63X with zoom 2X from lower magnification in supplemental figure S9, and (B) is zoom 4X from (A). Data is from three individual samples. Scale bar, 10µm (enlarged insets).
Supplementary figures

Fig. S1. Scheme for generation of T\textsubscript{H}17 clones. PBMCs were isolated from normal donors and stimulated for 16 hours with either C\textsubscript{H} or C\textsubscript{A} associated C. acnes strains. Cytokine secretion was determined using IL-17 cytokine secretion capture assay. After IL-17 staining, cells were further stained with α-CD4 antibodies and the CD4\(^+\) IL-17\(^+\) cells sorted under sterile conditions and cloned in Terasaki plates. On day 7, C. acnes-specific clones were selected using T cell proliferation assays (30) followed by a further 6-day expansion in 24 well plates in T cell media supplemented with 100 U/ml IL-2 and 2ng/ml IL-23. On day 13, T\textsubscript{H}17 cell clones were either frozen, and/or used immediately in subsequent functional experiments.

Fig. S2. Tissue resident memory T cell markers expressed by T\textsubscript{H}17 clones. (A and B).
Normalized count expression of tissue resident memory T cell genes, CXCR6, ITGAE (CD103), KFL2 and S1PR1 expression in \textsubscript{AM}T\textsubscript{H}17 compared to \textsubscript{n-AM}T\textsubscript{H}17 clones as determined by RNA-seq after 6h (A) and 12h (B) stimulation with α-CD3/CD28 antibodies are shown. *\(p<0.05\), **\(p<0.01\), ***\(p<0.001\) by 2-tailed Students’s t test.

Fig. S3. \textsubscript{AM}T\textsubscript{H}17 antimicrobial signatures revealed by RNA-seq. (A and B). Normalized count expression of antimicrobial-related genes, transcriptional factors, and IL17-associated receptor genes in \textsubscript{AM}T\textsubscript{H}17 compared to \textsubscript{n-AM}T\textsubscript{H}17 clones as determined by RNA-seq after 6h (A) and 12h (B) stimulation with α-CD3/CD28 antibodies are shown. *\(p<0.05\), **\(p<0.01\), ***\(p<0.001\) by 2-tailed Students’s t test.

Fig. S4. Secretion of antimicrobial molecules by \textsubscript{AM}T\textsubscript{H}17 (A) Flow cytometry of a representative \textsubscript{AM}T\textsubscript{H}17 clone S26 stimulated with α-CD3/CD28 antibodies and stained for granulysin, granzyme B and perforin. Data is representative of three independent experiments.
(B) Secretion of cytotoxic molecules by \textsubscript{AM}T\textsubscript{H}17 compared to \textsubscript{n-AM}T\textsubscript{H}17 clones as measured by ELISA. *\(p<0.05\), **\(p<0.01\), ***\(p<0.001\) by 2-tailed Students’s t test.
**Fig. S5. Effects of neutralizing histone H2 and H4 on $\text{AMTH}_17$ antimicrobial activity.** (A) Supernatants derived from activated $\text{AMTH}_17$ and $\text{n-AMTH}_17$ clones were incubated with $\alpha$-H2B and $\alpha$-H4 neutralizing antibodies for 1h and used for CFU assay against $C.\ acnes$ strain HL005PA1. Data is representative of three independent experiments. $****p<0.0001$ by repeated measures 1-way ANOVA for treatment groups compared to $C.\ acnes + \text{n-AMTH}_17$ control. (B-D) Confocal microscopy of $\text{AMTH}_17$ clone S26 stimulated with PMA for 30 minutes, fixed, and stained with (B) DAPI (blue) (C) Histone H2B (green) (D) an overlay of DAPI and Histone H2B. Original magnification: $\times 63$.

**Fig. S6. Histones H2B expression in $\text{AMTH}_17$ clones.** Western blotting analysis of histone H2B protein expression in (A) supernatants and (B) lysates derived from activated $\text{AMTH}_17$ and $\text{n-AMTH}_17$ clones. (C) Secretion of histone H2B by $\text{AMTH}_17$ compared to $\text{n-AMTH}_17$ clones as measured by ELISA. $****p<0.0001$ by repeated measures 1-way ANOVA for $\text{AMTH}_17$ supernatants compared to $\text{n-AMTH}_17$ Cl. S38 control. (D and E) Secretion of IFN-$\gamma$, IL-4 and histone H2B by $\text{T}_{H1}$ and $\text{T}_{H2}$ cell lines as measured by ELISA. $****p<0.001$ by repeated measures 1-way ANOVA for $\text{AMTH}_17$ clone compared to $\text{T}_{H1}$ cell line. (F) Several $\text{T}_{H1}$ and $\text{T}_{H2}$ cell lines were stimulated with PMA and supernatants used for CFU assays against $C.\ acnes$ strain HL096PA1. Observed CFU activity is shown. $****p<0.001$ by repeated measures 1-way ANOVA for $\text{AMTH}_17$ S26 clone compared to a $\text{T}_{H2}$ cell line.

**Fig. S7. Characterization of histone H2B expression on $\text{T}_{H1}$ and $\text{T}_{H2}$ cell lines.** (A-B) $\text{T}_{H1}$ (A) and $\text{T}_{H2}$ cell line (B) were stimulated with PMA for 2 hours as previously described and incubated with PKH-labeled $C.\ acnes$ (red) (1:1). Cells were fixed, stained with DAPI (blue) and
α-histone H2B isotype control antibodies (green). Confocal staining images are shown. Original magnification: x63.

**Fig. S8. AM\(_{17}\) extracellular structures are prominently coated with Histone H2B. (A-B)** n-AM\(_{17}\) clone S13 (A) and AM\(_{17}\) clone S16 (B) were stimulated with PMA for 2 hours as previously described (42) and incubated with PKH-labeled *C. acnes* (red) (1:1). Cells were fixed, stained with DAPI (blue) and α-histone H2B isotype control antibodies (green). Confocal staining images are shown. Original magnification: x63.

**Fig. S9. AM\(_{17}\) release extracellular traps that entangle *C. acnes*.** Scanning electron microscopy of the interaction of AM\(_{17}\) and n-AM\(_{17}\) clones with *C. acnes* at different time points. (A-B) AM\(_{17}\) clones were stimulated with PMA and *C. acnes* for 60 minutes. (C-E) AM\(_{17}\) clones stimulated with α-CD3/CD28 antibodies for 20, 30 and 60 minutes respectively. (F) n-AM\(_{17}\) clone stimulated with PMA and *C. acnes* 30 minutes. (G) n-AM\(_{17}\) clone stimulated with PMA and *C. acnes* + DNase 90 minutes. (H) AM\(_{17}\) clone stimulated with PMA and *C. acnes* + DNase 90 minutes. (I) Neutrophil stimulated for 30 minutes with PMA and *C. acnes* positive control (42).

**Fig. S10. Histone H2B and IL-17 expression in acne lesions.** Representative section from skin biopsy specimens of normal and acne lesions stained by the immunoperoxidase method with monoclonal antibodies specific for histone H2B, IL-17 and corresponding isotype controls (n=3). Multiple histone H2B and IL-17-positive cells (brown) can be seen scattered around the dermis. Original magnification: x40.
**Fig. S11. Colocalization of CD4^IL-17^ T cells in acne lesions.** High power confocal images of IL-17 (red), CD4 (cyan), and nuclei (DAPI, blue) in acne lesions of two donors (D1 and D2). Merge indicate CD4^T cell secreting IL-17 within acne lesions. Dashed-line boxes identify the area further studied at higher power. The images are projections of confocal z stacks generated from sections of 10µm thickness. Scale bar, 10µm (enlarged insets). Original magnification: ×63.

**Fig. S12. Colocalization of IL-17^H2B^ T cells in acne lesions.** (A) Confocal images of IL-17 (red), histone H2B (green), and nuclei (DAPI, blue) in acne lesions. Dashed-line boxes identify the area further studied at higher power. (B). Higher power magnification of the delineated regions marked in (A) showing H2B (green) and DAPI (blue) only. White arrows indicate T cell extracellular traps in proximity to CD4^IL-17^H2B^ triple-positive cells within acne lesions. TETs are visualized as fibrous structures containing DNA (DAPI, blue) decorated with histone H2B (green) in the extracellular space. The images are projections of confocal z stacks generated from sections of 10µm thickness. Magnification, (A) 63X with zoom 2X from lower magnification in supplemental figure S11, and (B) is zoom 4X from (A). Data is from three individual samples. Scale bar, 10µm (enlarged insets).

**Fig. S13. Colocalization of IL-17^H2B^ T cells in acne lesions.** (A) High power confocal images of acne lesions from donor D1 labeled with isotype control antibodies; CD4 isotype (mIgG1; cyan), IL-17 isotype (mIgG2b; red), histone H2B isotype (rabbit IgG; green), and nuclei (DAPI, blue). (B) High power confocal images of acne lesions from donor D2 labeled with isotype control antibodies; CD4 isotype (mIgG1; cyan), IL-17 isotype (mIgG2b; red), histone H2B isotype (rabbit IgG; green), and nuclei (DAPI, blue). The images are projections of confocal z stacks generated from sections of 10µm thickness. Scale bar, 10µm (enlarged insets). Original magnification: ×63.
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Table S2. Common genes expressed in the AM\(_T\)H\(_{17}\) clones after 12h stimulation. Specific AM\(_T\)H\(_{17}\) gene signatures with a twofold or more expression in comparison to the n-AM\(_T\)H\(_{17}\) clones and that highly correlated with CFU activity were overlapped with an antimicrobial gene list from the Gene Cards database. 78 common genes at the 12h time point are listed. The top 20 genes are listed in figure 3.
REFERENCES


**Fig. 1.** AMT17 secrete T17-associated cytokines and are antimicrobial against C. acnes and other bacterial strains. (A) Observed CFU activity against C. acnes strain HL005PA1 after 4 h incubation with AMT17 clone S26 and n-AMT17 clone S35 supernatants. (B) Observed CFU activity against several bacterial strains after 24 h incubation with AMT17 clone S26 and n-AMT17 clone S35 supernatants. Data represents the mean ± SEM. n>3. **p<0.0001 by repeated measures 1-way ANOVA for treatment groups compared to n-AMT17 supernatants in panel D and C. acnes in panel E. (C) AMT17 and n-AMT17 clones were stimulated with α-CD3/CD28 for 5h and IL-17 and IFN-γ expression determined by flow cytometry. n>3. (D-E) Cytokine levels in AMT17 clones (S26, S27, S28) and n-AMT17 clones (S35, S38, S44) as determined by ELISA. Data are shown as mean ± SEM. n>3 (D and E). *p<0.05, **p<0.01, ***p<0.001 by 2-tailed Students’s t test.
Fig. 2. AM\textsubscript{T17} are CD4\textsuperscript{+} TEM and TEMRA cells and demonstrate antimicrobial activity as early as six hours. (A) AM\textsubscript{T17} and n-AM\textsubscript{T17} clones were stimulated with α-CD3/CD28 and stained with antibodies to CD4, CD45RA and CCR7. The AM\textsubscript{T17} clones consisted of primarily CD4\textsuperscript{+}CD45RA\textsuperscript{+}CCR7\textsuperscript{−}RA (TEM) and CD4\textsuperscript{+}CD45RA\textsuperscript{−}CCR7\textsuperscript{−} (TEMRA) whereas the n-AM\textsubscript{T17} clones consisted mainly of TEM and +CD45RA CCR7\textsuperscript{−} (TCU). Data is representative of four independent experiments using clones derived from four different donors. (B and C) Analysis of memory markers in AM\textsubscript{T17} clones (S5, S16, S26, S28) and n-AM\textsubscript{T17} clones (S10, S13, S35, S38) by flow cytometry (n=4). ****p<0.0001 by repeated measures 1-way ANOVA for TEM compared to TEMRA, TEMRA, and TN. (D) Several AM\textsubscript{T17} and n-AM\textsubscript{T17} clones were stimulated with α-CD3/CD28 and supernatants used for CFU assays against \textit{C. acnes} strain HL096PA1. The AM\textsubscript{T17} clones were subsequently stratified into High, Medium, and Low based on the results of the CFU assays. ****p<0.0001 by repeated measures 1-way ANOVA, Low, Medium and High killer AM\textsubscript{T17} compared to n-AM\textsubscript{T17}. (E) Observed antimicrobial kinetics of supernatants derived from activated AM\textsubscript{T17} clones against several \textit{C. acnes} strains (HL110PA1, HLA110PA3, HL043PA1, HL096PA1 HL005PA2, and ATCC6919) in CFU assays. Data are shown as mean ± SEM. n>3. ***p<0.0001 by repeated measures 1-way ANOVA for treatment groups compared to \textit{C. acnes} control.
Fig. 3. Antimicrobial transcripts are highly expressed in AM.TH17. (A and B) AM.TH17 genes with a log2-Fold-change (FC) >2 and positively correlated with % antimicrobial activity (r>0.5) were overlapped with an antimicrobial gene list from the Gene Cards database. (C-D) Heatmap of the top 20 highest correlated genes with % antimicrobial activity found in the AM.TH17 clones with Low (sky blue), Medium (yellow) and High (purple) antimicrobial activity against C. acnes at 6h (C) and 12h (D). Annotation for % antimicrobial activity and correlation coefficient values for each sample and gene are displayed on top (dark blue) and on the left (green). Gene expression values are displayed as Z-scores of log10 normalized counts.
Fig. 4. Antimicrobial gene expression in \( \mu \)M17 clones highly correlate with both protein secretion and antimicrobial CFU activity. (A-D) Correlation plots of \( \text{GNLY} \), \( \text{PRF1} \), and \( \text{GZMB} \) expression in stimulated \( \mu \)M17 as determined by RNA-seq. Specific \( \mu \)M17 gene signatures with a twofold or more expression in comparison to the \( \mu \)M17 clones and that highly correlated with \( C. \) acnes CFU activity (A and C) and ELISA protein secretion (B and D) are shown for the 6h and 12h time points. \( p \) value by Student’s \( t \) test (n=15).
Histones H2B is a component of \( \alpha_\text{M}_{\text{T}_{\text{H}17}} \) antimicrobial activity. (A) Supernatants derived from activated \( \alpha_\text{M}_{\text{T}_{\text{H}17}} \) clone S26 were incubated with \( \alpha \)-granulysin neutralizing antibody or control IgG for 1h prior and used in CFU assay against \( C. \text{acnes} \) strain HL005PA1. Data are shown as mean ± SEM. \( n>3. \) ** \( p<0.0001 \) by repeated measures 1-way ANOVA for treatment groups compared to \( C. \text{acnes} \) control. (B-E) Correlation plots of HIST2H2BE gene expression in \( \alpha_\text{M}_{\text{T}_{\text{H}17}} \) as determined in RNA-seq against CFU assays and ELISA protein secretion after 6h (B and C), and 12h (D and E). \( p \) value by Students t test (\( n=20 \)). (F) Observed CFU activity against \( C. \text{acnes} \) strain HLA11PA3 after 4 h incubation with recombinant histones H2B, H4 and heat inactivated controls. Data shows average CFU from three independent experiments ** \( p<0.0001 \) by repeated measures 1-way ANOVA for treatment groups compared to \( C. \text{acnes} \) control. (G) Supernatants derived from activated \( \alpha_\text{M}_{\text{T}_{\text{H}17}} \) clone S26 were incubated with \( \alpha \)-H2B neutralizing antibody or control IgG for 1h prior and used in CFU assay against \( E. \text{coli} \). Data are representative of 3 independent experiments. ** ** \( p<0.0001 \) by repeated measures 1-way ANOVA for treatment groups compared to \( E. \text{coli} \) control. (H) \( S. \text{aureus} \) after 24h incubation with recombinant histone H2B and H4. Data shows average CFU from three independent experiments ** ** \( p<0.0001 \) by repeated measures 1-way ANOVA for treatment groups compared to \( S. \text{aureus} \) control.
Fig. 6. Th17 extracellular structures are prominently coated with Histone H2B. (A-B) Th17 clones S13 (A) and Th17 clone S16 (B) were stimulated with PMA for 2 hours as previously described (42) and incubated with PKH-labeled *C. acnes* (red) (1:1). Cells were fixed, and stained with DAPI (blue) and α-histone H2B (green). Confocal staining images are shown. White arrows indicate T cell extracellular traps and ensnared *C. acnes*. Magnification 63X.
Fig. 7. Antimicrobial T\(\gamma\)17 release extracellular traps that entangle C. acnes. Scanning electron microscopy of the interaction of AM T\(\gamma\)17 and C. acnes at different time points. (A) AM T\(\gamma\)17 clones were stimulated with PMA for 30 minutes. (B and C) PMA and C. acnes for 30 minutes. (D) \(\nu\)-CD3/CD28 for 30 minutes. (E and F) C. acnes 30 minutes. (G) PMA and C. acnes for 40 minutes, and (H) PMA, C. acnes and DNase for 40 minutes (42). Extended and released TETs can be seen attached to bacteria.
Fig. 8 Expression of T cell extracellular traps in acne lesions. (A). Confocal images of IL-17 (red), histone H2B (green), and nuclei (DAPI, blue) in acne lesions. Dashed-line boxes identify the area further studied at higher power. (B). Higher power magnification of the delineated regions marked in (A) showing H2B (green) and DAPI (red) only. White arrows indicate T cell extracellular traps in proximity to CD4+IL-17+H2B+ triple-positive cells within acne lesions. TETs are visualized as fibrous structures containing DNA (DAPI, blue) decorated with histone H2B (green) in the extracellular space. The images are projections of confocal z stacks generated from sections of 10µm thickness. Magnification, (A) 63X with zoom 2X from lower magnification in supplemental figure S9, and (B) is zoom 4X from (A). Data is from three individual samples. Scale bar, 10µm (enlarged insets).
Fig. S1. Scheme for generation of T\textsubscript{H}\textsubscript{17} clones. PBMCs were isolated from normal donors and stimulated for 16 hours with either C\textsubscript{H} or C\textsubscript{A} associated C. acnes strains. Cytokine secretion was determined using IL-17 cytokine secretion capture assay. After IL-17 staining, cells were further stained with α-CD4 antibodies and the CD4\textsuperscript{+} IL-17\textsuperscript{+} cells sorted under sterile conditions and cloned in Terasaki plates. On day 7, C. acnes-specific clones were selected using T cell proliferation assays (30) followed by a further 6-day expansion in 24 well plates in T cell media supplemented with 100 U/ml IL-2 and 2ng/ml IL-23. On day 13, T\textsubscript{H}\textsubscript{17} cell clones were either frozen, and/or used immediately in subsequent functional experiments.
Fig. S2. Tissue resident memory T cell markers expressed by T<sub>H17</sub> clones. (A and B). Normalized count expression of tissue resident memory T cell genes, CXCR6, ITGAE (CD103), KFL2 and S1PR1 expression in n-AM<sub>H17</sub> T<sub>H17</sub> compared to AM<sub>H17</sub> T<sub>H17</sub> clones as determined by RNA-seq after 6h (A) and 12h (B) stimulation with α-CD3/CD28 antibodies are shown. *p<0.05, **p<0.01, ***p<0.001 by 2-tailed Students’ t test.
Fig. S3. AMT\textsubscript{H}17 antimicrobial signatures revealed by RNA-seq. (A and B). Normalized count expression of antimicrobial-related genes, transcriptional factors, and IL17-associated receptor genes in AMT\textsubscript{H}17 compared to n-AMT\textsubscript{H}17 clones as determined by RNA-seq after 6h (A) and 12h (B) stimulation with α-CD3/CD28 antibodies are shown. *p<0.05, **p<0.01, ***p<0.001 by 2-tailed Student's t-test.
Fig. S4. Secretion of antimicrobial molecules by αCD3/CD28 stimulations and staining for granulysin, granzyme B and perforin. Data is representative of three independent experiments. (B) Secretion of cytotoxic molecules by αCD3/CD28-stimulated nAMTH17 clones as measured by ELISA. *p<0.05, **p<0.01, ***p<0.001 by 2-tailed Student's t test.
Fig. S5. Effects of neutralizing histone H2 and H4 on \( \alpha_{\text{AMT}} \text{Th} 17 \) antimicrobial activity. (A) Supernatants derived from activated \( \alpha_{\text{AMT}} \text{Th} 17 \) and \( \alpha_{\text{AMT}} \text{Th} 17 \) clones were incubated with \( \alpha \)-H2B and \( \alpha \)-H4 neutralizing antibodies for 1h and used for CFU assay against \( C. \text{acnes} \) strain HL005PA1. Data is representative of three independent experiments. **\( p < 0.0001 \) by repeated measures 1-way ANOVA for treatment groups compared to \( C. \text{acnes} + \alpha_{\text{AMT}} \text{Th} 17 \) control. (B-D) Confocal microscopy of \( \alpha_{\text{AMT}} \text{Th} 17 \) clone S26 stimulated with PMA for 30 minutes, fixed, and stained with (B) DAPI (blue) (C) Histone H2B (green) (D) an overlay of DAPI and Histone H2B. Original magnification: \( \times 63 \).
**Fig. S6.** Histones H2B expression in αAMTh17 clones. Western blotting analysis of histone H2B protein expression in (A) supernatants and (B) lysates derived from activated αAMTh17 and nαAMTh17 clones. (C) Secretion of histone H2B by αAMTh17 compared to nαAMTh17 clones as measured by ELISA. **p<0.0001 by repeated measures 1-way ANOVA for αAMTh17 supernatants compared to nαAMTh17 Cl S38 control. (D and E) Secretion of IFN-γ, IL-4 and histone H2B by Th1 and Th2 cell lines as measured by ELISA. ****p<0.001 by repeated measures 1-way ANOVA for αAMTh17 clone compared to Th1 cell line. (F) Several Th1 and Th2 cell lines were stimulated with PMA and supernatants used for CFU assays against C. acnes strain HL096PA1. Observed CFU activity is shown. ****p<0.001 by repeated measures 1-way ANOVA for αAMTh17 S26 clone compared to a Th2 cell line.
Fig. S7. Characterization of histone H2B expression on T_{H1} and T_{H2} cell lines. (A-B) T_{H1} (A) and T_{H2} cell line (B) were stimulated with PMA for 2 hours as previously described and incubated with PKH-labeled C. acnes (red) (1:1). Cells were fixed, stained with DAPI (blue) and α-histone H2B isotype control antibodies (green). Confocal staining images are shown. Original magnification: x63.
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Fig. S10 Histone H2B and IL-17 expression in acne lesions. Representative section from skin biopsy specimens of normal and acne lesions stained by the immunoperoxidase method with monoclonal antibodies specific for histone H2B, IL-17 and corresponding isotype controls (n=3). Multiple histone H2B and IL-17-positive cells (brown) can be seen scattered around the dermis. Original magnification: x40.
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