Keeping the peace: commensal *Cutibacterium acnes* trains CD4$^+$ T_H17 cells to trap and kill

Diane M. Thiboutot and Amanda M. Nelson

Department of Dermatology, Penn State College of Medicine and Hershey Medical Center, Hershey, Pennsylvania, USA.

Commensal or pathogenic bacterial communities of the skin interact with the host immune system to preserve homeostasis or sustain disease. In this issue of the *JCI*, Agak et al. substantially advance our conceptual understanding of T_H17 cell biology. The researchers identified IL-26–independent mechanisms by which CD4$^+$ T_H17 clones directly kill bacteria. These CD4$^+$ T_H17 clones share antimicrobial properties with cytotoxic T cells and granulocytes as evidenced by secretion of granulysin, granzyme B, and histone-laden DNA extracellular traps. Interestingly, these clones emerged following monocyte education by *Cutibacterium acnes* strains associated with healthy skin, but not those associated with acne. Overall, the antimicrobial mechanisms employed by these T_H17 subsets suggest a unique link between innate and adaptive immune responses.

The skin microbiome

Epithelia that interface with the external environment have distinctive microbial communities associated with health and whose disruption leads to barrier defects and inflammatory or autoimmune disease. Complex interactions exist between commensal or pathogenic communities and the host immune system that either maintain skin homeostasis or perpetuate disease. As various strains of bacterial species are identified and as our understanding of how these strains differentially influence the host immune response evolves, the functional distinctions between traditional innate and adaptive antimicrobial responses become blurred.

Cutibacterium acnes is the major commensal of sebaceous areas of skin where it is thought to keep pathogens at bay (1–3). Despite being a commensal, certain strains are associated with inflammation in acne, a disease of the pilosebaceous unit fueled by hormonal influences on the sebaceous gland. In this issue of the *JCI*, Agak et al. decipher T cell response to *C. acnes*, making a substantial conceptual advance in our understanding of T_H17 biology (4). The researchers demonstrate that strains of *C. acnes* associated with healthy skin (but not those associated with acne) specifically induce subpopulations of antimicrobial T_H17 ($_{AM}T_H$17) cells that secrete histone-rich extracellular traps (termed "TETs" for "T cell extracellular traps") capable of trapping and killing *C. acnes*. These findings support the premise that healthy skin commensals are critical to the education of our immune system and our overall defense against pathogens. These TETs were also found within the dermis of acne lesions in vivo, strongly suggesting that TETs assist in the host response to clear *C. acnes* following hair follicle rupture in acne (Figure 1).

The present study builds on the substantial progress over the last decade of our understanding of the skin microbiome and the role of *C. acnes* as a commensal organism, or as a causative factor in acne or prosthetic joint infection. Advances in sequencing led to the recognition that not all strains of *C. acnes* are created equal (5). Using skin microbiota samples collected from healthy adult volunteers and acne patients, Fitz-Gibbon et al. identified *C. acnes* ribotypes (unique 16S rDNA sequences) that associate with either healthy skin (ribotype 6) or acne skin (ribotypes 4 and 5) (5). Subsequently, Agak et al. determined that clinical isolates of *C. acnes* stimulated the production of IL-17 and IL-22 from peripheral blood mononuclear cells (PBMCs) and that IL-17$^+$ T_H17 cells were present in acne lesions, suggesting a role for this cytokine in acne vulgaris (6). Building on the cytokine-acne connection, Yu et al. determined that *C. acnes* phylotypes that associate with acne (phylotypes IA-2, IB-1, and IC) can induce 2- to 3-fold more IL-17 and IFN-γ in isolated PBMCs than strains associated with healthy skin (phylotypes II [RT6] and III), which secrete higher levels of IL-10 (7). These data clearly demonstrate that our immune system differentially responds to these unique strains. Delving further, Agak et al. discovered that *C. acnes* phylotypes differentially induce distinct phenotypes of T_H17 cells, including some that directly kill bacteria by an IL-26–independent mechanism (8).

Antimicrobial T_H17 clones secrete cytotoxic proteins

The mechanisms by which these $_{AM}T_H$17 clones killed bacteria remained elusive until now. In this issue of the *JCI*, Agak et al. generated T_H17 clones by stimulating PBMCs with *C. acnes* strains associated with either acne or healthy skin. These $_{AM}T_H$17 clones inhibited the growth of *C. acnes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *E. coli* colonies in vitro. The fact that bacteria were killed only by supernatants from $_{AM}T_H$17 clones activated by healthy strains of *C. acnes* implied that

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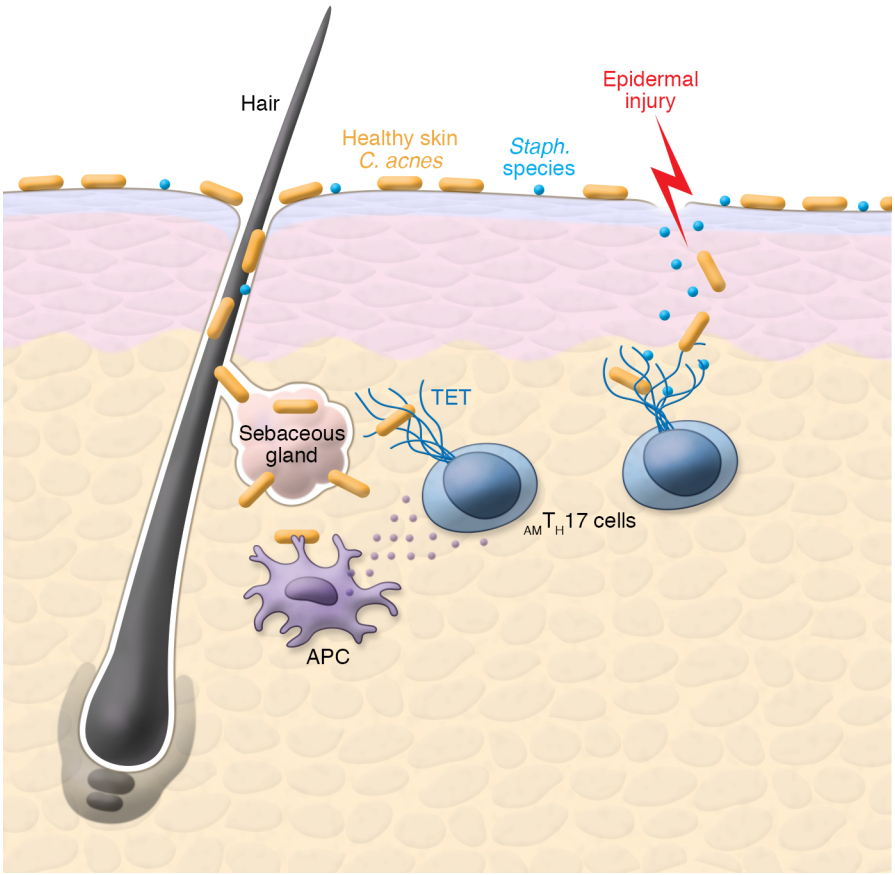

Figure 1. Model for how healthy skin commensals promote T_H17-mediated host defense. Healthy skin–associated *C. acnes* strains are detected by antigen-presenting cells (APCs), which then educate skin-residing $CD4^+$ T_H17 cells. Agak et al. (4) showed that subsets of these T_H17 cells have antimicrobial activities ($_{AM}T_H17$), secreting granzyme B, granulysin and perforin, and forming histone-studded DNA-based extracellular traps (TETs). The $_{AM}T_H17$ cells with effector memory T cell function that exist in relatively high proportion have antimicrobial activities against multiple pathogens (such as *Staphylococcus* species), strongly suggesting that the presence of $_{AM}T_H17$ cells in the skin increases our host defense to a broad range of insults.

these T cells produced soluble bactericidal products. Using a combination of transcriptomics, confirmation of protein secretion via ELISA, and antibody-depletion experiments, the authors determined that a portion of the antimicrobial activity was due to secretion of cytotoxic granulysin, granzyme B, and perforin (4). These cytotoxic proteins lyse tumor cells and infected cells and can also kill bacteria directly. Although most commonly associated with $CD8^+$ cytolytic T cells and natural killer cells, cytotoxin secretion has also been reported in $CD4^+$ cells (9, 10). These data provide additional evidence that certain subsets of T_H17 cells may bridge the gap between innate and adaptive immune responses.

Antimicrobial T_H17 clones secrete histone-laden extracellular traps

In a series of elegant experiments, Agak et al. recognized that inhibition of cytotoxins alone failed to completely abrogate the bactericidal effects of the $_{AM}T_H17$ clones. Reexamination of their transcriptomic data revealed that histone 2B and histone 4 transcripts were highly expressed (4). Histones are a major component of neutrophil extracellular traps (NETs) that can form α-helical structures and exhibit hydrophobic and cationic properties similar to other well-known antimicrobial peptides (11). Therefore, increased expression of histone proteins, and perhaps secretion of histones, could contribute to the antimicrobial activity. Using a combination of immunoblots, ELISAs, high-resolution confocal microscopy, and scanning electron microscopy, Agak et al. clearly demonstrated that $_{AM}T_H17$ clones were capable of forming and secreting TETs. These TETs contained entangled bacteria and localized to the dermis of acne lesions in proximity to $_{AM}T_H17$ cells. Disruption of the TET structure by DNase abrogated the antimicrobial activity against *C. acnes*. Further, TET formation was specific to T_H17 cells, as neither T_H1 nor T_H2 cells produced TETs upon stimulation and activation (4).

Secretion of DNA-based extracellular traps is likely an ancient, conserved, innate immune defense mechanism (12). In 2004, the discovery that NETs kill bacteria reshaped our collective thinking on host defense mechanisms, beyond the traditional microbe engulfment and secretion of cytokines, interferons, and antimicrobial peptides (11). Now we know that extracellular traps are part of the arsenal of several immune cells including mast cells, eosinophils, macrophages, and now T_H17 cells (4, 13).

Since the initial discovery of DNA extrusion from lymphocytes in 1972, it took almost 50 years for researchers to identify which lymphocyte population is capable of forming extracellular traps. Capitalizing on the fact that NETs are formed in patients with systemic lupus erythematosus (SLE), Rocha Arrieta et al. found that both T and B cell populations secreted DNA into the extracellular milieu in response to treatment of PBMCs with SLE serum and other inflammatory stimuli (14). Narrowing in on T cells, Costanza and colleagues demonstrated that murine $CD4^+$ T cells extrude DNA fibers (termed "threads") upon activation (15). However, the responsible T cell subset remained unknown. From the work of Agak et al., we learn that T_H17 cells, but

not T_H1 or T_H2, are able to extrude DNA threads that trap bacteria, now termed TETs (4). The capacity of TETs to exacerbate inflammation or act as autoantigens, as in the case of NETs, is unclear. Teasing apart the, likely, yin and yang activities of TETs will be of future interest.

What events lead to the generation of $_{AM}T_H17$?

Questions arise as to the timing and location of the interaction between bacteria and host immune cells leading to the development of these TET-producing $_{AM}T_H17$ subpopulations. Although the externally facing epidermis is blanketed with microbiota, the deeper layers beneath the healthy epidermis should be free from both commensal and pathogenic bacteria if the epithelial barrier is intact. However, bacterial components of unknown viability have been detected deep within healthy dermis (16, 17). Hair follicles that penetrate the dermis contain a distinct commensal population of bacteria compared with the skin surface (18, 19). Although the hair follicle exhibits immune privilege, it could serve as a site for the rendezvous between bacteria and immune cells, particularly in a diseased state like acne where the follicular epithelium and sebaceous glands are disrupted (Figure 1). Accordingly, little is known about the potential for bidirectional excursion of immune cells from the dermis through the intact follicular epithelium to contact luminal bacteria in the healthy state.

What is the functional role of $_{AM}T_H17$ clones?

The TET-producing $_{AM}T_H17$ clones are predominantly effector memory T (T_{EM}) cells or terminally differentiated T_{EM} (T_{EMRA}) cells, which suggests that these $_{AM}T_H17$ cells promote sustained skin homeostasis and protection against pathogens. T cells are integral to the skin's immune response to pathogens and skin microbiota help fine-tune the T cell response (20). The fact that these $_{AM}T_H17$ cells have widespread antimicrobial activity against other commensals and pathogens is intriguing and raises a myriad of questions. How does an adaptive T_H17 cell that was primed with *C. acnes* recognize and respond to other pathogens — is it through recognition of pathogen-associated molecular patterns, similarly to innate immune cells? Could these $_{AM}T_H17$ cells that were educated by a heathy skin commensal strain serve as another line of defense against breaching pathogens due to skin injury (Figure 1) or do they work to maintain the balance among commensal strains?

The relative contribution of $_{AM}T_H17$ cells within the intricate functional redundancy of the immune response remains to be determined not only in the context of acne but in other inflammatory or autoimmune diseases such as psoriasis, rheumatoid arthritis, SLE, multiple sclerosis, inflammatory bowel disease, and asthma. The findings by Agak et al. (4) provide another strand to the web of host-microbe interactions and T_H17 biology.

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Address correspondence to: Amanda Nelson, Penn State University College of Medicine, Department of Dermatology HU14, 500 University Drive, Hershey, Pennsylvania 17033, USA. Phone: 717.531.0003 ext. 284512; Email: anelson@pennstatehealth.psu.edu.

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