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IL-17: A Key Player in the *P. acnes* Inflammatory Cascade?

Diane M. Thiboutot[1], Alison M. Layton[2] and E. Anne Eady[2]

Recent advances in our understanding of inflammatory skin diseases now afford an opportunity to delve deeper into microbial/host interactions in acne. Agak et al. report that Propionibacterium acnes induces IL-17 expression in peripheral blood mononuclear cells and present new evidence that IL-17 + cells are found in the perifollicular infiltrate of comedones. Additional studies are needed to assess the clinical relevance of IL-17 in acne.

Plewig and Kligman (2000) wrote that ''Sebum is the fuel of the acne flame''. The question is ''How did the fire get started?''.

In this issue, Agak et al. (2014) present a series of experiments that support a role for IL-17 in the pathogenesis of acne. They demonstrate that *Propionibacterium acnes* induces IL-17 expression in human peripheral blood mononuclear cells (PBMCs) from healthy individuals *in vitro* and that supernatants from cultures of *P. acnes* incubated with PBMCs induce naive CD4+CD45TA+ T cells to differentiate into Th17 cells (expressing Th17,

RORα, and RORc) and Th1 cells (expressing IFNγ). By using a panel of neutralizing antibodies, they determined that IL-1β, IL-6, and transforming growth factor-β (TGFβ) regulate *P. acnes*–induced IL-17 responses as they do in other systems. Furthermore, the authors suggest that the clinical relevance for these findings is supported by the identification of IL-17+ cells in perifollicular infiltrates in biopsies of typical closed comedone-type acne lesions. Before concluding that these findings are relevant in the pathogenesis of acne, links need to be made between the *in vitro* and *in vivo* data. For example, is

P. acnes present universally in follicles surrounded by an infiltrate containing IL-17-positive cells and what type of cells are they? In addition to providing potential insight into acne pathogenesis, these data raise questions regarding the multiplicity of mechanisms by which *P. acnes* interacts with immune cells *in vivo*, the sequence of events that initiates and terminates inflammatory responses in acne and the possible role of IL-17. Furthermore, demonstration of the ability of all *trans* retinoic acid (ATRA) and vitamin D to suppress *P. acnes*–induced generation of Th17 cells suggests a potential new mechanism of action whereby retinoids and vitamin D might modulate acne inflammation physiologically or even pharmacologically.

How does *P. acnes* wage war in acne?
P. acnes is a commensal organism that colonizes the pilosebaceous follicles of people with and without acne. Although not a classical pathogen, *P. acnes* have the capacity to contribute to the genesis of inflammatory acne via multiple pathways (Table 1). Several *in vitro* studies demonstrate that *P. acnes* whole cells or cell fractions stimulate cytokine and matrix metalloproteinase release from immune cells, keratinocytes, and sebocytes (Kim *et al.*, 2002; Liu *et al.*, 2005; Nagy *et al.*, 2006; Lee *et al.*, 2010) (Figure 1a and b).

The mechanism by which *P. acnes* exerts its effects on these cells *in vivo* is unknown, perhaps via direct contact, secreted factors, or secondary events (Table 1). *P. acnes* resides mainly in the microaerophilc deeper portions of healthy follicles where it comes in contact with follicular keratinocytes and cells within the proximal region of the sebaceous duct. Within comedones, it multiplies within the sebum-filled lacunae that form inside of the cornified plugs. It can be envisioned that *P. acnes* whole cells, cell fragments, and/or secreted factors may exert proinflammatory effects on follicular keratinocytes. *P. acnes* comes in direct contact with the cells within the dermis following follicular rupture, a late finding in the development of inflammatory acne lesions (Plewig and Kligman, 2000). Soluble factors, however, may escape

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COMMENTARY

Clinical Implications

- Data on IL-17 expression in closed comedones and induction by *Propionibacterium acnes* are novel.
- Before translating this information into treatment considerations, clinical relevance must be established.
- Key studies are needed to link the *in vitro* effects of *P. acnes* on IL-17 expression in monocytes to the progression of inflammation in acne lesions over time.

from the duct once the permeability barrier is disrupted, which would occur much sooner than follicular rupture.

As pointed out by Agak *et al.* (2014), the mechanism by which *P. acnes* influences the development of Th17 cells in the inflammatory infiltrate of acne is not known. The authors expand upon a hypothesis put forth previously by Farrar and Ingham regarding the processing of immunogenic *P. acnes* proteins released into the follicle by Langerhans cells, which would then be transport to lymph nodes to activate naive T cells, with subsequent migration of Th17 cells back to the skin. Within this scenario, a major question would be whether *P. acnes* must be ingested and processed by antigen-presenting cells before activating naive T cells to become Th17 cells. If antigen presentation by Langerhans cells is needed, then findings

generated from PBMCs *in vitro* might not reflect *in vivo* findings. We know that follicular rupture occurs late in acne lesion development, leading to the release of *P. acnes*, sebum, and cellular debris into the dermis, which intensifies inflammation greatly. Under these conditions, *P. acnes* or secreted cytokines may interact with immune cells within the dermis, also influencing Th17 development.

The relative importance of IL-17 compared with other cytokines induced *in vitro* by *P. acnes,* such as IL-8, IL-12, IL12p40, IL-6, TNFα, IFNγ, and IL-1β, is not known. In terms of relative abundance, Agak *et al.* (2014) demonstrate that following *P. acnes* induction of naive T cells 4.5% become Th1 cells, whereas 1.4% become IL-17-expressing Th17 cells. A similar question regarding the relative importance of IL-17 in psoriasis existed; it is only now

beginning to be answered in clinical trials of agents that target IL-17.

Surprisingly, there is still a large gap in our understanding of cytokine expression within acne lesions. Figure 1 compares the expression of cytokines and other selected mediators in acne lesions *in vivo* with the mediators that have been shown to be modulated by some or all strains of *P. acnes in vitro* (Ingham *et al.*, 1992; Chronnell *et al.*, 2001; Kim *et al.*, 2002; Jeremy *et al.*, 2003; Kang *et al.*, 2005; Liu *et al.*, 2005; Nagy *et al.*, 2005; Nagy *et al.*, 2006; Trivedi *et al.*, 2006; Lee *et al.*, 2010; Agak *et al.*, 2014). It is noteworthy that IL-6, IL-12, IFNγ, and TGFβ have not been studied in acne lesions; this information would be of additional importance given that acne has until now been considered to be primarily a Th-1-mediated disease. T cells cloned from acne lesions express IFNγ, which is consistent with a Th1 response (Mouser *et al.*, 2003). It would be of value to clone T cells from early and late acne lesions to identify and characterize the Th17 cells.

Where in acne lesion development might IL-17 have a role?

Advances in immunology continue to shift paradigms in our thinking about common skin diseases. Psoriasis was once thought to be a disorder of keratinocyte differentiation, with secondary
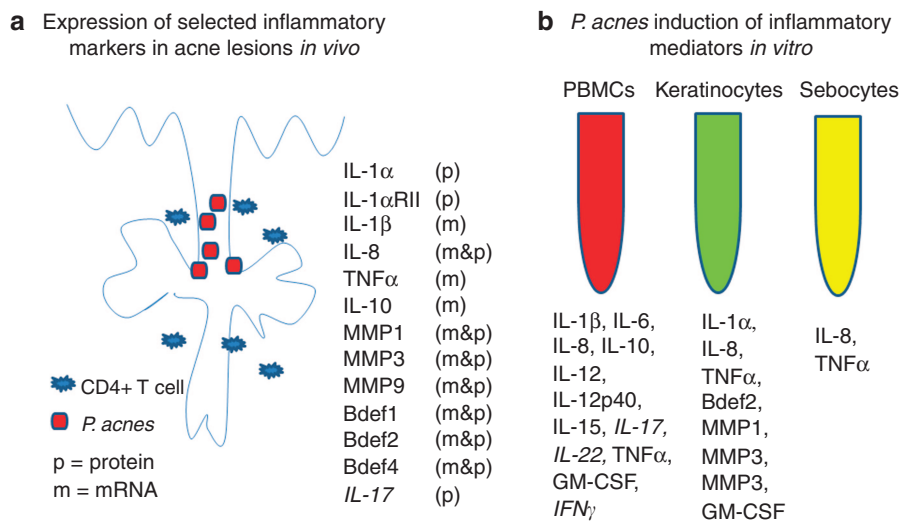

a Expression of selected inflammatory markers in acne lesions *in vivo*

IL-1α	(p)
IL-1αRII	(p)
IL-1β	(m)
IL-8	(m&p)
TNFα	(m)
IL-10	(m)
MMP1	(m&p)
MMP3	(m&p)
MMP9	(m&p)
Bdef1	(m&p)
Bdef2	(m&p)
Bdef4	(m&p)
IL-17	(p)

CD4+ T cell
P. acnes
p = protein
m = mRNA

b *P. acnes* induction of inflammatory mediators *in vitro*

PBMCs   Keratinocytes   Sebocytes

PBMCs: IL-1β, IL-6, IL-8, IL-10, IL-12, IL-12p40, IL-15, *IL-17*, *IL-22*, TNFα, GM-CSF, *IFNγ*

Keratinocytes: IL-1α, IL-8, TNFα, Bdef2, MMP1, MMP3, GM-CSF

Sebocytes: IL-8, TNFα

Figure 1. Comparison of cytokines expressed in acne lesions *in vivo* and in response to *Propionibacterium acnes in vitro*. (a) Cytokines expressed in acne lesions from patients (Ingham *et al.*, 1992; Chronnell *et al.*, 2001; Jeremy *et al.*, 2003; Kang *et al.*, 2005; Trivedi *et al.*, 2006; Agak *et al.*, 2014). **(b)** Cytokines expressed by various cell types in response to *P. acnes* stimulation *in vitro* (Kim *et al.*, 2002; Liu *et al.*, 2005; Nagy *et al.*, 2006; Lee *et al.*, 2010). Cytokines in italics are those identified in Agak *et al.* (2014).

Table 1. Mechanisms by which *P. acnes* may modulate inflammatory responses in acne

Mechanism	Significance
Production of tissue-destructive enzymes	Damaged follicle walls, allowing contents (including intact bacteria and auto-antigens) to escape into the dermis
Complement activation via the alternate pathway	Complement C3 deposition, an early event in lesion formation
Activation of pattern recognition receptors	Induction of innate immune responses and triggering the release of proinflammatory cytokines
Production of chemoattractants	Possible diffusion across intact follicle wall to attract leukocytes
T-cell mitogenic activity	Nonspecific upregulation of T cell–mediated immune responses
Adjuvant activity	Nonspecific upregulation of immune responses to unrelated antigens
Production of soluble antigens	Possible diffusion across intact follicle walls
Production of particulate antigens	Cell fragments induce a family of specific responses
Intracellular persistence	Persistent inflammation

inflammatory events. Clearly, this concept has changed over the years, and it has led to the discovery of novel biologic therapies that target components of the inflammatory cascade in psoriasis, including IL-17. Acne was thought to result from a combination of follicular hyperkeratinization, proliferation of *P. acnes*, and increased sebum production, with inflammatory events occurring subsequently. This paradigm began to shift with elegant studies from Cunliffe's group examining the inflammatory infiltrate in early acne lesions. Comedones clinically are noninflammatory lesions characterized by follicular hyperkeratinization. These lesions were found to contain IL-1-like material that was hypothesized to be released by keratinocytes, thus initiating an inflammatory cascade (Ingham *et al.*, 1992). A series of studies mapping the progression of inflammatory lesions from less than 6 hours up to 72 hours revealed that CD4+ lymphocytes, along with macrophages (CD68+), were the earliest immune cells to infiltrate sites of evolving inflammatory lesions (Norris and Cunliffe, 1988; Layton *et al.*, 1998). Furthermore, subclinical inflammation, consisting of the same cell types in lower numbers, can be found in otherwise normal follicles from unaffected skin in acne patients, suggesting that inflammation may precede keratinocyte hyperproliferaton (Jeremy *et al.*, 2003). If inflammation precedes comedone formation, it

may be less likely that *P. acnes* is driving this early influx of inflammatory cells, given that in acne only a minority (<20%) of healthy follicles are colonized with viable *P. acnes* (Leeming *et al.*, 1988).

Th17 cells were not known to exist at the time of the studies by Cunliffe's group. It would be interesting in view of Agak's report to speculate that the early infiltrating CD4+ cells might represent Th17 cells or Th1 cells drawn in toward secreted factors from *P. acnes*. In this case, could IL-17 represent the spark for the acne flame? If IL-1α triggers the nonspecific immune responses in acne, as Jeremy *et al.* (2003) suggest, *P. acnes* might augment this via Toll-like receptor activation. Concomitantly or subsequently, *P. acnes* may also initiate specific cell-mediated responses via the recruitment of different T-cell populations, perhaps depending on the type of lesion and its cytokine mileu, type of *P. acnes*, and the immune status of the individual. Clearly, we are a long way from knowing all of this, but perhaps it should not be surprising that acne lesions are so pleomorphic. A repeat of the challenging studies of lesion mapping as done by the Cunliffe group using today's technologies is clearly needed. A complete histological examination of acne lesions over the course of lesion development (including lesion resolution) and over a range of patient ages may help establish a role for IL-17 in acne. It is important to keep in mind that

factors other than *P. acnes* may also influence IL-17 secretion *in vivo*.

How do ATRA and vitamin D suppress *P. acnes*–induced IL-17 effects and what are the therapeutic implications?

ATRA and vitamin D decrease the expression of TLR2 on monocytes (Liu *et al.*, 2005). Studies from the Kim group demonstrate that *P. acnes* induction of IL-12, IL-1β, IL12p40, IL-6, and TNFα in PBMCs is blocked in the presence of neutralizing antibodies against TLR2 (Kim *et al.*, 2002; Liu *et al.*, 2005; Qin *et al.*, 2014). Th17 development is regulated by IL-6, TGFβ, and IL-1β. Because IL-6 and IL-1β are key players in Th17 cell differentiation, it is possible that ATRA or vitamin D exerts its suppressive effects on IL-17 induction via TLR2 inhibition, in addition to negative regulation of transcription of IL-17, RORα, and RORc. Clinical relevance for retinoic acid suppression of TLR2 is also supported by the finding that treatment of acne patients with oral isotretinoin temporally reduces the expression of TLR2 on PBMCs from patients during the course of treatment (Dispenza *et al.*, 2012). Little is known regarding a putative role for vitamin D in acne apart from reports indicating that vitamin D augments cathelicidin expression in sebocytes, which in combination with other antimicrobial peptides can kill *P. acnes* (Nakatsuji *et al.*, 2010). Additional studies regarding the importance of Th17 in acne would be needed to support the use of vitamin D in therapy. Data on the dose–response of IL-17 suppression by ATRA and vitamin D would also help determine whether these effects are relevant *in vivo*.

What new information does this study bring?

By putting the spotlight on IL-17 and Th17 cells, Agak *et al.* (2014) have raised the possibility that acne is not uniquely a Th-1 cell–mediated disease. Their observations suggest that the immune response in acne may be more complex than thought previously. In addition to showing that *P. acnes* can induce the production of IFNγ, a key cytokine marker for Th-1 cells, they have demonstrated that *P. acnes* can

COMMENTARY

modulate IL-17 and IL-22 production, as well as the expression of IL-17 receptors on PBMCs from healthy donors *in vitro*.

Looking ahead

This interesting and provocative work by Agak *et al.* (2014) poses many fascinating new questions relating to initiating events in acne pathogenesis and mechanisms of inflammation. Further *in vivo* work should determine whether some or all of these findings have relevance to the clinical setting, with a view toward informing the development of novel therapies.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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New Insights into Acne Pathogenesis: *Propionibacterium Acnes* Activates the Inflammasome

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The precise contribution of the commensal bacterium *Propionibacterium acnes* (*P. acnes*) in the inflammatory response associated with acne vulgaris remains controversial. In this issue Qin *et al.* show that *P. acnes* induces robust IL-1β secretion in monocytic cells by triggering the activation of the NLRP3 inflammasome. *In vivo*, the encounter of *P. acnes* and macrophages in the peri-follicular dermis could locally result in the release of substantial amounts of IL-1β and therefore exacerbate inflammation. Such findings suggest that molecules targeting IL-1β and/or the NLRP3 inflammasome may constitute new treatment possibilities for acne vulgaris.

Acne vulgaris is a common inflammatory skin disease affecting ∼80% of individuals at some time during their lives (Williams *et al.*, 2012). The disease, affecting the pilo-sebaceous unit, is a multifactorial process involving both endogenous and exogenous factors, including increased sebum production, altered follicular keratinization, inflammation, and bacterial colonization of the pilo-sebaceous unit by *Propionibacterium acnes* (*P. acnes*), a common anaerobic Gram-positive commensal of normal skin. Increased sebum production and follicular hyperkeratosis are thought to be initial events leading to a change of the pilo-sebaceous milieu that favors the proliferation of *P. acnes*

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