

Advances in the Understanding of the Pathogenesis of Inflammatory Acne

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ABSTRACT

Acne vulgaris (AV) is the most common skin disorder. It was traditionally thought that AV lesions developed after abnormal desquamation of the keratinocytes that line the sebaceous follicle, leading to hyperkeratinization and microcomedone formation. However, in recent years there has been a paradigm shift with regard to understanding the pathogenesis of AV, and it is now viewed as a primary inflammatory skin disorder. Research has implicated the presence of subclinical inflammation in the normal skin of acne patients, even before microcomedone formation. This article will review the novel concepts that play a role in the new pathogenesis of acne vulgaris.

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INTRODUCTION

Acne vulgaris (AV) is a chronic inflammatory disease of the pilosebaceous unit, and it is the most pervasive skin disorder regardless of gender, skin color, or ethnicity.[1-3] For decades it was thought that AV lesions initially developed after abnormal desquamation of the keratinocytes that line the sebaceous follicle, creating hyperkeratinization and microcomedone formation.[4-6] The pathological process in AV was then facilitated by an increase in circulating androgens at the onset of puberty, which stimulated the production of sebum in the pilosebaceous unit.[4-6] The combination of hyperkeratinization and the increase in circulating androgens then created a milieu that was conducive for the colonization of *Propionibacterium acnes,* resulting in various inflammatory molecules and chemotactic factors that initiate and perpetuate inflammatory cascades.[4-6]

However, a paradigm shift has occurred with regard to understanding the pathogenesis of AV. A seminal study demonstrating subclinical inflammatory cascades in AV was conducted by Norris and Cunliffe in 1988, and they observed lymphocytes and polymorphonuclear leukocytes prior to and concurrently with hyperkeratinization and microcomedone formation.[7] Moreover, in 1998, Layton et al found that CD4+ lymphocytes and macrophages (CD68+) were the earliest immune cells to infiltrate sites of nascent, subclinical inflammatory AV lesions.[8]

A New Acne Vulgaris Paradigm Emerges

In 2003, Jeremy et al published a landmark study regarding the pathogenesis of AV that produced a paradigm shift.[9] The investigators biopsied clinically normal follicles from the uninvolved skin of AV patients, the nascent lesions from AV patients, and the skin of healthy controls. After the biopsies were performed, cellular, vascular, and proliferative markers for inflammation were evaluated from the 3 groups.

Jeremy et al found that although CD3+ and CD4+ T cells were elevated in the uninvolved skin of AV patients, the elevation of these cells was not equivalent to the elevation in the papules of AV patients. The number of macrophages in the uninvolved skin of AV patients was also significantly increased and comparable to those in the papules of AV patients. E-selectin, vascular adhesion molecule 1, and interleukin-1 (IL-1) levels were also upregulated in the uninvolved skin of AV patients. The investigators concluded that vascular endothelial cell activation and the involvement of inflammatory responses are integral to the earliest stages of AV lesion development, and occur during hyperkeratinization.

The study conducted by Jeremy et al played a crucial role in deconstructing the dogma that the pathogenesis of AV commences with hyperkeratinization and comedogenesis. A subsequent study conducted by Do et al provided additional evidence that AV is an inflammatory skin disorder instead of an hyperproliferative disorder of the sebaceous follicle.[10] Using digital photographs and spatial alignment software, Do et al photographed 25 subjects with untreated facial AV every 2 weeks for 12 weeks. The investigators discovered that although 54% of inflammatory lesions were preceded by comedones, 28% of inflammatory lesions were preceded by normal-appearing skin. Consequently, Do et al further demonstrated that cellular inflammatory events occur at every stage of AV, from subclinical manifestations to the clinical presentation of active lesions.

The Role of *Propionibacterium acnes* in the Pathogenesis of Acne Vulgaris

Toll-Like Receptors

The paradigm shift regarding comedogenesis has initiated a reexamination of the involvement of *P. acnes* in the pathogenesis of AV. Although considerable evidence delineates the role of *P. acnes* in AV, the exact mechanisms by which it contributes to AV are currently in the process of being reevaluated. Studies have shown that *P. acnes* activates cytokine responses via toll-like receptors (TLRs), which recognize pathogen-associated molecular patterns on microorganisms and elicit immune responses.

In 2002, Kim et al established an association between *P. acnes* and TLR-2.[11] In that study, the investigators found that macrophages presenting TLR-2 were present in the acne lesions, around pilosebaceous follicles, and they increased during the evolution of the disease. In fact, *P. acnes* was able to induce nuclear factor-κB (NF-κB) activation from transfecting of TLR-2 into a non-responsive cell line. Moreover, in monocytes, *P. acnes* induced IL-12 and IL-8 protein production that was inhibited by anti-TLR-2 blocking antibodies. In a murine model, *P. acnes* initiated IL-12 p40 promoter activity via TLR-2, and IL-6 was elicited too. Kim et al felt that their data suggested that *P. acnes* triggers inflammatory cytokine responses in AV by activation of TLR-2.

A 2005 study conducted by Jugeau et al built on the study of Kim et al and further demonstrated the role that TLRs play in response to *P. acnes.*[12] The investigators found that the in vivo expression of TLR-2 and TLR-4 is increased in the acne lesions. In vitro tests also demonstrated that an increase in TLR-2 and TLR-4 expression occurred in human keratinocytes during the first hours of incubation with *P. acnes.* Additionally, Jugeau et al found that keratinocytes had an increased in vitro expression and secretion of metalloproteinase-9 (MMP-9) when incubated with *P. acnes.*

A 1992 study found that high levels of the pro-inflammatory cytokine IL-1α were expressed in acne lesions, and those findings were corroborated by Selway et al in 2013 and framed within the paradigm of TLRs playing a role in the inflammatory process that engenders AV.[13] Selway et al found TLR-2 to be expressed in basal and infundibular keratinocytes, and its activation elicited the release of IL-1α from primary human keratinocytes in vitro.[14] The in vitro exposure of micro-dissected human sebaceous glands to pathogen associated molecular patterns specific for TLR-2 also resulted in the increased expression of IL-1α.

Nucleotide-Binding Oligomerization Domain-Like Receptors

In addition to activating TLRs, *P. acnes* has been shown to activate nucleotide-binding oligomerization domain-like receptors, or NLRs, which are an important class of inflammasome genes that trigger inflammation and anti-microbial responses. Qin et al stimulated human monocytes with *P. acnes,* and found that *P. acnes*-induced NLRP3 activation that resulted in enhanced IL-1β secretion.[15] The investigators also determined that monocytes stimulated with *P. acnes* upregulated caspase-1 expression that resulted in further IL-1β secretion.

Additionally, the investigators noted a higher cellular expression of NLRP-3 and active caspase-1 in the dermis surrounding the pilosebaceous follicles in acne lesions compared with normal skin controls, and also a higher prevalence of CD68+ monocytes/macrophages in acne lesions compared with normal skin controls. Qin et al determined that *P. acnes* triggers a key inflammatory mediator, IL-1β, via NLRP-3 and caspase-1 activation, indicating a role for inflammasome-mediated inflammation in acne pathogenesis. A second study, conducted by Kistowska et al, has also demonstrated that NLRP-3 and IL-1β are integral to the inflammatory process induced by *P. acnes.*[16]

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Proteinase-Activated Receptors

P. acnes has been shown to produce exogenous proteases, and Lee et al investigated the function of these proteases in the induction of inflammatory cascades. The Lee et al study found that *P. acnes* protease and proteinase-activated receptor-2 (PAR-2) activity were increased on keratinocytes in AV.[17] Furthermore, keratinocytes that had increased PAR-2 activity stimulated the mRNA expression of IL-1α, IL-8, tumor necrosis factor- α (TNF-α), human beta defensin-2, LL-37, MMP-1, MMP-2, MMP-3, MMP-9, and MMP-13. The results of this study indicate that PAR-2 plays an important role in the pathogenesis of AV by inducing inflammatory mediators in response to *P. acnes* proteases. The study also indicates that some of the inflammatory mediators that are augmented by PAR-2 activity are integral to AV prior to the presence of *P. acnes*, so *P. acnes* is merely enhancing their response.

Nuclear Factor-κB

An article by Kim et al, published in *Dermatology,* was referenced earlier regarding *P. acnes* inducing NF-κB activation via TLR-2. A subsequent study by Kang et al also demonstrated that NF-κB and activator protein-1 are activated in acne lesions.[18] Kang et al found that TNF-α and IL-1β secretion, which are the resultant effect of NF-κB activation, will further amplify the NF-κB signaling pathways that originally led to their production and stimulate nearby cells for additional pro-inflammatory responses.

For example, TNF-α and IL-1β have been shown to up-regulate adhesion molecules ICAM-1 and VCAM-1 on endothelial cells.[19,20] Consequently, Kang et al hypothesized that ICAM-1, VCAM-1, and E-selectin expression levels on the luminal surface of endothelial cells are increased in inflammatory acne papules due to TNF-α and IL-1β induction.

Gene Array Profiling

In the wake of mounting evidence that AV is initially driven by abnormal, subclinical inflammatory responses and also after various biomarkers in the disease progression of AV have been identified, Trivedi et al performed gene expression profiling of acne patients.[21] Skin biopsies were obtained from an inflammatory papule and from the normal skin of 6 patients with AV, as well as from the normal skin of 6 subjects without AV. The biopsies demonstrated that 211 genes were upregulated in the lesional skin of AV subjects compared with the non-lesional skin of AV subjects and healthy controls.

Trivedi et al found that a significant proportion of upregulated genes are involved in pathways that regulate inflammation and initiate inflammatory cascades. The upregulated genes included MMP-1, MMP-3, IL-8, human beta-defensin 4, and granzyme B. The investigators concluded that matrix metalloproteinases, inflammatory cytokines, and antimicrobial peptides play a salient role in AV lesions.

Although the Trivedi et al gene expression profiling of acne patients established that multiple inflammatory cascades were involved in the pathogenesis of AV, the investigators observed that the normal skin of AV patients did not elicit the plethora of up-regulated genes as biopsied lesional skin. In fact, there were no gene expression differences between the normal skin of subjects with AV patients and without AV in the array analysis. These results were most likely due to the nominal inflammation involved in the small, 5 mm biopsies that were taken from the AV patients.

Acne Vulgaris and Scarring

In addition to cellular inflammatory mechanisms playing a role from subclinical comedogenesis to the clinical presentation of active lesions, research has shown that cellular inflammatory mechanisms are involved in AV resolution and scarring. Lee et al conducted a histopathological analysis of atrophic acne scars from AV patients, and found cellular infiltrates from transforming growth factor-β, (MMP-1), MMP-2, MMP-9, and MMP-13 in 77% of the scars.[22]

In an effort to differentiate the cell-mediated immune responses in patients who were prone to AV scarring vs AV patients who were not prone to AV scarring, Holland et al investigated various cellular and vascular biomarkers from the biopsies of inflamed lesions on the backs of AV patients.[23] The lesions were 6 hours to 7 days in duration.

Holland et al observed that patients who did not have AV scarring had an effuse influx of CD4+ T cells, macrophages, and Langerhans cells early in the lesions' development, and a significant number of these cells expressed HLA-DR. In the patients without AV scarring, the investigators also noted significant angiogenesis and vascular adhesion molecule expression in the early phase of their lesion development.

Conversely, the patients with scarring had significantly less CD4+ T cells, Langerhans cells, and a lower cellular HLA-DR expression in the early development of their lesions. Moreover, patients with scarring had higher angiogenesis molecule expression after 48 hours, and they experienced a later influx of macrophages, and increased cellular HLA-DR expression. Holland et al concluded that patients with scarring had an initial cellular response to AV that was weaker and less effective, but that it was more protracted throughout the resolution of AV lesions.

Types of Propionibacterium acnes

As the understanding of the pathogenesis of AV has expanded, so has the understanding of multiple facets of *P. acnes,* including its various genotypes. *P. acnes* has been subdivided into type I, type II, and type III. Within type I, there are 2 subtypes, IA and IB, whose distinction was initially based on serologic differentiation of cell wall carbohydrates and phage typing and later confirmed by analysis of recA, tly, and CAMP gene sequences.[24,25] *P. acnes* type III was identified in a 2008 article in which the investigators found isolates belonging to a novel recA cluster of *P. acnes* that was distinct from types I and II.[26]

P. acnes type IA has an extremely high association with acne, and it has been shown to be phenotypically resistant to multiple antibiotics, including tetracycline, clindamycin, and erythromycin, because of resistance conferring mutations in the 16S ribosomal RNA gene and the 23S rRNA gene.[27] In contrast, *P. acnes* type 1B is not specifically associated with AV, which challenges the traditional concept that all *P. acnes* contributes to AV pathogenesis. When comparing the different *P. acnes* types for pro-inflammatory expression, Jasson et al found that *P. acnes* type III had the highest pro-inflammatory potential due to its up-regulation of PAR-2, TNF-α, MMP-13, and tissue inhibitor of metalloproteinases-2.[28]

P. acnes resistance to antibiotics is a major concern for clinicians. A growing body of evidence indicates that antibiotic resistance and AV pathogenesis are associated with particular types or subtypes of *P. acnes.* For example, resistance to erythromycin was described as early as 1972 and, since then, widespread resistance among *P. acnes* to macrolides, lincosamines, and tetracyclines has been reported in several countries.[29,30] Acne vulgaris patients who do not respond to antibiotics may carry a strain of *P. acnes* with diverse virulence potential and antibiotic resistance patterns. These findings provide an explanation for the difficulties in predicting the clinical effects of antibiotic treatment for AV.

CONCLUSION

A paradigm shift has occurred in our understanding of the pathogenesis of AV since it has moved from being viewed as primarily a hyperproliferative disorder of the sebaceous follicle to that of an inflammatory skin disorder. We also have a new perspective for the role of *P. acnes* in AV as well as the sequence of events in evolution of acne lesions. The fact that not every *P. acnes* type causes clinical acne is a revolutionary idea that shows how far we have come from the original idea of the infectious origin of the disease caused by *P. acnes.* Moreover, we have now accepted the presence of subclinical inflammation and therefore consider AV as a primary inflammatory process rather than a secondary inflammation to *P. acnes.* Additionally, inflammatory processes continue even after the resolution of papules and pustules, leading to persistent hyperpigmentary changes and finally scarring. All these changes in our understanding of acne pathogenesis may eventually lead to disappearance of nomenclature such as "non-inflammatory lesions" for comedones, and replacement of post-inflammatory hyperpigmentation with persistent inflammatory hyper-pigmentation.

DISCLOSURES

Dr. Kircik has served as an advisor, investigator, consultant, and speaker for Allergan, Bayer, Galderma, Promius Pharma, Quinnova, Stiefel/GSK, LeoPharma, Taro, Valeant, and Warner-Chilcott.

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