THE ROLE OF SKIN MICROBIOME IN THE PATHOGENESIS OF ACNE VULGARIS

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Summary

Acne vulgaris is a highly prevalent inflammatory disease, which affects predominantly young adults. The infectious factor, along with the hypersecretion of sebum with altered lipid composition, the abnormal proliferation and differentiation of keratinocytes in the hair follicle, as well as the inflammasome activation with the infiltration of immune cells into the perifollicular dermis are considered to play an essential part in the pathophysiology of acne vulgaris. It has been shown that Cutibacterium acnes is the most prevalent and abundant bacterial species on the skin with the highest presence in sebaceous gland-rich skin. Furthermore, Cutibacterium acnes has many interactions with key events involved in the pathogenesis of acne, including: host inflammation, augmentation of lipogenesis, comedo formation and biofilm production. This article's purpose is to emphasize the correlations between the skin microbiome and acne vulgaris by describing the complex effects that Cutibacterium acnes and other bacterial species exert on the sebaceous follicle.

Keywords: *acne vulgaris, skin microbiome, Cuti-bacterium acnes, Staphylococcus epidermidis.*

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Introduction

The number of microbial cells that colonize the human body is ten times higher than the number of human cells and it is estimated that the microbes of the skin and gut are vital to the metabolic, hormonal, and immunologic equilibrium of the host [1, 2, 3].

The term 'microbiome' refers to a multitude of microorganisms, including viruses, bacteria and fungi, but also their metabolites, genes and the environment surrounding them [1, 2, 3]. The term 'microbiota' is more limited, describing the group of commensals and symbiotic microorganisms, but also the pathogenic ones found in a fixed environment [1, 2, 3].

Host-microbiome interplay is frequently involved in the pathogenesis of chronic dermatologic diseases such as acne vulgaris, rosacea, hidradenitis suppurativa, atopic dermatitis, psoriasis vulgaris, chronic wounds and many others [4, 5, 6, 7, 8, 9].

Acne vulgaris is a highly prevalent inflammatory disease of the human sebaceous follicle, that affects approximately 85% of young adults [10]. Four important factors have been thought to contribute to the pathogenesis of acne: the hypersecretion of sebum with altered lipid composition, the abnormal proliferation and differentiation of keratinocytes in the hair follicle leading to comedogenesis, the infectious factor with the implication of *Cutibacterium acnes* (*C. acnes*) and other microorganisms, as well as the inflammasome activation with the infiltration of immune cells into the perifollicular dermis [10]. These pathogenic factors are expressed in unique proportions for each individual affected by acne vulgaris. Moreover, the factors influence each

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other producing complex vicious circuits. The host- microbiome interplay in acne vulgaris is essential in the pathogenesis and it is best reflected by the results of advanced molecular studies. There were observed significant differences of the microbiomes in inflammatory and non-inflammatory lesions, regarding bacterial diversity and activity [5].

The infectious pathogenesis of acne vulgaris was intensely debated over the past few years. There were described two types of skin microbiome: the resident microbiome and the transient one. The resident microbiome consists of commensal microbes that live in homeostasis with the host and includes *C. acnes* and *Staphylococcus epidermidi*s [11]. The transient microbiome consists of pathogen microbes from the environment, which temporarily live on the skin and includes *Staphylococcus aureus* [11]. It has been suggested that a microbial imbalance or dysbiosis compared with the normal distribution in healthy tissues, is involved in the pathophysiology of inflammatory acne [11].

All the complex concepts and controversies have led us to perform a literature review on the role of skin microbiome in the pathogenesis of acne vulgaris.

The skin microbiome

The skin is the human body's largest organ, colonized by a heterogenous communities of microorganisms, including bacteria, fungi, viruses, most of which are harmless or even beneficial to their host. Innate and adaptative cutaneous immune responses can modulate the skin microbiota, but the microbiota also functions in educating the immune system [4].

Bacteria represent the most dominant members of the cutaneous microbiome. More than 40 bacterial types have been identified on the skin, mainly belonging to: Actinobacteria (Corynebacterineae, Propionibacterineae), Firmicutes (Staphylococcaceae), Proteobacteria and Bacteroidetes [12, 13, 14].

The proportions of these bacteria vary depending on individuals, body sites, as well as skin micro-environments. Environmental factors such as temperature, occupation, ultraviolet radiation exposure, humidity or the use of antibiotics, soaps, cosmetics can influence microbial colonization of the skin [15, 16, 17].

The microbial density and composition also change with age. In neonates, the microbiota depends on the route of delivery, and in infancy, dominant is Firmicutes [18]. The microbiota of the sebaceous sites is formed during puberty when hormonal changes activate the sebaceous glands [19]. The bacterial composition and diversity is also influenced by gender so women's hand surfaces carry a more diverse set of microorganisms than men's [20].

Skin sites are divided into three categories: moist areas (sole, popliteal fossa, axillae, inguinal area), dry sites (forearm), and sebaceous sites (head - especially on the forehead, nasal and alar crease, retro-auricular; upper trunk; genital) [3, 4]. The bacteria especially found in moist area are *Staphylococcus* and *Corynebacterium* spp. [3,4]. Dry skin sites present in various proportions the four major phyla- Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes [3,4]. Sebaceous sites have the lowest bacterial diversity because few microorganisms can tolerate the environment. *Cutibacterium* spp. can survive in anaerobic, lipid-rich conditions and are the main isolates from sebaceous areas [3,4].

Sebaceous follicles are the target for acne. The microbiome of the pilosebaceous units is similar with the surface composition and comprises two major residents namely *C. acnes* and *Staphylococcus epidermidis,* but it can comprise in variable quantities other *Cutibacterium* spp. like *C. granulosum* and *C. avidum*, as well as species belonging to *Staphylococcus*, *Corynebacterium*, *Pseudomonas* and the commensal fungi *Malassezia* [21, 22]. These microbes have the ability to form communities known as biofilms. In the pilosebaceous follicle, a biofilm matrix can act as a biological adhesive to restrict sebum passage into the infundibulum and promotes retention and accumulation of corneocytes in the lumen, resulting in a keratinaceous plug and leading to comedo formation [21, 22].

Cutibacterium acnes

In 1896, *C. acnes* was first described by Paul Gerson Unna when observing histological sections of comedones and was initially named as *Bacillus acnes*; later, in 1922 was first classified in

the genus *Corynebacterium* and in 1933 the name was changed again in *Porpionbacterium acnes*. In 2016, metagenomics and genomic investigations resulted in new reclassification of *P. acnes* in ***Cutibacterium*** acnes, but frequently the name of *P. acnes* continues to be used to reduce the confusion between *Cutibacterium* and *Corynebacterium* [10, 23, 24]. In this article the new nomenclature of *Cutibacterium acnes (C.acnes)* will be used.

C. acnes is involved in key events of acne pathogenesis, including: host inflammation, augmentation of lipogenesis, comedo formation, biofilm production. Recently, studies using the latest molecular methods show that imbalance between the multitude of skin microorganisms and different strains of *C. acnes*. The development of acne as well as its severity may be associated with a loss of diversity of *C. acnes* strains compared with healthy individuals [25, 26, 27].

C. acnes is the most prevalent and abundant bacterial species on the skin with the highest presence in sebaceous gland-rich skin [10].

In the study of Leyden JJ *et al*., the facial and scalp skin contained the highest density of *C. acnes* ($\sim 10^{5-106}/cm^2$), followed by the torso and upper limbs, and the lowest density of *C. acnes* is at the lower limbs ($\sim 10^2/cm^2$) [28]. The density of *C. acnes* also varies with age, so it is very low on the skin of children before puberty, but increases with age-from adolescence to adulthood, and then decreases after the age of 50 years [28, 29, 30].

C. acnes is also an important skin commensal. It hydrolyses triglycerides, releases free fatty acids and maintains low skin pH. The low skin PH inhibits the colonization of pathogenic bacteria, such as *Streptococcus* and *Staphylococcus aureus* [4, 31, 32].

Studies have reported that certain strains of *C. acnes* express linoleic isomerase, leading to an increase in trans-10, cis-12 conjugated linoleic acid [33, 34, 35, 36]. This activation of lipid mediators could trigger endoplasmic reticulum stress (and subsequent apoptosis), thus contributing to the pathogenesis of acne lesions. This led to the hypothesis that the replacement of *C. acnes* strains related to the inflammatory process in acne skin with those strains that are not associated with inflammation, might reduce the degree of acne [33, 34, 35, 36]. A study published in 2019 showed that this replacement led to a significant reduction in non-inflamed lesions [36].

C. acnes classification based on genetic variations includes three major genetic phylotypes I, II, and III [10, 23]. Based on multilocus sequence typing, *C. acnes* was subdivided into phylogenetic classes IA-1, IA-2, IB-1, IB-2, IB-3, IC, II, and III [10, 23]. Moreover, *C. acnes* strains have been classified by 16S rRNA gene sequencing into ten major ribotypes [10, 23]. These classifications are very useful, since they facilitate the study of correlations between particular *C. acnes* strains and disease activity [23]. Strains IA-2, IB-1 and IC seem to be associated with acne [3, 14, 23, 37, 38] while strain II is more frequent in healthy skin [3, 14, 23, 37, 38]. Strains IA-1, IB-2, and IB-3 were identified in both [14, 23, 3, 37, 38]. Type III strains are rarely present on the face and more abundant on the back, being associated with the condition of progressive macular hypomelanosis [23, 39, 40].

RT1, RT2 and RT3 were found in both healthy and acne individuals with no significant differences [10]. RT4, RT5 and RT8 were found in acne patients, while RT6 was found mostly in healthy individuals [10].

1. Host inflammation

C. acnes activates the innate immunity, interacting with Toll-like receptors (TLRs), TLR-2 in particular, protease-activated receptors (PARs), antimicrobial peptides (AMPs), and matrix metalloproteinases (MMP) [41,42]. It upregulates the production and secretion by human sebocytes, keratinocytes and macrophages of pro-inflammatory cytokines, including IL-1α , IL-1β, IL-6, IL-8, IL-12, TNF-α (tumor necrosis factor alpha), GM-CSF (granulocyte-macrophage colony-stimulating factor), β-defensin, interferon and other polypeptides, thereby initiating and/or exacerbating inflammatory responses [41, 42].

C. acnes also activates the two pathways of the complement: classical and alternative pathways, with the increase in C3a and C5a, leading to vascular permeability and lymphocyte infiltration [43, 44].

C. acnes is able to modulate keratinocyte differentiation by inducing a3, b1, a6s, filaggrin and aVb6 integrin expression on keratinocytes, changes observed in the development of acne lesions [45]. *In vitro* studies have shown that *C. acnes* can induce the stimulation of T cells (Th1/Th17) and the production of IL-17 [46]. $CD3^+$ cells were identified near *C. acnes*-positive comedones, cells that were absent from the surrounding inflamed lesions of acne [47]. These findings support the role of *C. acnes* in the initiation of inflammation in acne vulgaris [47].

On the other side, *C. acnes* also induces the secretion of staphylococcal lipoteichoic acid which could reduce the inflammation by inhibiting TLR-2 expression in keratinocytes and inducing expression of miR-143 [48, 49].

C. acnes produces multiple enzymes (proteases, lipases, phosphatases, porphyrins, hyaluronidases), while it is also able to activate host cells to produce matrix metalloproteinases (MMPs) [50, 51, 52]. Therefore, the microorganisms injure sebaceous glands, hair follicles and the dermal extracellular matrix and exacerbate inflammation [50, 51, 52].

A recent study reported that *C. acnes* can produce a pore forming toxin, the CAMP factor (Christie-Atkinson-Munch-Peterson factor), that induces sebocyte death, resulting in an amplified inflammation of the sebaceous glands [53, 54]. In addition, another study reported that CAMP factor 1 of *C. acnes* stimulated keratinocytes *in vitro* by direct interaction with TLR-2 [55].

C. acnes also secrets porphyrins that can generate reactive oxygen species and can induce keratinocyte inflammation [56]. In addition to these findings, a study from 2013 by McDowell A *et al.* suggested that a CAMP – factor targeted therapy could be the subject of future research in order to establish what may be its exerted effect on *C. acnes* strains [54].

Hyaluronidase is an enzyme with two variants in the *C. acnes* population that differ in their ability to degrade hyaluronic acid and could be involved in the pro-inflammatory responses observed in acne [57]. First variant is observed in *C. acnes* type IA strains and can be associated with acne, while the second variant was identified in *C. acnes* type IB and II strains and is associated especially with soft tissue infections. [57] HA fragments interact with CD44 or TLR-2 and can induce the inflammatory response [57].

Lipase is a *C. acnes* virulence factor that causes increased amounts of short chain fatty acids, especially free oleate, which promotes the adhesion of *C. acnes* and initiates biofilm formation [10].

Zouboulis CC *et al.* (2019) showed that short-chain fatty acids (SCFAs) produced by *C. acnes* in hypoxic, lipid-rich conditions have a pro-inflammatory effect on keratinocytes [58]. These short-chain fatty acids are shown to influence the behaviour of sebocytes and keratinocytes through two mechanisms: the inhibition of histone deacetylase activity and the activation of fatty acid receptors [58]. Epidermal keratinocytes express more proinflammatory genes, breaking the epithelial tolerance to these commensal microorganisms [31, 58].

2. Augmentation of lipogenesis

Moreover, *C. acnes* has been involved in lipogenesis, as it stimulates the sebaceous glands through the corticotropin-releasing hormone pathway [59]. CRH expression has been described in acne; a study of facial skin biopsies of patients diagnosed with acne reported a higher expression of CRH in sebocytes of affected skin compared with normal skin [60]. CRH increases sebaceous lipid synthesis and induces IL-6 release and IL-8 release by sebocytes, mediated by the CRH receptor [61].

3. Comedogenesis

An essential factor in the pathogenesis of acne is abnormal follicular keratinization with exacerbated proliferation of acroinfundibular keratinocytes that promote comedones [10]. The most important comedogenic factors are free fatty acids. They are released from sebum triacylglycerols by the action of *C. acnes* lipase [10]. Some studies have also found that interleukin-1α (IL-1α), which has been detected in open comedones, plays an important role in

comedogenesis [10]. Free oleic acid improves calcium influx into epidermal keratinocytes. Increases keratinocyte proliferation and induces abnormal keratinization associated with increased IL-1α release [10]. Free palmitic acid acts as a stimulating ligand of TLR-2 and activates the NLRP3 inflammasome [10]. Free palmitic acid, oleic acid, and DAGs (diacylglycerols) act as danger signals that activate the innate immune response [10].

4. Biofilm production

Another pathological feature of *C. acnes* is the ability to develop biofilms, protecting themselves from the host's immune system, antimicrobials or other environmental factors [3, 37]. Microorganisms attach to a surface, multiply and produce an extracellular protective matrix, a process regulated by bacterial signalling through *quorum sensing* [10]. While in comedones there were described biofilms of *C. acnes* in a proportion of 16%, in active acne lesions biofilms were present in 86% of cases [62, 63].

Other acne vulgaris-associated microorganims

Several other bacteria colonize the surface of the skin, some of which are important in maintaining skin health, while others can exacerbate certain diseases [23].

A study conducted by N. Jusuf *et al.* in 2020 reported that there are differences of microbiomes found in non-inflammatory and inflammatory lesions of acne vulgaris. *C. acnes*, *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus haemolyticus*, *Leuconostoc mesentroides* and *Staphylococcus aureus* were isolated in all types of acne lesions but with significant quantitative differences [38]. On the other side, *Micrococcus luteus*, *Kocuria varians* and *Staphylococcus vitulinus* were present only in non-inflammatory lesions, while *Staphylococcus cohnii*, *Staphylococcus arlettae* and *Dermacoccus nishinomyaensis* were isolated only from inflammatory lesions [38].

Some coagulase-negative staphylococcal species like *Staphylococcus epidermidis* or *Staphylococcus hominis* can be present in both healthy and acne skin [23]. In the last years, light has been shed upon the potential role of *Staphylococcus epidermidis* in the pathophysiology of acne [64]. Wang *et al.* (2016) observed that *Staphylococcus epidermidis* could control the proliferation of *C. acnes* by releasing succinic acid which inhibits the surface TLRs of keratinocytes and suppresses *C. acnes*- induced IL-6 [48].

Other studies have reported that *C. granulosum* is more abundant in the comedones and pustules and displays increased virulence phenotypes compared to *C. acnes* [65, 66].

Malassezia is one of the most abundant fungi of the skin. Song *et al.* (2011) and Numata *et al.* (2014) observed that *Malassezia restricta* and *Malassezia globosa* can be isolated from young acne patients [67, 68]. Moreover, Akaza *et al.* (2012) observed that the lipase activity of *Malassezia* is 100 times greater than the lipase activity of *C. acnes* [69]. *Malassezia* can hydrolyze triglycerides into free fatty acids which can interfere with the normal keratinization of hair follicular ducts [70, 71, 72, 73]. It can also chemoattract polymorphonuclear cells and promote the release of pro-inflammatory cytokines [70, 71, 72, 73].

Demodex folliculorum and *Demodex brevis* are ubiquitously found on the normal skin, especially in the pilosebaceous follicles of the face. *D. folliculorum* is found in the hair follicle, while *D. brevis* is found predominantly in the sebaceous glands. *Demodex* spp. may contribute to the development of acne by follicular obstruction, leading to distension, hyperkeratosis and inflammation [74, 75, 76].

Antibiotic resistance of microorganisms associated with acne vulgaris

Antibiotic resistance is an important problem across medicine. Antibiotherapy, either topical or systemic, is frequently prescribed in acne vulgaris and may be associated with a variety of adverse reactions including bacterial resistance, skin dysbiosis, gut dysbiosis and others [77, 78, 79, 80, 81, 82, 83, 84]. Several reports highlight the

fact that antibiotic resistance of *C. acnes* is rising, including towards tetracycline-class antibiotics [77, 78, 79, 80, 81, 82, 83, 84]. Moreover, after long term antibiotherapy, it was observed that the skin microbiota is depleted especially in *Staphylococcus epidermidis* [77, 78, 79, 80, 81, 82, 83, 84].

Conclusions

While the microbiome of each individual is unique and complex, researchers have observed the association of particular *C. acnes* strains with inflammatory and non-inflammatory lesions of acne vulgaris. Moreover, skin microorganisms can display variable virulence patterns depending on external or host-related factors, alternating from beneficial to pathogenic. The complex interactions between different microorganisms as well as the host-microbiome interplay in acne vulgaris patients are currently under intense research. The results could contribute to the development of new therapies to modulate the proliferation of beneficial strains and promote skin homeostasis.

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Conflict of interest
NONE DECLARED

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