# Acne Pathogenesis: What We Have Learned Over the Years

8

Clio Dessinioti

## Contents



**Core Messages**

- Past knowledge is essential for future research and therefore should not be underestimated
- In this chapter we will discuss what we have learned regarding the four major factors that influence acne pathogenesis (increased sebum production, follicular hyperkeratinization, *P. acnes* proliferation, and inflammation) over the years until 2000
- The increased sebum production in acne patients may be due to an hyperresponsiveness of the target organ (the pilosebaceous unit) to androgens
- The culture of sebocytes made it possible to better study the complex pathways of androgen control on the sebaceous gland
- Ductal hypercornification results from an increased rate of keratinocyte proliferation and/or a reduced separation of ductal corneocytes
- Additional factors that may influence comedogenesis include abnormalities of the sebaceous lipids, local androgens, retinoids, comedone cycling, and cytokines (IL-α)
- The exact nature and sequence of events in acne initiation have been a matter of debate. Inflammation has been classically considered as a secondary event

C. Dessinioti
Department of Dermatology,
Andreas Syngros Hospital, National and Capodistrian University of Athens, Athens, Greece
e-mail: cliodes@hotmail.com

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- *P. acnes* secretes various biologically active molecules like enzymes, proinflammatory cytokines, and chemotactic factors, which play a role in the initiation and perpetuation of the local inflammatory response

## 8.1 Introduction

A better understanding of the pathophysiology of acne has been made possible through years of research in the field. Studies of the past have opened the way for new research ideas and have provided the foundations for further scientific progress.

In this chapter we will summarize what we have learned on acne pathogenesis over the years until 2000. There are four major pathogenetic factors that have been implicated in acne pathogenesis, namely sebaceous gland hyperplasia with seborrhea, altered follicular growth and differentiation, *Propionibacterium acnes* colonization of the pilosebaceous unit, and inflammation [1]. Each factor will be addressed separately in the following sections.

### 8.1.1 Increased Sebum Production

#### 8.1.1.1 The Sebaceous Gland and Sebum Production

Seborrhea had been identified as a sine qua non for acne development as early as 1964, and sebum production was shown to be greater in acne patients compared to matched controls [2, 3].

Sebum is a mixture of lipids, most of which are synthesized de novo by the sebaceous gland, and it provides hydrophobic protection against overwetting and heat insulation in mammals. Sebum composition is remarkably species specific [4, 5]. The unique composition of human sebum has been shown in animal studies by Nikkari [4]. The lipid composition of sebum was investigated and early results showed lower quantities of triglycerides and higher alcohol esters in the surface lipids of acne patients [6] and more squalene and wax esters in acne patients than controls [7], while others have failed to demonstrate any differences in surface lipid composition [8–10]. Differential composition of lipids from different follicles was documented by using skin surface biopsies, in the study of Thielitz et al [11].

Sebum production and sebaceous gland activity is high at birth. Indeed, the neonatal adrenal gland is primarily a "fetal" adrenal gland consisting of an enlarged zona reticularis, the androgen-producing zone, and producing high levels of dehydroepiandrosterone (DHEA). Increased DHEA levels, in turn, stimulate sebaceous glands to produce sebum, until around 1 year of age when DHEA levels disappear following the decrease of the fetal adrenal gland. During adrenarche (at the age of 6–7 years in girls and 7–8 years in boys), the secretion of androgens by the adrenal glands (DHEA, DHEAS) begins to increase, and the reactivation of the sebaceous glands takes place [12].

Prof. J.S. Strauss and Prof A.M. Kligman were among the first researchers who begun working on the basic fundamentals of acne [13, 14]. Strauss and his colleagues developed a technique for measuring sebum excretion rate by absorbent paper [3, 15] and were one of the first to demonstrate the hormonal dependence of the sebaceous gland [14, 16]. Also, the work of Montagna, Ebling, Strauss, Pochi, and coworkers provided evidence that the pilosebacous unit is hormonally controlled [16–20].

The increased sebum production in acne patients may be due to an increased blood level of androgens and/or a hyper-responsiveness of the target organ (the pilosebaceous unit) to androgens [1].

Until 2000, it had been shown that the sebaceous gland possesses all the enzymes required for the conversion of DHEAS into active androgens (dihydrotestosterone, DHT) and estrogens [21–23]. These enzymes include 3β-hydroxysteroid dehydrogenase, 17β-hydrosteroid dehydrogenase, and 5α-reductase.

There are two isoenzymes of 5α-reductase, and the type 1 isoenzyme is predominant in the

sebaceous gland. This enzyme is responsible for the conversion of testosterone to the most potent androgen, DHT [24]. Increased activity of type 1 5α-reductase was shown in sebaceous glands isolated from acne-prone regions of the skin compared with nonacne-prone regions [24]. There is a differential response of sebocytes to androgens in vitro depending on the anatomic localization of their origin [25]. Facial sebocytes exhibit in vitro a stronger 5α-reductase expression than other cultured cells derived from adult skin [26] and their proliferation was stimulated by 5α-dihydrotestosterone [25]. Therefore, regional differences in the activity of this enzyme and consequently in the local production of DHT may be critical for increased sebum production and acne development [24]. Testosterone and DHT then interact with nuclear androgen receptors that have been localized to the basal layer of the sebaceous gland and the outer root sheath keratinocytes of the hair follicle [27, 28]. Moreover, the sebaceous glands of acne patients have increased number of such androgen receptors [29].

Initially, experimental animal models were used to study the pathophysiology of the sebaceous gland [30]. However, since acne is an exclusively human disease and the sebaceous gland differentiation is species specific, human models were necessary [4]. Early studies have been performed on whole human skin plugs, either been incubated in vitro [31–33] or grafted on to nude mice [34]. The isolation of viable human sebaceous glands by Kealey et al [35] and the establishment of the human sebocyte culture model in vitro by Xia et al [36] revolutionized research on sebocyte function. Thus, new insight was provided on sebocyte differentiation and sebocyte markers [37, 38]. Free fatty acids were shown to be synthesized by sebocytes without bacterial influence [37] and to play an active part on sebocyte proliferation [39].

Androgens have been proven to be one of the main factors in acne pathogenesis as they enhance follicular keratosis and influence sebum production [40–42].

The exact mechanisms by which androgens increase the size and secretion of sebaceous glands remain unknown [43]. Nevertheless, the culture of sebocytes made it possible to better study the complex pathways of androgen control on the sebaceous gland. Over the years, modifications of the technique of Xia et al. (1980) facilitated reproducible cultivation of human sebocytes in vitro [25, 26, 44–46]. Human sebocytes, however, could be maintained only for 3–6 subcultures with decreasing numbers of proliferating and increasing numbers of differentiated cells, accumulating neutral fat droplets until they died [36, 47]. This way, multiple donors were necessary in order to obtain adequate cell amounts for laboratory experiments, and even then, the short life span of the cells did not permit prolonged studies. The establishment of a human immortalized sebaceous gland cell line termed SZ95 by Zouboulis et al. overcame these constraints and opened the way for future research on the physiology of the sebaceous gland and its role in acne. The SZ95 cell line was shown to retain the morphologic, phenotypic, and functional characteristics of human sebocytes, including synthesis of the sebaceous lipids squalene, wax esters, triglycerides, and free fatty acids, even after 25–40 passages [47].

Apart from androgens, other hormones including insulin, hydrocortisone, and thyroid-stimulating hormone influence cultured sebocytes [48].

In addition, retinoids were shown to influence sebaceous gland growth and differentiation. Retinoic acid receptors γ and α and retinoid X receptor α have been detected in human sebocytes at the mRNA level [49]. Isotretinoin (13-*cis* retinoic acid) demonstrates an independent regulation of proliferation, lipid synthesis, and terminal differentiation of human sebocytes in vitro [37, 38].

#### 8.1.1.2 The Role of Androgens

The important role of androgens in acne has been substantiated by both clinical and research evidence (Table 8.1) [43].

There are studies of the role of DHEA, the major adrenal androgen, in prepubertal acne. The production of sebum correlated significantly with serum levels of DHEAS in prepubertal boys and girls. Also, the serum levels of

**Table 8.1** Evidence supporting the role of androgens in acne pathogenesis

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| Early acne in prepubertal patients is associated with elevated serum DHEAS |
| Men with androgen insensitivity do not produce adult levels of sebum and do not develop acne |
| Androgen excess due to hyperplasias/carcinomas of the adrenals or the gonads is often associated with the development of acne |
| Androgen excess has been reported in female patients with acne vulgaris, usually associated with other clinical signs of hyperandrogenism such as hirsutism, alopecia, or menstrual disturbances |
| Systemic administration of testosterone and DHEA increases the size and secretion of the sebaceous glands |
| Severe acne may be associated with high serum androgen levels |
| Anti-androgen therapy is beneficial in female acne |

**Table 8.2** Evidence supporting the hyper-responsiveness of the pilosebaceous unit to androgens in acne patients

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| Normal serum levels of testosterone and other androgens are usually found in acne patients |
| Not all sebaceous gland follicles are similarly affected by acne which predominates on the face, chest, and back, despite a constant serum level of androgens |
| The response of sebocytes to DHT and testosterone varies depending on their anatomic localization: sebocytes from the leg have a lower or no response at all to DHT and testosterone, while sebocytes from the face show a dose-dependent increase in proliferation |
| Female patients with clinical and laboratory androgen excess may have no acne |

DHEAS in prepubertal girls with comedonal or inflammatory acne were significantly higher compared with controls [50]. This data indicates that adrenal androgens are a major determinant of sebaceous gland activity during the prepubertal period [51].

Hyperplasia or carcinomas of the gonads or the adrenals, which result in elevated androgen levels, are often associated with the development of acne. Moreover, high androgen levels have been reported in patients with acne vulgaris, usually associated with other clinical signs of hyperandrogenism such as hirsutism, alopecia, or menstrual disturbances [40, 42, 52–58]. Conversely, androgen excess has been found in women with persistent or severe acne without other clinical evidence of hyperandrogenism [54, 59].

Additional evidence supporting the role of androgens in acne includes the findings that androgen-insensitive men (with nonfunctional androgen receptors) do not produce adult levels of sebum and do not develop acne [60] and that the systemic administration of testosterone or DHEA increases the size and secretion of sebaceous glands [61]. Also, anti-androgen therapy is highly successful in the management of female acne, highlighting the key role of androgens in acne etiology [62, 63].

As already mentioned, the increased sebum production in acne patients may be due to an increased blood level of androgens and/or a hyperresponsiveness of the target organ (the pilosebaceous unit) to androgens. There are many studies showing elevation of free testosterone, DHEA, and androstenedione [54, 64–66], although most patients with acne do not suffer from endocrinologic abnormalities. Also, the severity of acne has not been correlated with elevated androgen levels [67]. This raises the question of whether there is an increased local production of androgen within the sebaceous gland of patients with acne, which may then influence sebum production [68]. It was found that skin with acne converted testosterone to DHT at a rate 2–20 times greater than normal skin.

The end-organ sensitivity of the pilosebaceous unit to androgens could explain the normal serum levels of testosterone and other androgens usually found in acne patients (Table 8.2) [13, 68]. Also, not all sebaceous gland follicles are similarly affected by acne which predominates on the face, chest, and back, despite a constant serum level of androgens [69]. What is more, the response of sebocytes to DHT and testosterone varies depending on their anatomic localization. Thus, sebocytes from the leg have a lower or no response at all to DHT and testosterone, while sebocytes from the face show a dose-dependent increase in proliferation [25]. In support of this findings, female patients with clinical and laboratory hyperandrogenism may have no acne [69].

Although the role of androgens in the pathogenesis of acne cannot be refuted, an association

between acne severity and the degree of androgen excess has not been consistently reported. In 1989, Levell et al. showed a weak relationship between total acne count and level of free DHT, but no correlation between other androgens or SHBG levels and acne severity [70]. Walton et al. showed a positive correlation between levels of androstenedione and DHEAS and acne score and a negative correlation between SHBG levels and acne score [71]. Schmidt et al. also showed a positive correlation between androstenedione and acne severity [72]. On the other hand, Sheehan-Dare et al. demonstrated no relationship between clinical markers of androgenicity (excessive body hair, irregular menstrual bleeding, alopecia) and acne severity [73]. Also, the severity of acne in adult women (>17 years old) was not positively correlated with any clinical or laboratory markers of androgenicity in the study of Cibula et al [67].

### 8.1.2 Follicular Hyperkeratinization

Ductal hypercornification may be due to an increased rate of keratinocyte proliferation and/or a reduced separation of ductal corneocytes due to increased cohesion between keratinocytes [13, 74, 75].

The microcomedone is the initial lesion in acne and may be present in normal-appearing skin of acne patients, as has been demonstrated by biopsies [76]. Keratinocyte hyperproliferation of both comedones and microcomedones compared with normal follicles has been demonstrated immunohistochemically by the use of the antibody Ki-67. Also, cellular proliferation was greater in normal follicles from acne-affected areas (acne-prone follicles) compared with areas not affected by acne (not acne prone) [74].

Ductal hypercornification centers on the interplay of various factors, including local androgens, retinoids, local cytokines, abnormalities of the sebaceous lipids, and comedone cycling.

Certain sebaceous lipids, such as squalene oxide and free fatty acids, are higher in acne patients than controls and may contribute to comedone formation [77, 78]. Similarly, a deficiency of linoleic acid may be an additional comedogenic factor [79, 80]. In addition, local cytokines may play a role, and interleukin-1α (IL-1 α) has been shown in vitro to cause comedone formation, while this process was inhibited by the addition of IL-1α receptor antagonist to the growth medium. It was suggested that changes in sebum excretion or composition may result in the production of IL-1 by follicular corneocytes, thus influencing comedogenesis [81]. These findings provide some evidence for the involvement of endogenous inflammation processes in acne initiation [5].

The potential role of androgens in controlling ductal hyperproliferation has been studied. It has been reported that keratinocytes are capable of converting testosterone to DHT, as 5α-reductase type 1 activity was demonstrated in infrainfundibular segments of follicles. Activity of this enzyme varies within regions of the pilosebaceous unit. Infrainfundibular keratinocytes demonstrate greater activity of this enzyme compared to interfollicular epidermal cells, thus showing greater capacity for producing androgens compared with the epidermis. Androgens in turn may influence follicular hyeprkeratinization [82, 83]. This data is supported by the clinical observation that anti-androgen therapy with combined oral contraceptives reduces the number of comedones [84].

Both oral and topical retinoids suppress comedogenesis by 98 and 60 %, respectively, after 4 months of treatment [85–87].

Comedo cycling may be an important factor in the development and resolution of comedogenesis and it provides an explanation to the clinical observation that many open and closed comedones resolve spontaneously [85]. Pilosebaceous follicles and comedones have showed different expression of cycling cells and proliferation markers, suggesting that the duct may also undergo cycling like the hair follicle [88].

There was no convincing evidence until 2000 to support a role for *Propionibacterium acnes* (*P. acnes*) in comedogenesis; formalin-killed *P. acnes* cells did not induce normal human keratinocytes to produce IL-1α in vitro [89, 90].

### 8.1.3 *Propionibacterium acnes* and Inflammation

*Propionibacterium acnes* (*P. acnes*) is a Gram-positive anaerobic bacterium found in the normal human cutaneous flora. When it was first isolated in 1896, it was thought to be the direct cause of acne. However, in the early 1960s the role of *P. acnes* in causing acne was refuted as it was shown that it also resides on normal human skin and that surface *P. acnes* levels were similar between patients with acne and controls [91, 92]. Also, numbers of viable *P. acnes* within follicles do not correlate with the severity of inflammation and some inflamed lesions do not contain viable bacteria [93]. A possible explanation was offered as early as 1978; it was proposed that specific changes in the follicular microenvironment may allow follicular colonization by *P. acnes* [94]. Later, a key role was attributed to *P. acnes* in acne pathogenesis when antibiotics that reduced skin surface *P. acnes* (such as erythromycin and clindamycin) were shown to clinically improve acne [92, 94]. Moreover, the fact that clinical failure of oral erythromycin was associated with the presence of resistant *P. acnes* strains in some patients delineated the key role of this bacterium in acne [95, 96]. Nevertheless, antibiotics possess anti-inflammatory properties that may, at least in part, account for their effectiveness in acne [97]. The aforementioned evidence suggested that *P. acnes* is not the direct cause of acne, but a significant contributing factor to the inflammatory stages of the disease.

*P. acnes* secretes various biologically active molecules like enzymes and chemotactic factors, which play a role in the initiation and perpetuation of the local inflammatory response. Also, it stimulates monocytes to produce proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), IL-1β, and IL-8 [98].

The exact nature and sequence of events in acne initiation has been a matter of debate. Inflammation has been classically considered as a secondary event. Evidence published until 2000 supported that inflammatory lesions arise from non-inflamed comedones, which are the clinical manifestation of abnormal ductal hypercornification [99]. However, as already mentioned, a role of the proinflammatory cytokine IL-1α in comedone formation has been demonstrated in vitro [81]. In vivo, comedones contain enough IL-1α activity to initiate a nonspecific inflammatory response if released into the dermis [100]. This data raises the question of whether inflammatory events occur pre- or post-hyperproliferation.

Another controversial issue is whether the initial cellular infiltrate is neutrophilic or lymphocytic.

In 1974, Kligman's study demonstrated that the initial infiltrate consisted of neutrophils and was followed by microscopic rupture of the follicle wall and subsequent formation of clinically apparent inflammatory lesions [13, 76]. *P. acnes* produces neutrophil chemoattractants that diffuse through the follicle wall and trigger the inflammation process. Moreover, it was proposed that inflammation results from a type IV hypersensitivity reaction to *P. acnes* or other comedonal components after the release of reactive oxygen radicals and enzymes by neutrophils and rupture of the sebaceous follicle wall [101, 102].

On the other hand, studies investigating early inflammatory events in acne lesion showed an initial CD4+ lymphocytic infiltrate as a primary inflammatory event [99, 103]. Also, a 1998 study concluded that the inflammatory cell and cytokine profile in papules is that of a delayed cellular reaction to an antigen or antigens, the nature of which is yet uncertain [103].

Until 2000, although several lines of evidence support the direct role of *P. acnes* in acne, little is still known about the mechanism by which this microorganism contributes to the pathogenesis of the disease.

## 8.2 The Role of Cutaneous Neuropeptides: Neurogenic Inflammation

Up to 2000, there is increasing evidence that the cutaneous sensory nervous system innervates multiple cell types and plays an important role in inflammation [104, 105]. After the activation of peripheral nerve endings by various stress-sensing stimuli, neuropeptides are released and result in

changes collectively termed as neurogenic inflammation [106, 107]. Neuropeptides, such as substance P, are produced from sensory neurons or from keratinocytes and mast cells in the skin [107].

Also, the presence and activity of proopiomelanocortin, corticotropin-releasing hormone, and corticotropin-releasing hormone receptor genes has been demonstrated in human skin and sebaceous glands [108, 109].

### Conclusion

Fascinating new data elucidating the pathophysiology of acne has recently been published and will be discussed in detail elsewhere in this book. However, none of these achievements would have been possible without the work done by predecessors.

Many questions on acne pathogenesis and resolution still remain unanswered. As history can teach us many lessons and techniques, both in the clinic and the laboratory, the profound knowledge of the past is a prerequisite for research and advancement in the future.

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