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Viewpoint

New developments in our understanding of acne pathogenesis and treatment

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Abstract: Interest in sebaceous gland physiology and its diseases is rapidly increasing. We provide a summarized update of the current knowledge of the pathobiology of acne vulgaris and new treatment concepts that have emerged in the last 3 years (2005–2008).
We have tried to answer questions arising from the exploration of sebaceous gland biology, hormonal factors, hyperkeratinization, role of bacteria, sebum, nutrition, cytokines and toll-like receptors (TLRs). Sebaceous glands play an important role as active participants in the innate immunity of the skin. They produce neuropeptides, excrete antimicrobial peptides and exhibit characteristics of stem cells. Androgens affect sebocytes and infundibular keratinocytes in a complex manner influencing cellular differentiation, proliferation, lipogenesis and comedogenesis. Retention hyperkeratosis in closed comedones and inflammatory papules is attributable to a disorder of terminal keratinocyte differentiation. *Propionibacterium acnes,* by acting on TLR-2, may stimulate the secretion of cytokines, such as interleukin (IL)-6 and IL-8 by follicular keratinocytes and IL-8 and -12 in macrophages, giving rise to inflammation. Certain *P. acnes* species may induce an immunological reaction by stimulating the production of sebocyte and keratinocyte antimicrobial peptides, which play an important role in the innate immunity of the follicle. Qualitative changes of sebum lipids induce alteration of keratinocyte differentiation and induce IL-1 secretion, contributing to the development of follicular hyperkeratosis. High glycemic load food and milk may induce increased tissue levels of 5α-dihydrotestosterone. These new aspects of acne pathogenesis lead to the considerations of possible customized therapeutic regimens. Current research is expected to lead to innovative treatments in the near future.

Key words: sebaceous gland – acne – cytokine – Toll-like receptor – PPAR – hyperkeratinization

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Biology of sebaceous glands

The sebaceous gland is a holocrine gland, and its secretion is formed by the complete disintegration of the glandular cells. Excreting sebum is the major function of sebaceous glands (1), and increased sebum excretion is a major concurrent event that parallels the development of acne lesions. With the development of human sebaceous gland experimental models for *in vitro* studies (2–5), considerable progress has been made in our understanding of many new

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aspects of the gland's function and control (6–8). These studies have illustrated why the view of the human sebaceous gland has turned from a 'living fossil of the skin' (9) to become the 'brain of the skin' (10), and are providing many new insights into the pathogenesis and treatment of sebaceous gland-associated diseases, such as acne vulgaris.

Neuropeptides and sebaceous glands

Neuropeptides (NPs) are a heterogeneous group of biologically active peptides that are present in neurons of both the central and peripheral nervous systems (11). The human sebaceous gland has been shown to express functional receptors for NPs, such as corticotropin-releasing hormone (CRH), melanocortins, β-endorphin, vasoactive intestinal polypeptide, NP Y and calcitonin gene-related peptide. These receptors modulate the production of inflammatory cytokines, proliferation, differentiation, lipogenesis and androgen metabolism in human sebocytes (6,12).

The NP substance P (SP) was found to express in dermal nerves around the sebaceous glands of acne patients (13). SP promotes both the proliferation and the differentiation of sebaceous glands *in vitro*, and increases immunoreactivity and RNA expression of proinflammatory factors (14). It induces the expression of neutral endopeptidase (CD10) in sebaceous germinative cells and of E-selectin in perisebaceous venules (15). Recently, ectopeptidases dipeptidyl peptidase IV (DP IV or CD 26) and aminopeptidase N (APN or CD13), which have been shown to be involved in the degradation of several NPs, especially SP (16), have been found to be highly expressed in human sebocytes *in vivo* and *in vitro* (17). Further studies showed unexpectedly that inhibitors of DP IV and APN can suppress proliferation and slightly decrease neutral lipids, but can also enhance terminal differentiation in SZ95 sebocytes. This suggests that ectopeptidases may be new targets to modulate certain sebocyte functions, and that ectopeptidase inhibitors may have potential therapeutic roles in acne pathogenesis (6,17).

The hypothalamic-pituitary-adrenal axis is traditionally regarded to be responsible for neuroendocrine responses of sebaceous gland to stress (18). The presence of CRH, its binding protein (CRHBP) and its receptors CRH-R1 and R2 in human sebaceous glands *in vivo* and SZ95 sebocytes *in vitro* has been confirmed (10,19–21). CRH can inhibit proliferation and induce synthesis of neutral lipids in SZ95 sebocytes, and testosterone antagonizes CRH by downregulating CRH-R expression in human sebocytes *in vitro*. In addition, growth hormone, which also enhances sebaceous lipid synthesis, modifies CRH-R expression by reducing mRNA levels of CRH-R1 and by enhancing CRH-R2 mRNA levels, enhancing the release of interleukin (IL)-6 and IL-8 in SZ95 sebocytes *in vitro* by an IL-1β-independent pathway (22). CRH was also found to enhance mRNA expression of Δ5-3β-hydroxysteroid dehydrogenase in human sebocytes *in vitro*, an enzyme that is responsible for androgen activation through the conversion of dehydroepiandrosterone to testosterone (20). Antalarmin, a CRH-R1 specific CRH inhibitor, reduced sebaceous neutral lipid synthesis (21). These *in vitro* data may be compatible with the significant increase in CRH expression in acne-involved compared with sebaceous glands not involved with acne (10). The interaction between androgen signalling and CRH-dependent signalling mechanisms, especially the differential regulation of CRH-R1 and CRH-R2, needs further study to provide elucidation in more detail.

The proopiomelanocortin (POMC) system also plays an important role, as a neuromediator system in controlling the sebaceous gland. The α-melanocyte-stimulating hormone (α-MSH) can stimulate sebocyte differentiation and lipogenesis (23,24). Human sebocytes express MC-1Rs and MC-5Rs *in vitro* and *in vivo* (22,25,26). While MC-1Rs was expressed in both undifferentiated and differentiated sebocytes, MC-5Rs was expressed only in differentiated sebocytes. Activation of MC5R apparently stimulates lipogenesis, which indicates that MC-1R expression is not obligatorily associated with the sebaceous cell differentiation process and lipogenesis (26). In contrast, MC-5Rs is a marker of sebocyte differentiation and responsible for the lipogenesis of sebocytes. Studies also showed that acne-involved sebaceous glands express higher levels of MC-1R than sebocytes of healthy glands (27), but no studies have been performed on MC-5R expression in acne. The latter data indicate that further research is required to establish the role of melanocortin receptors in the physiology and pathology of the sebaceous glands. Recent findings indicate that β-endorphin suppresses cell proliferation and induces lipid formation in SZ95 sebocytes *in vitro*, which may be mediated by the μ-opioid receptor, which is expressed in human sebocytes *in vivo* and *in vitro* (6).

Sebaceous gland and innate immunity

The pilosebaceous unit is an immunocompetent organ. Keratinocytes and sebocytes may act as immune cells capable of pathogen recognition and abnormal lipid presentation (28). Innate immunity molecules such as toll-like receptor (TLR) 2 and TLR4 (29), CD1d (28) and CD14 (6) are expressed in SZ95 sebocytes. Keratinocytes and sebocytes, as major components of the pilosebaceous unit, may act as immune cells and may be activated by *P. acnes* via TLRs and CD14 and through CD1 molecules and also may recognize altered lipid content in sebum, followed by the production of inflammatory cytokines. In addition, antimicrobial peptides, such as defensin-1, defensin-2 and cathelicidin, showed expression and immunoreactivity in

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the sebaceous gland (13,30). Human β-defensin-2 (hBD-2) is also expressed upon exposure to lipopolysaccharides (LPS) and *P. acnes* (31).

The monounsaturated fatty acids (MUFA), mainly palmitic acid (C16:1) and oleic acid (C18:1), both of which are bactericidal against Gram-positive organisms (32), are produced by the sebaceous gland, as is sapienic acid, an important antimicrobial lipid. Stearoyl coenzyme A desaturase (SCD) 1, an enzyme responsible for the biosynthesis of MUFA, is also expressed by the sebaceous gland (33). The TLR-2 ligand macrophage-activating lipopeptide-2 stimulates both SCD and fatty acid desaturase-2 mRNA expression in SZ95 sebocytes (32).

Sebaceous gland and stem cells

Sebaceous gland cells derive from the basal progenitor cells of the sebaceous gland alveolus (34,35) and were until recently regarded to be updated by the reservoir of stem cells in the hair follicle bulge (36). This interdependence is, however, not obligatory (37): sebaceous glands are present in some mouse mutants that lack hair follicles (38,39), they can be maintained independently of the hair follicle bulge (40) and they can be induced in footpad epidermis, an anatomic region normally devoid of hair follicles and sebaceous glands. Sebaceous glands have also been found to express skin stem cell marker bone morphogenetic protein-1 (41). The human sebocyte lines SZ95 and SebE6-E7 have the ability to differentiate into both sebocytes and interfollicular epidermal cells (8). This suggests that human sebocytes may represent a bipotential stem cell. Furthermore, the interaction between β-catenin and Indian hedgehog stimulates the proliferation of sebocyte precursors (42). Overexpression of Myc stimulates sebocyte differentiation, whereas overexpression of β-catenin stimulates interfollicular epidermal differentiation *in vitro* (8).

Other sebocyte properties

Histamine-1 receptor is expressed in SZ95 sebocytes and in human sebaceous glands, and diphenhydramine, a histamine-1 receptor antagonist, significantly decreases squalene levels, indicating that histamines and anti-histaminics could potentially directly modulate sebocyte function (43). Peroxidated squalene induced the production of inflammatory mediators in HaCaT keratinocytes and induced upregulation of PPARγ, mRNA and protein expression in a dose-dependent manner (44).

Liver X receptors (LXRs) are members of the nuclear receptor superfamily, which plays a critical role in cholesterol homeostasis and lipid metabolism. Expression of LXRα and LXRβ was detected in SZ95 sebocytes (45), and LXR ligands enhance the expression of LXRα, but not of LXRβ, inhibit cell proliferation and stimulate lipid synthesis (14,45). They also decrease the expression of cyclooxygenase-2 and inducible nitric oxide synthase that was induced by LPS treatment, a function that indicates the important roles of LXRα in differentiation and inflammatory signalling in sebaceous glands (14).

The physiological role of non-neuronal acetylcholine (ACh) and its receptors (AChR) in epidermal physiology has been studied recently (46). The undifferentiated and mature sebocytes express different AchR subunits (47), which imply that sebocyte differentiation, sebum production or sebum composition may be altered by endogenously produced ACh acting in a paracrine manner or stimulated exogenously by nicotine. Presence of AChR and nicotinic activity are also found in the infundibulum of the pilosebaceous unit and can promote infundibular epithelial hyperplasia and follicular plugging, suggesting an important role for the cholinergic system in acne vulgaris (48), and a possible etiological role for nicotine uptake by smoking in acne and especially hidradenitis suppurativa (47).

Hormones

Androgens play an essential role in acne pathogenesis. As most of the patients with acne have normal circulating androgen levels, acne severity does not correlate with serum androgen levels. It is postulated that androgens may play only a *permissive* role in priming or initiating acne development, or it may be the local overproduction of androgens in the skin and/or the high expression and responsiveness of androgen receptors that determines the formation of acne lesions.

The sebaceous gland has been shown to express all the necessary enzymes for the biosynthesis of testosterone *de novo* from cholesterol, from 5α-reduced substances ingested in dairy products (49), or in a shortcut from circulating dehydroepiandrosterone (50).

The main influence of androgen on acne pathogenesis concerns the proliferation/differentiation of sebocytes and infrainfundibular keratinocytes. The human models useful for *in vitro* studies include sebaceous gland organ culture, primary culture of sebocytes and immortalized human sebocyte cell lines (4,5,51).

1 Sebocyte proliferation: The stimulatory effect of testosterone and 5α-dihydrotestosterone (DHT) on sebocyte proliferation was observed in primary culture of human sebocytes and hamster sebaceous gland cells (52,53). In SZ95 sebocytes, testosterone and DHT may showed a stimulatory effect or no effect on cell proliferation (4,54). In cultured rat preputial cells, DHT suppressed cell proliferation (55). Of note, androgens are effective on human sebocytes *in vitro* in concentrations above the physiological ones.

2 Sebocyte differentiation and lipogenesis: In the sebaceous gland organ culture, testosterone and DHT at nearly physiological concentrations demonstrated no effect or inhibitory effect on cell division rates or lipogenesis (2). In SZ95 sebocytes, the combination of testosterone and linoleic acid exhibited a synergistic effect on sebaceous lipids (56). In hamster sebocytes, DHT augmented the formation of intracellular lipid droplets along with an increase in the accumulation of triglycerides (5). Interestingly, DHT treatment of hamster ear sebaceous glands *in vivo* had a profound effect on sebocyte proliferation, differentiation and induction of lipogenesis involving the upregulation of the sterol response-element-binding protein (SREBP) pathway, a key regulator of lipogenesis (54,57). SREBP expression has also been detected in human sebocytes (33).

3 Comedogenesis: Formation of microcomedones is caused by hyperproliferation/hyperkeratinization of the infrainfundibulum of the follicular canal. It remains to be determined if higher activity of the type I 5α-reductase detected in the follicular infrainfundibulum is related to the abnormal differentiation of keratinocytes (58). In addition to the isolated infundibulum culture, various animal models have been used for studies on comedogenesis, such as the rabbit ear assay, the Mexican hairless dog and the Rhino mouse. It is unclear if these models can truly reflect the human sebaceous follicles.

Taken together, despite the clinical evidence that androgens stimulate sebaceous lipids, the *in vitro* effect of androgens on proliferation and differentiation of sebocytes varies in different experiments. Further studies are needed to determine which cofactors are required for the display of androgen actions on sebocytes.

Hyperkeratinization

One of the most crucial initial events in the development of acne lesions is hyperkeratinization in the follicular infundibulum and sebaceous duct resulting in microcomedones (Fig. 1). Electronmicroscopically, the pattern of hyperkeratinization demonstrates retention hyperkeratosis with increased number and size of keratohyaline granules and accumulation of lipid droplets and folding of the retained squames on themselves as a result of pressure effects (59).

The pathogenesis of follicular hyperkeratinization is still unclear. IL-1α has been reported to induce hyperkeratinization in follicular infundibulum *in vitro* and *in vivo* (51). In addition, abnormal infundibular keratinization has been associated with a disorder in terminal differentiation of infundibular keratinocytes, which is related to increased filaggrin (filament aggregating protein) expression (60). Not only hyperkeratinization but also hyperproliferation can be observed within infundibular keratinocytes, with the

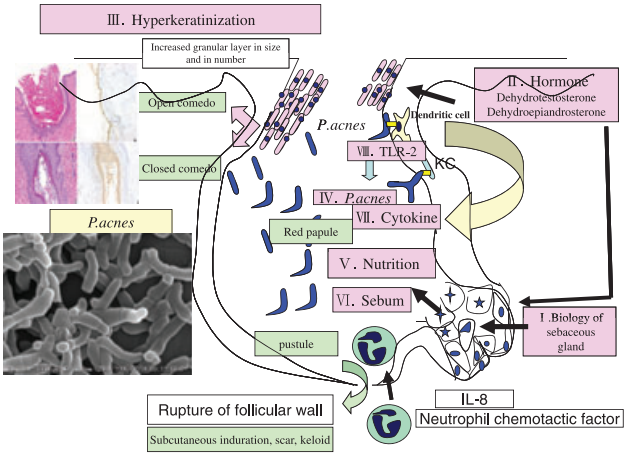

Figure 1. Pathogenesis of acne. The biology of sebaceous gland (I) has been elucidated recently. Hormonal factors (II) are involved in sebum excretion (VI) and hyperkeratinization in the infundibulum (III). Acne starts as microcomedones, which are generated by hyperkeratinization in the infundibulum, with increased in size and in number of granular layers. Microcomedones evolve into open or closed comedones. The follicular channel is colonized by *Propionibacterium acnes* (IV), develops and stimulates cytokine production (VII) via toll-like receptor (VIII), resulting in inflammatory lesions. Nutrition (V) may be involved in acne pathogenesis. IL-8, a neutrophil chemotactic factor, attracts neutrophils into the follicular walls. Once the follicular walls rupture, granulomatous lesions with subcutaneous induration, scarring and keloids are generated.

expression of the hyperproliferative markers keratin (K) 6 and K16 (61). IL-1α activates basal keratinocyte by autocrine production inducing K16 expression in suprabasal cells in the active state. This finding indicates that keratin and keratinocyte activation cycle are related to hyperproliferation of the keratinocytes lining the infundibulum (62).

Regarding the hormonal response, increased DHT may act on infundibular keratinocytes leading to abnormal hyperkeratinization (58). Follicular keratinization may be triggered by relative deficiency of linoleic acid and peroxides in sebum (32). Recently, *P. acnes* extracts have been implicated in the formation of the microcomedo (63).

However, the pathogenesis of closed and open comedo formation remains ambiguous. Studies on nevus comedonicus indicated that the disturbance of terminal differentiation in the follicular infundibulum may play a role in closed comedo formation (64), and fibroblast growth factor receptor (FGFR) 2 signalling also seems to be involved. Acneiform nevus, which is a variant of nevus comedonicus, has been shown to be associated with Ser252Trp-gain-of-function mutation of FGFR2, which also explains acne in Apert syndrome (65,66). Androgen-dependent FGFR2b-signalling has been proposed in the pathogenesis of acne (67). The Ser252Trp-FGFR2-mutation with increased FGFR2-signalling is associated with increased expression of IL-1α (68). In addition, cyst

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formation in acne usually occurs following closed comedo development. Terminal differentiation in filaggrin expression may be involved in cyst formation (69). Moreover, there is a debate on whether influx of inflammatory cells precedes hyperkeratinzation (70).

Bacteria

The significance of the involvement of *P. acnes* in acne pathogenesis is still controversial, mainly due to the fact that it belongs to the resident microbiota. The recently decoded genome of *P. acnes* again raised the question of the pathogenic potential of this bacterium (71). This possibility is further supported by the observation that *P. acnes* induces the expression of antimicrobial peptides and proinflammatory cytokines/chemokines from various cell types (14,31,72,73). Recent description of phylogenetically distinct *P. acnes* clusters (74) challenges our overall current understanding of the pathogenic nature of bacteria involved in acne pathogenesis and raises the possibility that certain *P. acnes* strains may cause an opportunistic infection worsening acne lesions. Indeed, phylogenetic clusters of *P. acnes* differ not only in the production of secreted proteins (74), but also in their ability to induce different immune responses in keratinocytes and sebocytes; the major difference being the ability to induce hBD-2 expression (31,73). Although hBD-2 has no direct antimicrobial effect on *P. acnes* (73), it does have synergistic activity with cathelicidin (14). Thus, the total antimicrobial activity in the pilosebaceous unit is likely due to several antimicrobial peptides – and additionally antibacterial lipids (32) – acting together.

Various antimicrobial peptides are expressed in healthy skin without any visible signs of inflammation, suggesting that (i) antimicrobial peptides may be induced in the absence of proinflammatory cytokines/chemokines and (ii) resident skin microbiota may facilitate antimicrobial peptide induction without inflammation. It was recently proposed that the beneficial effects of resident microbiota may come from their ability to induce antimicrobial peptide expression (75). The identification of *P. acnes* proteins, inducing solely antimicrobial peptide and not proinflammatory cytokine/chemokine expression, would promote stimulation of maintenance levels of antimicrobial peptides. Consequently, increased resistance to abnormal *P. acnes* colonization could be facilitated.

Sebum

Increased sebum production is a characteristic of acne patients even if it does not strictly correlate with the development of the lesions (76). Seborrhoea is, indeed, not a sufficient condition for the development of the pathology. Sebaceous gland growth and the consequent increased sebum excretion are phenomena experienced by all adolescents, but only in some cases does it seem to be associated with the incorrect regulation of lipid metabolism. Several variations in lipid metabolism have been described in acne patients, including a decreased amount of linoleic acid (77). Moreover, recent studies suggest that the desaturation of sebaceous fatty acids may contribute to acne development: an increase in the saturated/monounsaturated ratio together with a reduction in the enzymatic desaturation of C16:0 seems to correlate with the lesion counts and, therefore, with the clinical improvement (78). A hallmark of sebum in acne patients is the presence of lipoperoxides, mainly due to the peroxidation of squalene and a decrease in the level of vitamin E, the major sebum antioxidant (44). The generation of an inflammatory reaction seems to initiate the hyperkeratinization of the acroinfundibulum and the manifestation of acne lesions. In this context, both lipoperoxides and monounsaturated fatty acid (MUFAs) are capable of inducing alteration in keratinocyte proliferation and differentiation, whereas peroxides are capable of inducing production of pro-inflammatory cytokines and activation of peroxisome proliferator-activated receptors (PPARs) (44,78). Seborrhoea *per se* is not responsible for the development of acne, as demonstrated by the success of treatment with agents with no effect on sebum secretion rate that can inhibit the inflammatory process, such as antibiotics, topical retinoids, azelaic acid and benzoyl peroxide. Moreover, the occurrence of seborrheic dermatitis is associated with a change in the quality of sebum lipids, i.e. a decreased level of polyunsaturated fatty acids and vitamin E (79). Considering all these data, it is apparent that seborrhoea is not necessarily associated with the alteration of lipid composition and the oxidant/antioxidant ratio characteristic of the skin surface lipids of acne patients.

Nutrition

Acne is driven by hormones and growth factors [particularly insulin-like growth factor (IGF-1)] acting on the sebaceous glands and the keratinocytes lining the pilary canal. Dairy products (and perhaps some other foods) contain 5α-reduced steroid hormones and other steroid precursors (49) of DHT that drive sebaceous gland (and likely pilar keratinocyte) function. Dairy also contains about 60 other growth factors and micronutrients (80). Phytoestrogens in food seem to have no impact on acne. Drinking milk causes a direct rise in IGF-1 through a disproportionate elevation in blood sugar and serum insulin levels (81). High glycemic load foods also cause IGF-1-mediated elevations in DHT (82). IGF-1 levels during teenage years closely parallel acne activity and are likely synergistic with the steroid hormones (Fig. 2).

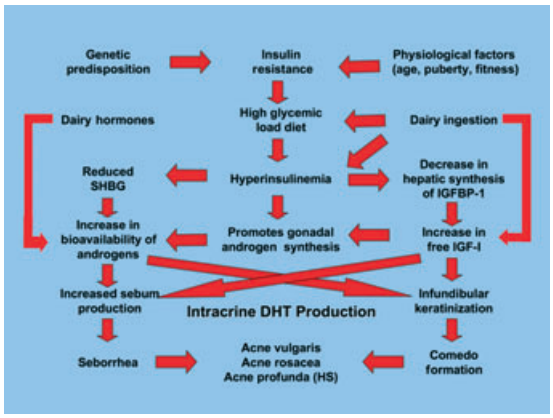

Figure 2. The acnegenic cascade: interactions among the numerous hormones, growth factors, enzymes and their targets.

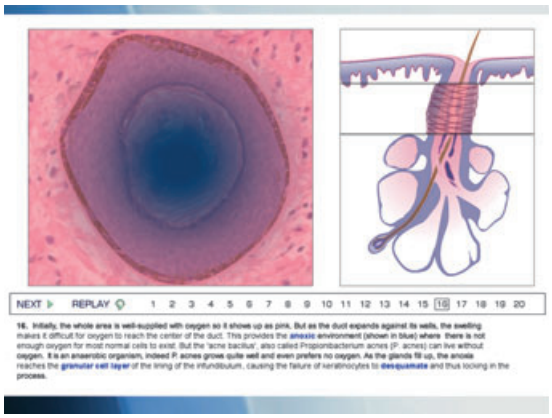

Figure 3. The formation of the anoxic duct. The expanding keratinocytic mass is constricted by the glassy membrane, with resulting anoxia represented as the bluish hue of deoxygenated haemoglobin (available at: http://www.acnemilk.com).

Vitamin A is needed for normal follicular function and is often deficient in teens. Dietary fatty acids influence inflammation, some pro-inflammatory, some anti-inflammatory, underlining the need for careful dietary selection for optimal control (83). Linoleic acid likewise has an ambivalent role in acne (84). Iodine, although not comedogenic, may enhance inflammation (85).

Acne can be improved by controlling hormones and inflammation, both of which are influenced by diet; so full acne control requires dietary control. Concurrent with standard anti-acne therapy, all dairy products and all high glycemic foods should be stopped for at least 6 months to evaluate the effect (86). Vitamin A supplementation may help reduce plugging of pores in deficient individuals. Foods containing ω-3 essential fatty acids (EFAs) and EFA supplements may help to control inflammation (83).

The pilary canal is plugged by what is best viewed as a straightforward mechanical effect. As the hormone-driven keratinocytes multiply, they are propelled towards the centre of the duct, which expands to accommodate the increasing bulk of the microcomedo until a point is reached beyond which the inelastic 'glassy membrane' that encloses the pilosebaceous duct can expand no further. Further production of keratinocytes into this closed system causes an increase in intraluminal pressure and this causes hypoxia centrally in the duct (Fig. 3). This produces an anoxic environment that favours development of intraductal *P. acnes* colonies, leads to rupture of the duct walls, release of the luminal antigens and the ultimate production or worsening of the inflammatory acne papule.

The inflammatory cascade that follows is produced by a Pandora's boxful of mediators, cytokines and chemokines causing the chemical and biological epiphenomena of acne; but no matter what occurs when the inflammation commences, the initiating factors are hormonal. Thus, no acne therapy is complete without a dietary and hormonal history and appropriate dietary and hormonal advice.

Cytokines

Cytokines are present in normal sebaceous glands, and they are affected by many factors (87) (Table 1). IL-1α, tumor necrosis factor (TNF)-α, IL-6 and IL-8 are released into supernatant in unstressed sebocyte culture (88). In a stressed environment, the amounts of released cytokines increase significantly. The treatment of cultured sebocytes with *P. acnes* and LPS significantly upregulated the expression of proinflammatory cytokines (31). While LPS stimulated CXCL8, TNF-α and IL-1α, *P. acnes* stimulated CXCL8 and TNF-α only. *Propionibacterium acnes* had no effect on IL-1α. There was also a difference in the cytokine production curve over time after treatment between *P. acnes* and LPS. Arachidonic acid and calcium ionophore enhanced the level of IL-6 and IL-8, but that of IL-1β and TNF-α was not affected (88).

Table 1. Current aspects of cytokine production in normal skin, acne lesions and associated factors

	IL-1α	IL-6	IL-8	CXCL8	TNF-α
Normal person, healthy skin		–	++		
Acne patient, uninvolved skin		+	++		
Acne patient, involved skin		++	+++		
Propionibacterium acnes stimulation	No effect			↑	↑
LPS treatment	↑			↑	↑
Increased PAF-R expression			↑		
ectopeptidase	↑[1]				
CRH		↑	↑		
α-MSH			↓		

IL, interleukin; TNF, tumor necrosis factor; LPS, lipopolysaccharide; PAF-R, platelet-activating factor receptor; CRH, corticotropin-releasing hormone; α-MSH, α-melanocyte-stimulating hormone.
[1]IL-1 receptor antagonist is significantly upregulated in cultured sebocytes in the presence of dipeptidyl peptidase IV and aminopeptidase N inhibitors.

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In *in vivo* studies, IL-6 was barely detectable in the sebaceous gland of healthy skin (88). In acne patients, a weak expression was found in uninvolved skin and a stronger one in acne-involved skin. IL-8 exhibited a stronger expression in sebocytes of acne patients' skin than in those of healthy controls (88).

Corticotropin-releasing hormone enhances the release of IL-6 and IL-8 from sebocytes *in vitro* by an IL-1β-independent pathway (10). In the POMC system, α-MSH peptide suppressed IL-1β-induced release of IL-8, a central pro-inflammatory mediator in the pathogenesis of acne inflammation (22).

Cultured sebocytes express functional platelet-activating factor receptors (PAF-R), and PAF-R are involved in regulating the expression of inflammatory mediators (26), including cyclooxygenase-2, prostaglandin E and IL-8 (10). Ectopeptidases found in sebocytes also modulate cytokine regulation. Inhibition of these ectopeptidases leads to an upregulation of endogenous IL-1 receptor antagonist (15).

It is very important that cytokine regulation of sebocytes, which play a key role in acne pathophysiology (70), is modulated by various factors mentioned above. Targeting these cytokine-regulating factors may have a potential in the future effective treatment of acne.

Toll-like receptors

Toll-like receptors are transmembrane proteins that are crucial players in the innate immune response to microbial and other invaders. Ten TLRs have recently been described in humans (72). TLRs are mainly expressed on immune cells, such as monocytes, macrophages, dendritic cells and granulocytes. TLR stimulation mimics the action of IL-1α and promotes the production of proinflammatory cytokines, prostaglandins, leukotrienes (LT) and chemokines (72). Intriguingly, selected IL-1 receptor associated kinases (IRAK-1, 2, M and 4) are bifunctional. They can be recruited to the TLR complex and thus mediate TLR signalling but can also associate with protein partners involved in T- and B-cell receptor-mediated signalling pathways and so can be critical mediators for both innate and adaptive immune responses (89–91). Conceivably, these molecules may be viable targets for designing new therapeutic strategies for various human inflammatory diseases.

Chemokine/cytokine synthesis in human monocytes is induced through activation of TLR2 by *P. acnes* (72), but the expression of active TLR2 and 4 and of CD14 in human keratinocytes (28) has also implicated *P. acnes* and TLRs in the development of acne inflammation. It is suggested that, by using this pathway, the innate immune system is able to recognize microbial components and then induce cytokine/chemokine synthesis in acne (32).

The sebaceous gland has usually been thought to simply provide physical protection to the external skin surface, but its numerous functions are gradually becoming understood (54). Human SZ95 sebocytes were found to express constitutively TLR2, TLR4, CD14, IL-1α, IL-1β, IL-6 and IL-8. The latter, augmented by exposure to components of Gram-negative (LPS) and Gram-positive (lipoteichonic acid) bacteria (29). In addition, human sebocytes produce antimicrobial lipids (32), antimicrobial peptides (hBD-2, psoriasin and cathelicidin) (12,31,92), which exhibit synergistic activities and induce proinflammatory cytokines/chemokines via TLR- and CD14-dependent mechanisms. Certain *P. acnes* species can stimulate SZ95 sebocytes to produce the endogenous TLR4 agonist hBD-2 (31), and sebaceous cathelicidin can even kill *P. acnes* (14). These findings indicate a direct functional induction of innate immunity in human epithelial cells, and especially in sebocytes, without the involvement of inflammatory cells, while recruitment of the latter to the involved sites can potentiate the inflammatory events in acne (54). This prominent new and previously unsuspected pattern recognition by the sebaceous glands establishes a functional link between lipids, lipid metabolism, antimicrobial proteins and epithelial innate immunity that can be important in acne pathogenesis. Therefore, the pharmacological regulation of TLR and CD14 expression may provide a novel target for the treatment of inflammatory acne lesions.

Overview of acne pathogenesis

The pathogenesis of acne, the most common skin disease, which manifests in the pilosebaceous follicle, is currently attributed to multiple factors such as increased sebum production, alteration of the quality of sebum lipids, regulation of cutaneous steroidogenesis, androgen activity, interaction with NPs, exhibition of pro- and anti-inflammatory properties, follicular hyperkeratinization and the proliferation of *P. acnes* within the follicle (6,32).

The increased sebum excretion is a major concurrent event associated with the development of acne. Neutral and polar lipids produced by sebaceous glands serve a variety of roles in signal transduction and are involved in biological pathways (6). Additionally, fatty acids act as ligands of nuclear receptors such as the PPARs. Sebaceous gland lipids exhibit direct pro- and anti-inflammatory properties, whereas the induction of 5-lipoxygenase and cycloxygenase-2 pathways in sebocytes leads to the production of proinflammatory lipids (88).

Furthermore, hormones like androgens control the sebaceous gland size and sebum secretion. In cell culture, androgens only promote sebocyte proliferation, whereas PPAR ligands are required for induction of differentiation and lipogenic activity (56). On the other hand, keratinocytes and sebocytes may be activated by *P. acnes* via TLR,

CD14 and CD1 molecules (31). Pilosebaceous follicles in acne lesions are surrounded with macrophages expressing TLR2 on their surface. TLR2 activation leads to transcription factor nuclear factor triggering and thus production of cytokines/chemokines, phenomena observed in acne lesions. Furthermore, *P. acnes* induces IL-8 and IL-12 release from TLR2 positive monocytes (72).

Acne research continues to deliver new pieces to the puzzle, helps us to understand acne pathogenesis and assists the development of new drugs against acne. New compounds which are able to inhibit LTB4 synthesis, antagonize PPAR or inhibit ectopeptidases (10) offer new ways to treat acne. PPAR regulation may be a pathway to modify sebaceous lipogenesis.

Future prospective drugs based on acne pathogenesis

Rational use of available treatment options based on the type and severity of acne lesions is presently a key component of successful acne therapy (93). However, increasing understanding of acne pathophysiology slowly gives rise to the anti-acne therapeutic agents and regimens of the future (32) (Fig. 4).

Isotretinoin

Oral isotretinoin is the most effective drug available for the treatment of acne. It directly suppresses sebaceous gland activity leading to significant reduction in sebaceous lipogenesis, normalizes the pattern of keratinization within the sebaceous gland follicle, inhibits inflammation, and – in a secondary manner – reduces growth of *P. acnes* (27). In addition, it normalizes the expression of tissue matrix metalloproteinases and their inhibitors (94). It is most active in the treatment of severe recalcitrant nodulocystic acne and in the prevention of acne scarring. However, despite its undeniable effectiveness, isotretinoin is not a curative drug (88). Its discontinuation may be followed by recurrence in the absence of appropriate maintenance treatment. Identifying the appropriate acne patient for isotretinoin treatment is nowadays important, because the compound has already drawn the attention of the European Committee on Proprietary Medicinal Products, which released a European directive to ensure harmonization of isotretinoin treatment throughout the European Union (95). Both the European guidelines and the iPLEDGE isotretinoin distribution programme of the (Food and Drug Administration) are aimed at preventing the use of the drug in women during pregnancy.

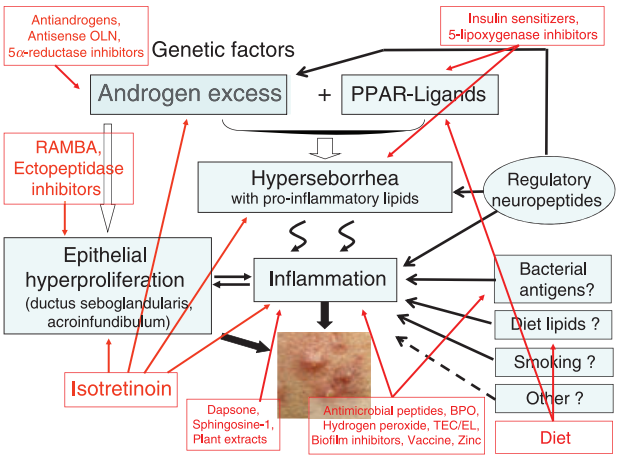

Figure 4. Future prospective drugs targeting elements of acne pathogenesis.

In addition to our better understanding of its activity (88), other developments in the application of this potent compound include the lower risk of side-effects using low-dose long-term regimens (0.1–0.3 mg/kg/day daily or intermittent use) and a micronised formulation, which exhibits similar efficacy and is associated with a lower risk of adverse events (54).

Retinoic acid metabolism blocking agents

Retinoic acid metabolism blocking agent (RAMBA) are compounds which block the catabolism of endogenous vitamin A. Oral talarozol, a potent retinoic acid 4-hydrolase inhibitor, was effective on comedones and inflammatory lesions in a pilot study with 17 acne patients after 12 weeks of treatment (96). Topical RAMBA modulates keratinization in the mouse and can induce a dose-dependent reduction in IL-1α mRNA in human skin.

Ectopeptidase inhibitors

Inhibitors of dipeptidylpeptidase IV and AP N stimulate the expression of IL-1 receptor antagonist, thus they could be expected to reduce primarily comedogenesis and, secondarily, inflammation (17). In the SZ95 sebocyte cell line, the DP IV inhibitors Lys[Z(NO2)]-thiazolidide and Lys[Z(NO2)]-pyrrolidide and the APN inhibitors actinonin and bestatin suppressed proliferation, enhanced terminal differentiation and slightly decreased total neutral lipid production. The antiinflammatory cytokine IL-1 receptor antagonist, which also restores epithelial cell differentiation, was significantly upregulated in SZ95 sebocytes and HaCaT keratinocytes in the presence of IL-1 receptor inhibitors. Furthermore, the inhibitors suppressed proliferation and IL-2 production of *P. acnes*-stimulated T cells *ex vivo* and enhanced the expression of the immunosuppressive cytokine transforming growth factor-beta 1. Therefore, the inhibitors of dipeptidylpeptidase IV and AP N may be able to reduce both comedogenesis and inflammation (17).

Antibiotics/anti-inflammatory agents

Oral antibiotics have been suggested to improve inflammatory acne by inhibiting the growth of *P. acnes*. However,

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several antibiotics exhibit para-antibiotic anti-inflammatory properties, assisting the improvement of acne through decreased leucocyte chemotaxis and alteration of cytokine production (32). Tetracyclines, erythromycin and nadifloxacin reduce reactive oxygen species formation by neutrophils and, therefore, acne inflammation (97). New antibiotics for the treatment of acne include limecycline, a second-generation tetracycline and roxithromycin, a macrolide that exhibits anti-inflammatory and anti-androgenic activities (97).

Ribosomally synthesized antimicrobial peptides have very wide bacteriotoxic spectra, and bacterial resistance to these peptides seems to be a rare phenomenon. Among them, indolicidin served as a template to omiganan (98). Omiganan is the most advanced molecule in the front line of clinical applications of antimicrobial peptides. Its interaction with membranes has been shown to play a fundamental role.

Products that form peroxide radicals appear to induce a significant alteration to the microenvironment of the pilosebaceous unit. A new 5% solubilized BPO-formulation consisting of small-size particles exhibits enhanced follicular penetration of benzoyl peroxide and improved clinical efficacy (99). A stabilized hydrogen peroxide cream seems to exhibit effects similar to benzoyl peroxide, but shows a better tolerability (100). A similar effect is released by combining two individually inactive compounds, triethyl citrate and ethyl linoleate, directly on the skin (82). Dapsone gel 5% was effective on inflammatory lesions with minimal systemic absorption (101). Nanoparticular sphingosine-1-phosphate inhibits Langerhans cell migration and the cellular release of pro-inflammatory cytokines, so is a candidate for anti-inflammatory treatment of mild acne (102).

The plant extracts from *Azadirachta indica*, *Sphaeranthus indicus*, *Hemidesmus indicus*, *Rubia cordifolia* and *Curcuma longa* have shown anti-inflammatory activity *in vitro* by suppressing the activity of *P. acnes*-induced reactive oxygen species and pro-inflammatory cytokines (103).

Treatments that target specific components of the *P. acnes* biofilm, e.g. recombinant human DNAse I, which can inhibit biofilm formation (104) may have a role as future anti-acne drugs. The development of vaccines targeting microbial products of *P. acnes* could also represent an alternative strategy to conventional antibiotic therapy (105).

Antiandrogens

Topical therapy with cyproterone acetate (CPA) in a new vehicle, solid lipid nanoparticles, which facilitates the penetration of the compound into the follicular canal, represents an additional therapeutic opportunity (106). Such a non-systemic treatment with CPA would be available for both men and women (107).

Two new non-androgenic-progestin-containing cyclical oral contraceptives with strong anti-androgenic activity (drospirenone 3 mg) with reduced concentrations of ethinyl estradiol (20 and 30 μg) have been shown effective in acne vulgaris (108) and may replace the classic CPA/ethinyl estradiol and chlormadinone acetate/ethinyl estradiol oral contraceptives, thanks to comparatively less side-effects.

Insulin-sensitizing agents

Insulin sensitizer treatment has been associated with a reduction in serum androgen levels and an improvement in serum lipids. Insulin resistance and compensatory hyperinsulinaemia may play a crucial role in the pathophysiology of peripheral hyperandrogenism, including the development of acne, as elevated serum insulin is thought to induce hyperandrogenism (54). Metformin and thiazolidinediones (rosiglitazone, pioglitazone) can decrease both fasting and stimulated plasma insulin levels and reduce insulin resistance through interaction with PPARγ. Troglitazone, another member of the thiazolidinedione family, has been withdrawn from use because of liver toxicity. Although pioglitazone and rosiglitazone have an improved safety profile in terms of liver toxicity, reports of increased cardiovascular morbidity should exclude these agents from anti-acne treatment. In addition, they may induce hypoglycemia in normoglycemic subjects (109). This leaves only metformin as an anti-acne drug with clinical potential (110), for improvement of both hirsutism and acne in females with polycystic ovary syndrome (111).

5α-reductase inhibitors

It was hoped that the 5α-reductase inhibitors finasteride and dutasteride would be useful for the treatment of androgen-dependent female acne. Unfortunately, in women with normal serum-free testosterone levels, no clinical improvement can be achieved using these molecules. It was hypothesized that some of these women might have excessive activity of the enzyme 5α-reductase in peripheral tissue. However, the inhibition of 5α-reductase activity alone has been shown to be insufficient to reduce overall sebocyte activity and improve acne lesions (112). In addition, dutasteride carries a contraindication warning for women in the USA.

5-lipoxygenase inhibitor

5-lipoxygenase controls the synthesis of LTB4, a natural PPARα ligand (113). Zileuton, an oral 5-lipoxygenase inhibitor, was shown to improve inflammatory acne (54) and to directly inhibit sebum synthesis in a transient manner with a potency similar to that of low-dose isotretinoin (32).

Diet

Dairy intake is clearly associated with acne in several studies, and clinical improvement has been demonstrated

in a small study using a low glycemic load diet in young males. Further studies are needed to elucidate the role of dietary interventions in acne therapy (114) and are hampered by the lack of availability of blind dietary substitutes for dairy.

Antisense oligonucleotides

The selective inhibition of androgen receptor by antisense siRNA oligonucleotide molecules, in combination with formulations that may improve compound penetration (115), could be a novel strategy for the blockade of the androgen receptor, and could open innovative, specific therapeutic possibilities in androgen-associated acne with much reduced risk of systemic side-effects. Inhibition of the expression of androgen receptor by antisense oligonucleotides reduces *in vitro* the enhanced proliferation of sebocytes challenged by testosterone and DHT (116).

Conclusions and perspective

Acne is a chronic obstructive and inflammatory disorder affecting the pilosebaceous follicles mainly in adolescents. Acne pathogenesis is gradually being elucidated. Sebaceous glands and their ductal infundibula are not merely the cutaneous appendageal tissues supplying sebum to retain humidity in the epidermis, but also serve as the stage for important immunological phenomena, including innate immunity, NP production, synthesis of antimicrobial peptides and expression of stem cell characteristics.

A current hypothesis of acne pathogenesis in genetically predisposed adolescents consists of the combined action of androgens and PPAR ligands on the pilosebaceous unit, which results in increased sebocyte proliferation and enhanced sebum excretion and quantitative sebum alterations. The androgenic stimulation, potentiated by synergistic growth factors, NPs and IL-1α, leads to abnormal ductal and infundibular hyperkeratinization. Ectopeptidases and *P. acnes* proliferation and the resulting increase in bacterial TLR2 ligands in the follicular canal may increase IL-1α production and IL-1β secretion, which induces IL-6, IL-8 and IL-12 production in infundibular keratinocytes and macrophages, resulting in inflammation and rupture of follicular walls and the induction of tissue matrix metalloproteinases that induce scar formation.

Prospective new acne treatments may – in the future – address the normalization of abnormal keratinization in the follicular infundibulum, the inhibition of IL-1α, IL-1α receptor antagonism, the inhibition of inflammatory mediators such as 5-lipoxygenase, the inhibition of leukocyte chemotaxis, the antagonism of pro-inflammatory cytokines, the inhibition of the production of reactive oxygen species, the improvement of anti-androgenic effectiveness, the enhanced production of endogenous antimicrobial peptides and possibly the manipulation of Langerhans cell migration and the expression and activity of TNFα, integrin and TLR2. Nanotechnology may facilitate follicular targeting of such treatments.

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