

REVIEW ARTICLE

Acne sans *P. acnes*

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Abstract

Acne vulgaris is a common disease that carries an enormous financial and psychosocial impact. Androgens, excessive sebum production, ductal hypercornification, changes in the microbial flora, as well as inflammation and immunological host reactions are considered the major contributors to acne pathogenesis. Despite extensive research on acne pathogenesis, the exact sequence of events and their possible mechanisms leading to the development of a microcomedone and its transformation into an inflamed lesion has remained unclear.

There is a significant amount of *in vitro* evidence suggesting a possible pathogenetic role for *Propionibacterium acnes* in comedogenesis as well as inflammation in inflammatory acne. However, the microbiological data from non-inflamed as well as inflamed acne lesions, cultured individually, do not entirely support the hypothesis that these micro-organisms are actually responsible for their initiation. There appears to be comedones and inflamed lesions in which there is no clear evidence of *Propionibacterium acnes* involvement. Considering this microbiological data, alongside the *in vitro* evidence, we have tried to delineate the possible sequence of events and their mechanisms, leading to the development of a microcomedone and its transformation into an inflamed lesion. Based on the available literature we have analysed the evidence of both non-inflamed as well as inflamed acne lesions occurring in the absence of *Propionibacterium acnes* from the pilosebaceous follicles. We propose that the development of an inflamed acne lesion depends on an imbalance between the pro-inflammatory and anti-inflammatory pathways rather than the incitement of inflammation by *Propionibacterium acnes*.

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Conflict of interest

None.

Introduction

Acne vulgaris is a multifactorial, pleomorphic skin disease of the pilosebaceous follicles (PSFs) characterized by a variety of non-inflamed (open and closed comedones) and inflamed (macules, papules, pustules and nodules) lesions. Microcomedones (earliest subclinical lesions) are thought to be the precursor lesions that can then develop into non-inflamed and/or inflamed lesions. Although a common disease, the aetiology of acne is not yet fully elucidated and is thought to be a multifactorial process. Androgens, excessive sebum production, hyper-proliferation and abnormal differentiation of the follicular infundibulum, changes in the microbial flora, as well as inflammation and immunological host reactions are considered the major contributors to acne pathogenesis.

The controversial role of *Propionibacterium acnes* in the initiation of non-inflamed and inflamed acne lesions

The role of *Propionibacterium acnes* (*P. acnes*) in acne has long been a very controversial topic. The microbiological data obtained from comedones, cultured individually, suggest that

P. acnes may not be involved in the initiation of these lesions. This data has been recently reviewed by Shaheen and Gonzalez.¹ However, there is a considerable amount of *in vitro* data suggesting a possible pathogenetic role for this micro-organism in comedogenesis. Nevertheless, none of the previous reviews on acne pathogenesis has tried to incorporate the existing microbiological data with the *in vitro* evidence to explain the possible role of this micro-organism in the evolution of non-inflamed acne lesions.^{2–4}

Likewise, the involvement of *P. acnes* in the initiation of inflammation in inflamed lesions is still a matter of debate. Two distinct phenotypes of *P. acnes* (type I and II), corresponding to phylogenetically distinct clusters or lineages, have been identified.⁵ Interestingly, *P. acnes* type I was isolated from the majority of acne lesions in one study, favouring the hypothesis that a specific *P. acnes* phenotype might be more common in acne patients.⁵ Similarly, based on multilocus sequence analysis of various housekeeping and virulence factor genes, Lomholt and Kilian⁶ also identified three phylogenetically different groups of *P. acnes* (I–III). They also found a subdivision of group I (I-1) to be significantly associated with moderate to severe acne. The difference in

the production of various virulence factors was thought to be responsible for this association.⁶ This viewpoint has been recently supported by the finding that strain specific differences in the genome of various *P. acnes* groups do exist.⁷ This along with a wealth of other *in vitro* evidence (discussed later), showing various ways in which *P. acnes* can be involved in inflammation, suggests a possible role for this organism in inflammatory acne. However, even with such compelling data, this by no means proves that *P. acnes* is involved in the initiation of inflamed acne lesions. The question regarding the involvement of *P. acnes* in the initiation of inflammation cannot be answered without looking at the microbiological data obtained from inflamed acne lesions, cultured individually. This data, as reviewed by us recently, showed that *P. acnes* is never isolated from 100% of inflamed lesions and that various investigators also found a proportion of these lesions to be sterile.¹ It is important to understand that although the organism is isolated from the skin surface, its normal habitat is the PSF.⁸ As acne is a disease of the PSFs, inflammation in an inflamed acne lesion cannot be assumed to be initiated by *P. acnes* particularly when the PSF is confirmed to be sterile or not colonized by this micro-organism.

***Propionibacterium acnes* may be a bystander in the development of inflamed acne lesions**

Based on the observation that patients with severe acne, compared with mild acne and normal controls, have higher levels of antibodies to *P. acnes*, it has been proposed that hypersensitivity to *P. acnes* may be responsible for the variation in acne severity.^{9,10} However, in both these studies no statistical analysis was done to ascertain whether or not there was any significant difference in the antibody titres between normal controls, mild/moderate acne and patients with severe acne. Provision of this data could have been more informative in drawing conclusions regarding the role of these antibodies in acne pathogenesis (i.e. whether pathogenic or consequential). In contrast, Ingham *et al.*¹¹ found no significant difference in the antibody titres to *P. acnes* in patients with mild or moderate acne compared with normal controls. They only found patients with severe acne, compared with normal controls, to have significantly higher titres of antibodies to this micro-organism. Furthermore, patients with severe acne do not harbour significantly larger numbers of *P. acnes* than those with milder disease.^{12,13} Thus, it is possible that the increased antibody titres to *P. acnes* in severe acne patients, is due to an increased exposure of these individuals to the immunogen as a result of their disease *per se*. Likewise, cell-mediated immunity to *P. acnes* cannot be solely held responsible for the initiation of inflammation in all acne patients as it has been shown to occur late in the chain of events.¹⁴ Moreover, the improvement seen with the use of antibiotics, such as tetracycline, may partly be explained by their anti-inflammatory effects.¹⁵ Together this evidence suggests that *P. acnes* may be merely a bystander and not an active participant in the development of inflamed acne lesions.

Although a number of different reviews have been published on acne pathogenesis, none of them gave a clear cut sequence of events and mechanisms leading to the development of a non-inflamed lesion followed by its progression to an inflamed one.²⁻⁴ The aim of this review is to propose a possible stepwise mechanism to explain this process. We have also tried to incorporate the *in vitro* evidence, regarding the pathogenic potential of *P. acnes*, to explain its possible role in the evolution of non-inflamed as well as inflamed acne lesion. Moreover, in line with the microbiological data, we have retained the viewpoint that both comedogenesis and inflammation may occur in the absence of *P. acnes*.

Comedogenesis

Comedones result from abnormal proliferation and differentiation of ductal keratinocytes.¹⁶ Hyper-proliferation has been confirmed by demonstration of an increase in the Ki-67 labelling of ductal keratinocytes.¹⁷ This fact is further substantiated by the presence of keratins 6 and 16 (keratin markers of hyper-proliferation) in comedones.¹⁸

Androgens, PPARs and seborrhoea

The role of androgens in acne vulgaris and the beneficial effect of anti-androgen therapy are well established. Acne development begins at the time of adrenarche when the adrenal glands start producing dehydroepiandrosterone sulphate (a precursor for testosterone).^{19,20} Androgen-insensitive subjects do not produce sebum and do not develop acne.²¹ Acne patients have also been found to have a higher density of androgen receptors²² and increased activity of type I 5 α -reductase enzyme²³ which might support the hypothesis of end-organ sensitivity in these patients. In addition, anti-androgen therapy reduces sebum excretion rate (SER) and improves acne.²⁴

The risk of acne vulgaris in relatives of patients with acne, as compared with controls, is significantly higher suggesting hereditary influences.²⁵ Interestingly, evidence of direct genetic association of acne with androgen abnormalities has been observed. CYP17-34C/C homozygote Chinese men have been found to be at a significantly increased risk of developing severe acne.²⁶ This gene encodes cytochrome P450c17 α which is one of the key enzymes in androgen biosynthesis. Moreover, neonatal acne has been found to be associated with familial hyperandrogenism.²⁷ It seems likely that acne is a polygenic disorder and that different genes, accounting for either increased concentration and/or sensitivity to androgens, may be responsible for the development of acne in an individual.

As mentioned earlier, acne patients have increased end-organ sensitivity to circulating androgens. Although androgens have a proliferative effect on cultured human²⁸ and SZ95 sebocytes,²⁹ they have only a minimal effect on SZ95 sebocyte differentiation.³⁰ PPAR ligands, on the other hand, have been shown to be the master regulators of lipid metabolism.^{30,31} In addition to the androgen receptors, PPARs are abundantly present in human sebaceous

glands.³² Taken together, these findings may explain the increase in sebaceous gland size with associated seborrhoea seen in acne patients at puberty.

Acne patients are also known to have a low sebaceous linoleic acid (LA, C₁₈Δ^{9,12}) level,³³ which returns to normal with a concomitant decrease in SER, after treatment with anti-androgens.³⁴ These results indicate that the proportion of LA in sebum is influenced by SER. A low concentration of linoleate in sebum has been proposed to cause follicular hyperkeratosis and decreased barrier function.³⁵ It is, therefore, understandable that an increased sensitivity to androgens, in acne patients, may lead to a low sebaceous linoleate concentration as a consequence of increased SER, resulting in comedogenesis. Indeed, the severity of acne has been found to be related to the rate of sebum excretion.³⁶ Adding more weight to the LA theory is the fact that topical LA has been shown to cause a significant reduction in the size of follicular casts and microcomedones in acne patients.³⁷

In addition, androgens have been shown to significantly stimulate the proliferation of keratinocytes co-cultured with beard dermal papilla cells via the production of insulin-like growth factor-1 (IGF-1) by the dermal papilla cells.³⁸ Thus, it is possible that androgens may also influence epithelial turnover in the follicular infundibulum via the production of IGF-1, which acts as a paracrine growth factor. Interestingly, serum IGF-1 levels have been found to be positively correlated with the number of comedones in females with clinical acne.³⁹ IGF-1 has also been shown to stimulate lipid production in human SEB-1 sebocytes by an increased expression of sterol response element-binding protein-1, a transcription factor that regulates numerous genes involved in lipid biosynthesis.⁴⁰ Thus androgens can cause seborrhoea in acne patients not only by their direct proliferative action on the sebocytes but also via IGF-1, which stimulates lipogenesis in the sebaceous glands.

Interleukin-1 α

Results from essential fatty acid deficient mice have shown mRNA levels for epidermal IL-1 α to be elevated several-fold over controls.⁴¹ Disruption of the skin permeability barrier and the body's attempt to repair it was postulated to be responsible for this increase. Elevated IL-1 α has been reported in comedones in acne patients.⁴² Furthermore, IL-1 α has been demonstrated to cause hypercornification of the follicular infundibulum, which can be blocked by IL-1 receptor antagonists.⁴³ Therefore, we propose that low sebaceous LA levels (an essential fatty acid), in acne patients, may cause disruption of the cutaneous permeability barrier of the follicular infundibulum. This may lead to increased epidermal IL-1 α production, resulting in comedogenesis.

In summary, it is highly probable that androgens may play an important role in comedogenesis. They may not only stimulate keratinocyte proliferation but may also lead to seborrhoea by their direct and indirect action (via IGF-1) on the sebocytes. These two androgen mediated effects may, therefore, explain the development

of comedones in acne patients. Our proposed mechanism may also explain the microbiological data which suggests that comedogenesis can occur in the complete absence of *P. acnes* from the PSFs.¹

Propionibacterium acnes colonization of comedones

Keeping in mind the above-mentioned hypothesis regarding the role of androgens in the initiation of comedogenesis, the fact that *P. acnes* has been found to colonize significantly more comedones compared with unaffected follicles^{44,45} can be explained as follows:

Follicular micro-environment

The micro-environment of individual PSFs, whether normal or acne affected, is thought to be important for colonization by micro-organisms.^{46,47} Follicular pH, water availability and oxygen as well as carbon dioxide tension are some of the possible parameters that might differ from one PSF to another and may determine microbial colonization.⁴⁶

Innate immunity

More recently, innate immunity has received a lot of attention because of its potential role in protecting surface epithelia from microbial colonization and invasion. Apart from secreting lipid-rich sebum onto the skin surface, sebocytes can also provide an innate immune defensive function. This has been supported by the observation that various antimicrobial peptides (e.g. cathelicidin, Psoriasin, human β defensin (hBD)-1 and 2 and histone H4) are produced by these cells.⁴⁸⁻⁵¹ These antimicrobial peptides have been found to have antimicrobial activity against *P. acnes*, *in vitro*.^{48,51,52}

It is prudent to mention herein that the actual concentration of these antimicrobial peptides released by human sebaceous glands remains unknown.⁵¹ It is possible that the concentration of these antimicrobial peptides having an antimicrobial activity *in vitro*, is not achievable *in vivo*. However, these antimicrobial peptides demonstrate additive or synergistic functions when combined.^{48,53} It is therefore possible that the total antimicrobial activity in sebocytes is due to all antimicrobial peptides acting together.

Although antimicrobial peptides are extensively studied, other protection systems also exist. Free fatty acids (FFAs) are ubiquitous on the human skin surface, possessing intrinsic antimicrobial activity predominantly against gram-positive bacteria.^{54,55} Moreover, they can further strengthen the cutaneous innate immunity by upregulation of hBD-2 by sebocytes.⁵² Lastly, synergistic antimicrobial activity of hBD-2 and lauric acid (C12:0) has been demonstrated against *P. acnes*, *in vitro*.⁵²

When all these findings are considered together we can hypothesize that in addition to the follicular micro-environment the synergistic antimicrobial activity of the various antimicrobial components secreted from sebocytes, may also determine colonization of the normal and acne affected PSFs. This innate defence mechanism may be defective in acne patients and, along with a

favourable follicular micro-environment, may be responsible for the significantly greater colonization of comedones (by *P. acnes*) compared with the unaffected follicles.^{44,45} Changes in the skin surface FFAs can be postulated as one of the possible mechanisms for such a defect. It is, therefore, possible that a lower LA level in acne patients may be one of the factors responsible for a defective innate immune mechanism in these patients.³³ However, this matter is still unresolved and more research is needed to test this hypothesis.

Potential of comedogenesis after *Propionibacterium acnes* colonization

After colonization, *P. acnes* can potentiate comedogenesis by various mechanisms (Fig. 1). It is known that *P. acnes* produces lipases which hydrolyse triglycerides, thereby releasing FFAs. These FFAs have been found to be comedogenic in the rabbit ear model.⁵⁶ Oxidized squalene is another substance that has been found to be comedogenic in the rabbit ear model.⁵⁷ *P. acnes*, through its production of porphyrins, may act as a catalytic agent in the oxidation of squalene.⁵⁸ This, along with the fact that keratinocytes stimulated by *P. acnes* have been shown to produce significantly more IL-1 α compared with unstimulated keratinocytes, might signify other potential pathways through which *P. acnes* may be involved in comedogenesis.⁵⁹

Compared with normal controls, aberrant $\alpha 6$ integrin expressions have been demonstrated around clinically normal follicles and early inflamed lesions of acne patients.⁶⁰ Integrins are postulated to be important in the proliferation and differentiation of keratinocytes.⁶¹ *P. acnes* has been shown to induce the expression

of $\beta 1$, $\alpha 3$, $\alpha 6$, $\alpha V\beta 6$ integrins and filaggrin on epidermal cells, *in vitro*.⁶² This may be another possible mechanism by which *P. acnes* may aggravate comedogenesis.

Lastly, *P. acnes* has been shown to increase the production of IGF-1 by keratinocytes.⁶³ This can then activate IGF-1R, mostly located in the basal layer of the epidermis and also induced by *P. acnes*, leading to proliferation of the keratinocytes and an increase in filaggrin expression through a paracrine pathway.⁶³ This can potentially be another mechanism by which *P. acnes* can potentiate comedogenesis.

Inflammation

Sub-clinical inflammation

The next question which needs to be answered is exactly what initiates inflammation, if not *P. acnes*? Compared with the control follicles obtained from non-acne patients, clinically normal skin of acne patients has been shown to have increased numbers of T cells and macrophages in the perifollicular and papillary dermis.⁶⁰ Furthermore, expression of E-selectin and vascular adhesion molecule-1 was also found to be upregulated, with the dermal concentration of IL-1 α reported to be three times higher in this clinically normal skin.⁶⁰ All these data suggest that sub-clinical inflammation exists in acne prone areas, even if it appears clinically normal, in people suffering with acne.

Many cells, including keratinocytes and fibroblasts, function as sources of IL-1.⁶⁴ In Ser252Trp-FGFR2 mutated osteoblasts (mesenchymally derived cells) in patients with Apert syndrome, an

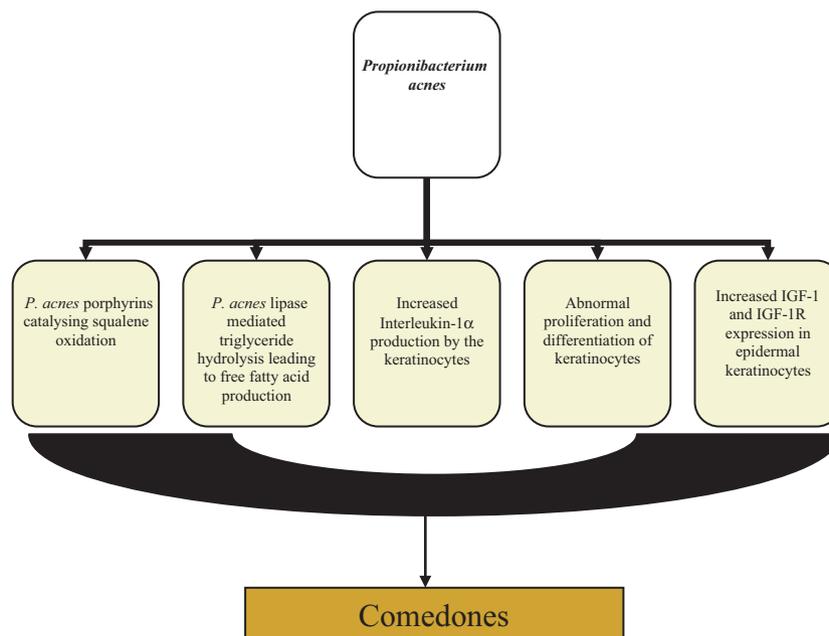


Figure 1 Possible mechanisms for the involvement of *Propionibacterium acnes* (*P. acnes*) in comedogenesis. IGF-1, Insulin-like growth factor-1; IGF-1R, Insulin-like growth factor-1 receptor.

increase in the expression of IL-1 α has been reported.⁶⁵ Androgens are thought to be involved in FGF2 production (by dermal fibroblasts) which then binds mesenchymal isoform FGFR2c receptors.⁶⁶ It is possible that increased androgen sensitivity in acne patients may lead to increased FGF2 production by dermal fibroblasts. FGF2 produced as a result of this stimulation may lead to increased IL-1 α production by these fibroblasts via an autocrine effect. This may explain the higher dermal concentration of IL-1 α , noted in the clinically normal skin of acne patients.⁶⁰

The IL-1 α is a pro-inflammatory cytokine which can upregulate the expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1.⁶⁷ Therefore, it is possible that the increased expression of IL-1 α , both in the dermis as well as epidermis (mechanism explained under comedogenesis), will not only contribute to comedogenesis but may also result in the initiation of non-specific sub-clinical inflammation, demonstrated in the clinically normal skin of acne patients.⁶⁰ More recently, upregulation of IL-6 and TNF- α in cultured human sebocytes after addition of Dihydrotestosterone has also been demonstrated.⁶⁸ These pro-inflammatory cytokines may also play a role in the sub-clinical inflammation mentioned above.

Clinical inflammation

The transition from sub-clinical to clinical inflammation might depend on an imbalance between pro-inflammatory and anti-inflammatory pathways that are activated as a result of this local stress.

Activation of the cutaneous equivalent of the central hypothalamic-pituitary-adrenal axis The hypothalamic-pituitary-adrenal (HPA) axis plays a crucial role in terminating the stress response and buffering tissue damage, in response to any systemic stress.⁶⁹ This process involves production and release of corticotropin-releasing hormone (CRH) followed by production and secretion of proopiomelanocortin (POMC) derived peptides (adrenocorticotrophic hormone, ACTH and α -melanocyte stimulating hormone, α -MSH). ACTH induces production and secretion of the powerful anti-inflammatory protein cortisol, which terminates the stress response and buffers tissue damage. The presence of corticotropin-releasing hormone (CRH), its binding protein (CRHBP) and corticotropin-releasing hormone receptor type 1 (CRHR-1) and 2 (CRHR-2) has been confirmed in human sebaceous glands, *in vivo*, suggesting that a complete CRH system exists in sebocytes.⁷⁰ Moreover, a functional CRH- proopiomelanocortin (POMC)-corticosteroid axis organized similarly to the HPA axis has been demonstrated in epidermal melanocytes,⁷¹ dermal fibroblasts⁷² and human hair follicles.⁷³

The CRH expression in the hypothalamic cells can be modulated by various pro-inflammatory cytokines (Tumour necrosis factor- α (TNF- α), IL-1 β and IL-6).⁷⁴ These cytokines may also modulate its expression in the skin. Thus, the sub-clinical inflam-

mation evident in the clinically normal skin of acne patients may lead to increased cutaneous production of CRH. Indeed, CRH expression has been found to be greatly increased in acne involved skin compared with non-involved and normal skin.⁷⁰ As α -MSH peptides, produced locally, as a result of this CRH stimulation of the cutaneous CRH-POMC-corticosteroid axis also modulate the expression of its receptor (MC-1R),⁷³ this may explain the increased expression of MC-1R in the sebaceous glands of lesional skin of patients with acne vulgaris.⁷⁵

Although CRH can induce cutaneous inflammation by causing mast cell degranulation,⁷⁶ it might also act as an anti-inflammatory agent by increasing the local production of steroids. Likewise, α -MSH has also been shown to exert anti-inflammatory actions by inhibition of IL-1 mediated IL-8 secretion from SZ95 sebocytes⁷⁷ and by suppressing TNF- α and IL-1 β gene expression following an ischaemic cerebral event in mice.⁷⁸ Furthermore, it has also been demonstrated to induce the production of IL-10 by human peripheral blood monocytes *in vitro*.⁷⁹ IL-10 is a regulatory cytokine that acts to harness the release of several pro-inflammatory cytokines.⁸⁰

In brief, activation of the cutaneous CRH-POMC-corticosteroid axis may activate both pro-inflammatory and anti-inflammatory pathways that when working in conjunction with other pro-inflammatory pathways (discussed below) may determine the development of a clinically inflamed lesion.

Substance P and human β -defensins Substance P (SP), another important pro-inflammatory neuropeptide,⁸¹ has also been found to be over expressed in dermal nerves around the sebaceous glands of acne patients.⁸² Recently, IL-1 α was found to upregulate SP expression in the dorsal root neurons from mature rats.⁸³ This may be another pro-inflammatory pathway that is activated as a result of sub-clinical inflammation seen in the clinically normal skin of acne patients.

Further, antimicrobial peptides hBD-1 and hBD-2 have been found to be upregulated in lesional skin from acne patients.⁵⁰ hBD-2 does not only possess antimicrobial activities but also acts as a chemoattractant for mast cells⁸⁴ and induces histamine release and prostaglandin D2 production from these cells as well.⁸⁵ Moreover, human β -defensins are also chemotactic for immature dendritic cells and memory T cells.⁸⁶ Pro-inflammatory cytokines e.g. TNF- α can modulate hBD-2 expression and, therefore, may explain its upregulation in acne lesions.⁸⁷

It is evident that various pro-inflammatory and anti-inflammatory pathways are activated as a result of sub-clinical inflammation, seen in the clinically normal skin of acne patients. We hypothesize that an imbalance between these pathways may lead to the development of clinical inflammation in inflammatory acne (Fig. 2). Interestingly, an *in vitro* study has shown that acne patients produce significantly less IL-10 from monocytes, in response to *P. acnes* stimulation, as compared with healthy controls.⁸⁸ It is possible that the production of IL-10 by monocytes, in response to α -MSH, in acne patients may also be impaired: this

may be one of the anti-inflammatory pathways that can be defective in these patients.

Our proposed mechanism signifies that the presence of *P. acnes* in PSFs is not necessary for the development of clinical inflammation in acne patients. This may therefore explain the sterility or absence of *P. acnes* from a significant proportion of inflamed acne lesions as reported by various investigators.¹

Potential of inflammation by *Propionibacterium acnes* and other intrafollicular contents

As explained above, clinical inflammation in an acne lesion may develop in the complete absence of *P. acnes* from the PSFs. How-

ever, micro-organisms (from colonized follicles) and other intra-follicular contents may further intensify this inflammation.

Propionibacterium acnes and/or its products, after being released into the dermis, may intensify the inflammatory process by its antigenic,^{9,11,89} enzymatic,⁹⁰⁻⁹² complement activation^{93,94} and chemoattractant activities^{95,96} (Fig. 3). Moreover, *P. acnes* can also lead to the production of pro-inflammatory cytokines/chemokines by keratinocytes^{59,97} (IL-1 α , TNF- α , granulocyte/macrophage colony-stimulating factor and IL-8), sebocytes⁹⁸ (CXCL8, synonymous with IL-8) and peripheral blood mononuclear cells⁹⁹ (IL-1 β , TNF- α , and IL-8). Lastly, *P. acnes* can also enhance expression of antimicrobial peptide hBD-2 in sebocytes and keratinocytes.^{97,98} It

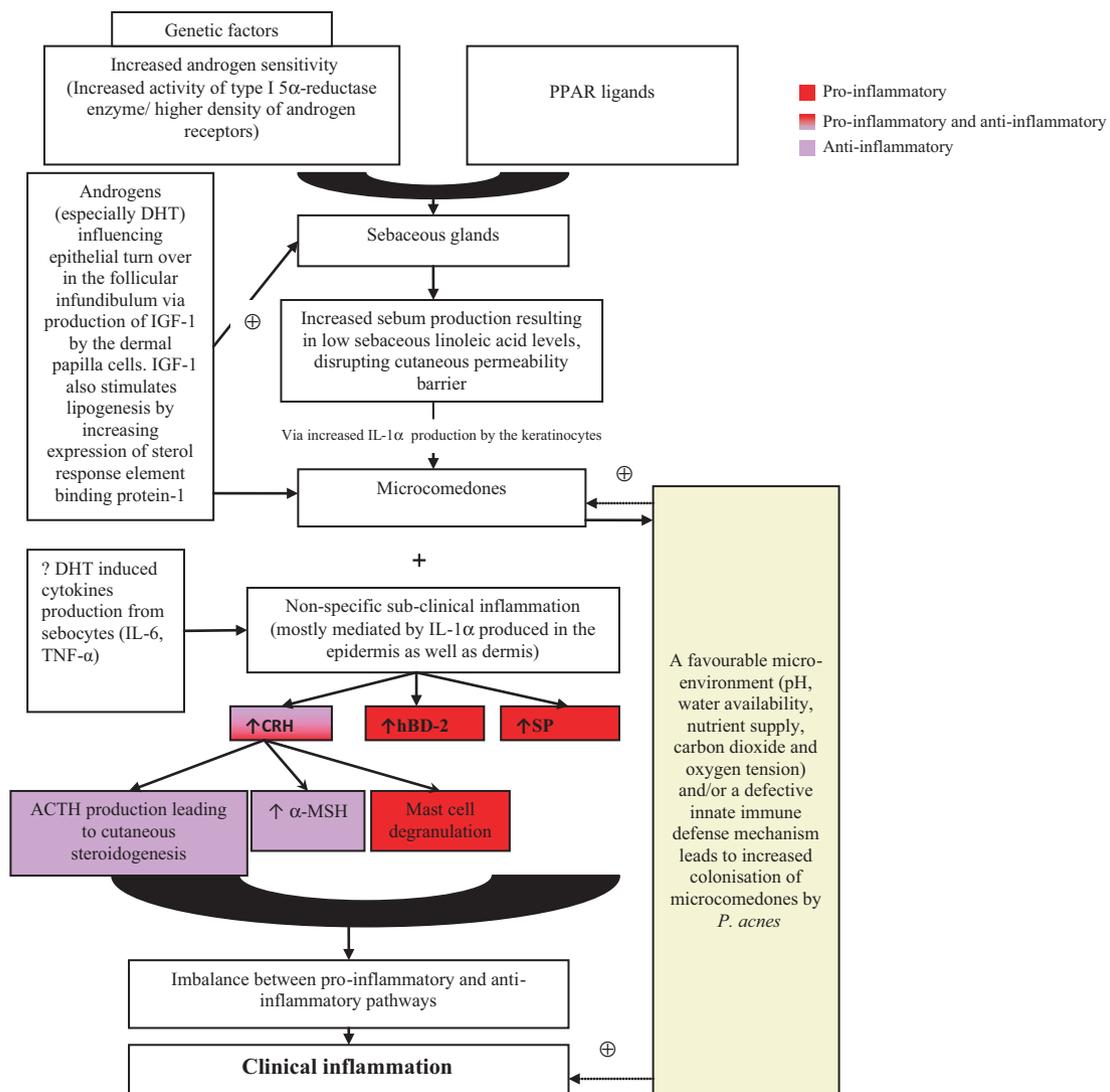


Figure 2 Proposed mechanism for the aetiopathogenesis of acne vulgaris and its association with *Propionibacterium acnes* (*P. acnes*). DHT, Dihydrotestosterone; IL, Interleukin; CRH, Corticotropin-releasing hormone; MSH, Melanocyte stimulating hormone; ACTH, Adrenocorticotropic hormone; hBD-2, Human beta defensin-2; SP, Substance P; TNF, Tumour necrosis factor; IGF-1, Insulin-like growth factor-1.

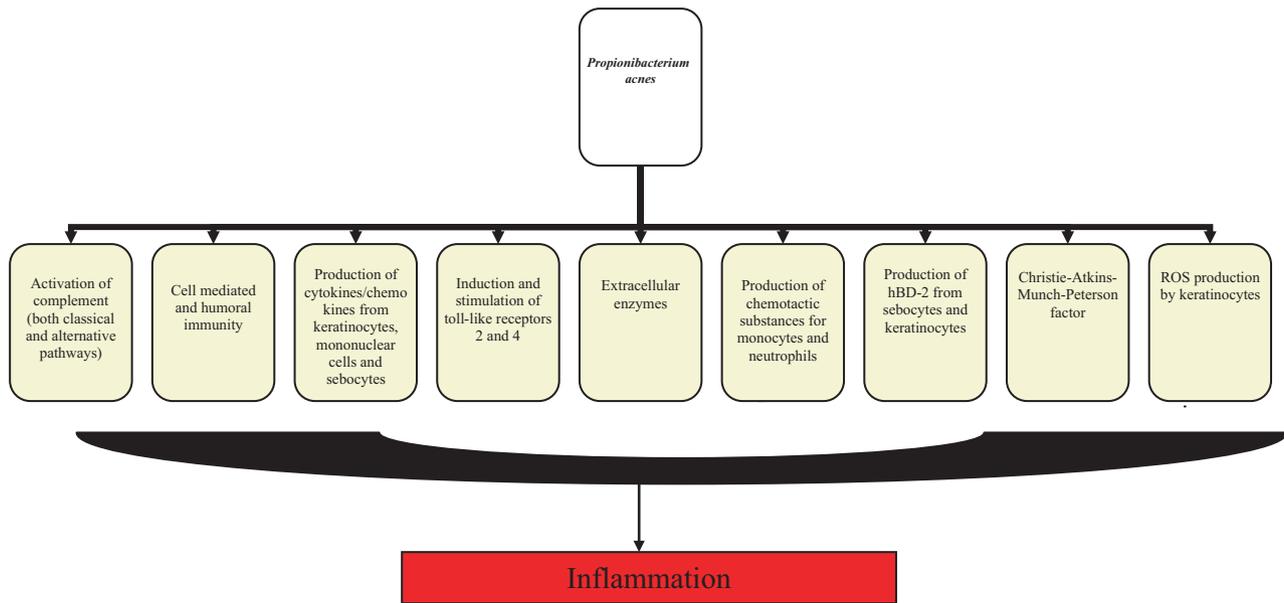


Figure 3 Possible mechanisms for *Propionibacterium acnes* (*P. acnes*) involvement in the intensification of the inflammatory response in acne. hBD-2, Human β defensin-2; ROS, Reactive oxygen species.

is obvious that hBD-2 (an antimicrobial peptide containing pro-inflammatory properties) and all the other pro-inflammatory cytokines/chemokines mentioned above, may also intensify inflammation in acne patients.

Propionibacterium acnes secretory protein called Christie-Atkins-Munch-Peterson factor and acid sphingomyelinase, which is released from the host cells in the presence of *P. acnes*, are cytotoxic to keratinocytes and macrophages, *in vitro*.¹⁰⁰ Furthermore, *P. acnes* can lead to the formation of reactive oxygen species, especially superoxide anions, by keratinocytes.¹⁰¹ These may be other potential mechanisms explaining the involvement of *P. acnes* in inflammatory acne. Last but not least, *P. acnes* can also exaggerate inflammation in acne by the induction¹⁰² and activation⁹⁷ of toll-like receptors 2 and 4. After rupture of the duct it is likely that other intrafollicular contents like FFAs, keratin and hairs may also contribute to the inflammation.

Conclusion

We conclude that androgens, sebaceous lipid abnormalities and key cytokines such as IL-1 α may play an important role in the initiation of comedogenesis. Although acknowledging the significant supportive evidence on the role of *P. acnes* in acne pathogenesis, we propose that *P. acnes* may not be central to the initiation of inflammation in inflamed acne lesions. Rather, an imbalance between the pro-inflammatory (CRH by causing mast cell degranulation, SP and hBD-2) and anti-inflammatory pathways (steriodogenesis and α -MSH production as a result of cutaneous CRH-POMC-corticosteroid axis activation) may be more important in this context. This hypothesis may be tested by comparing

the pro-inflammatory and anti-inflammatory effects of these different pathways in patients with either predominantly comedonal or inflammatory acne.

There is no doubt that the *in vitro* evidence for the role of *P. acnes* in acne pathogenesis is compelling. However, it cannot be taken as a proof of its involvement in the initiation of non-inflamed and inflamed acne lesions. We have attempted to take a balanced view of most of the available evidence, including the microbiological data, and shown that the central role of *P. acnes* in the initiation of acne lesions is not yet irrefutable. If *P. acnes* is not isolated from a significant number of acne-affected PSFs (whether inflamed or non-inflamed) the organism cannot be held responsible for the initiation of pathological changes in these lesions. The story on the role of *P. acnes* in acne pathogenesis must therefore be viewed as still evolving.

References

- 1 Shaheen B, Gonzalez M. A microbial aetiology of acne: what is the evidence? *Br J Dermatol* 2011; **165**: 474–485.
- 2 Kurokawa I, Danby F, Ju Q *et al*. New developments in our understanding of acne pathogenesis and treatment. *Exp Dermatol* 2009; **18**: 821–832.
- 3 Bhambrri S, Del Rosso JQ, Bhambrri A. Pathogenesis of acne vulgaris: recent advances. *J Drugs Dermatol* 2009; **8**: 615–618.
- 4 Lee DJ, Van Dyke GS, Kim J. Update on pathogenesis and treatment of acne. *Curr Opin Pediatr* 2003; **15**: 405–410.
- 5 McDowell A, Valanne S, Ramage G *et al*. *Propionibacterium acnes* types I and II represent phylogenetically distinct groups. *J Clin Microbiol* 2005; **43**: 326–334.
- 6 Lomholt HB, Kilian M. Population genetic analysis of *Propionibacterium acnes* identifies a subpopulation and epidemic clones associated with acne. *PLoS ONE* 2010; **5**: e12277.

- 7 Brzuszkiewicz E, Weiner J, Wollherr A *et al*. Comparative genomics and transcriptomics of *Propionibacterium acnes*. *PLoS ONE* 2011; **6**: e21581.
- 8 Leyden J, McGinley K, Vowels B. *Propionibacterium acnes* colonization in acne and nonacne. *Dermatology* 1998; **196**: 55–58.
- 9 Puhvel S, Barfatani M, Warnick M, Sternberg T. Study of antibody levels to corynebacterium acnes. In the serum of patients with acne vulgaris, using bacterial agglutination, agar gel immunodiffusion, and immunofluorescence techniques. *Arch Dermatol* 1964; **90**: 421–427.
- 10 Basal E, Jain A, Kaushal GP. Antibody response to crude cell lysate of propionibacterium acnes and induction of pro-inflammatory cytokines in patients with acne and normal healthy subjects. *J Microbiol* 2004; **42**: 117–125.
- 11 Ingham E, Gowland G, Ward R, Holland K, Cunliffe W. Antibodies to *P. acnes* and *P. acnes* exocellular enzymes in the normal population at various ages and in patients with acne vulgaris. *Br J Dermatol* 1987; **116**: 805–812.
- 12 Cove J, Cunliffe W, Holland K. Acne vulgaris: is the bacterial population size significant? *Br J Dermatol* 1980; **102**: 277–280.
- 13 Leyden J, McGinley K, Mills O, Kligman A. Propionibacterium levels in patients with and without acne vulgaris. *J Invest Dermatol* 1975; **65**: 382–384.
- 14 Gowland G, Ward R, Holland K, Cunliffe W. Cellular immunity to *P. acnes* in the normal population and patients with acne vulgaris. *Br J Dermatol* 1978; **99**: 43–47.
- 15 Webster G, Graber E. Antibiotic treatment for acne vulgaris. *Semin Cutan Med Surg* 2008; **27**: 183–187.
- 16 Cunliffe W, Holland D, Jeremy A. Comedone formation: etiology, clinical presentation, and treatment. *Clin Dermatol* 2004; **22**: 367–374.
- 17 Knaggs H, Holland D, Morris C, Wood E, Cunliffe W. Quantification of cellular proliferation in acne using the monoclonal antibody Ki-67. *J Invest Dermatol* 1994; **102**: 89–92.
- 18 Hughes B, Morris C, Cunliffe W, Leigh I. Keratin expression in pilosebaceous epithelia in truncal skin of acne patients. *Br J Dermatol* 1996; **134**: 247–256.
- 19 Lucky A, Biro F, Huster G, Leach A, Morrison J, Ratterman J. Acne vulgaris in premenarchal girls. An early sign of puberty associated with rising levels of dehydroepiandrosterone. *Arch Dermatol* 1994; **130**(3): 308–314.
- 20 Stewart M, Downing D, Cook J, Hansen J, Strauss J. Sebaceous gland activity and serum dehydroepiandrosterone sulfate levels in boys and girls. *Arch Dermatol* 1992; **128**: 1345–1348.
- 21 Imperato-McGinley J, Gautier T, Cai LQ, Yee B, Epstein J, Pochi P. The androgen control of sebum production. Studies of subjects with dihydrotestosterone deficiency and complete androgen insensitivity. *J Clin Endocrinol Metab* 1993; **76**: 524–528.
- 22 Schmidt J, Spona J, Huber J. Androgen receptor in hirsutism and acne. *Gynecol Obstet Invest* 1986; **22**: 206–211.
- 23 Thiboutot D, Knaggs H, Gilliland K, Lin G. Activity of 5-alpha-reductase and 17-beta-hydroxysteroid dehydrogenase in the infrainfundibulum of subjects with and without acne vulgaris. *Dermatology* 1998; **196**: 38–42.
- 24 Miller J, Wojnarowska F, Dowd P *et al*. Anti-androgen treatment in women with acne: a controlled trial. *Br J Dermatol* 1986; **114**: 705–716.
- 25 Goulden V, McGeown C, Cunliffe W. The familial risk of adult acne: a comparison between first-degree relatives of affected and unaffected individuals. *Br J Dermatol* 1999; **141**: 297–300.
- 26 He L, Yang Z, Yu H *et al*. The relationship between CYP17 -34T/C polymorphism and acne in Chinese subjects revealed by sequencing. *Dermatology* 2006; **212**: 338–342.
- 27 Bekaert C, Song M, Delvigne A. Acne neonatorum and familial hyperandrogenism. *Dermatology* 1998; **196**: 453–454.
- 28 Akamatsu H, Zouboulis CC, Orfanos CE. Control of human sebocyte proliferation *in vitro* by testosterone and 5-alpha-dihydrotestosterone is dependent on the localization of the sebaceous glands. *J Invest Dermatol* 1992; **99**: 509–511.
- 29 Zouboulis CC, Seltmann H, Neitzel H, Orfanos CE. Establishment and characterization of an immortalized human sebaceous gland cell line (SZ95). *J Invest Dermatol* 1999; **113**: 1011–1020.
- 30 Chen W, Yang CC, Sheu HM, Seltmann H, Zouboulis CC. Expression of peroxisome proliferator-activated receptor and CCAAT/enhancer binding protein transcription factors in cultured human sebocytes. *J Invest Dermatol* 2003; **121**: 441–447.
- 31 Rosenfield RL, Kentsis A, Deplewski D, Ciletti N. Rat prepubertal sebocyte differentiation involves peroxisome proliferator-activated receptors. *J Invest Dermatol* 1999; **112**: 226–232.
- 32 Trivedi NR, Cong Z, Nelson AM *et al*. Peroxisome proliferator-activated receptors increase human sebum production. *J Invest Dermatol* 2006; **126**: 2002–2009.
- 33 Morello AM, Downing DT, Strauss JS. Octadecadienoic acids in the skin surface lipids of acne patients and normal subjects. *J Invest Dermatol* 1976; **66**: 319–323.
- 34 Stewart M, Greenwood R, Cunliffe W, Strauss J, Downing D. Effect of cyproterone acetate-ethinyl estradiol treatment on the proportions of linoleic and sebaleic acids in various skin surface lipid classes. *Arch Dermatol Res* 1986; **278**: 481–485.
- 35 Downing D, Stewart M, Wertz P, Strauss J. Essential fatty acids and acne. *J Am Acad Dermatol* 1986; **14**: 221–225.
- 36 Cunliffe W, Shuster S. Pathogenesis of acne. *Lancet* 1969; **1**: 685–687.
- 37 Letawe C, Boone M, Piérard GE. Digital image analysis of the effect of topically applied linoleic acid on acne microcomedones. *Clin Exp Dermatol* 1998; **23**: 56–58.
- 38 Itami S, Inui S. Role of androgen in mesenchymal epithelial interactions in human hair follicle. *J Invest Dermatol Symp Proc* 2005; **10**: 209–211.
- 39 Cappel M, Mauger D, Thiboutot D. Correlation between serum levels of insulin-like growth factor 1, dehydroepiandrosterone sulfate, and dihydrotestosterone and acne lesion counts in adult women. *Arch Dermatol* 2005; **141**: 333–338.
- 40 Smith TM, Cong Z, Gilliland KL, Clawson GA, Thiboutot DM. Insulin-like growth factor-1 induces lipid production in human SEB-1 sebocytes via sterol response element-binding protein-1. *J Invest Dermatol* 2006; **126**: 1226–1232.
- 41 Wood L, Jackson S, Elias P, Grunfeld C, Feingold K. Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice. *J Clin Invest* 1992; **90**: 482–487.
- 42 Ingham E, Eady E, Goodwin C, Cove J, Cunliffe W. Pro-inflammatory levels of interleukin-1 alpha-like bioactivity are present in the majority of open comedones in acne vulgaris. *J Invest Dermatol* 1992; **98**: 895–901.
- 43 Guy R, Green M, Kealey T. Modeling acne *in vitro*. *J Invest Dermatol* 1996; **106**: 176–182.
- 44 Leeming J, Holland K, Cunliffe W. The microbial ecology of pilosebaceous units isolated from human skin. *J Gen Microbiol* 1984; **130**: 803–807.
- 45 Leeming J, Holland K, Cunliffe W. The pathological and ecological significance of microorganisms colonizing acne vulgaris comedones. *J Med Microbiol* 1985; **20**: 11–16.
- 46 Holland K, Cunliffe W, Roberts C. The role of bacteria in acne vulgaris: a new approach. *Clin Exp Dermatol* 1978; **3**: 253–257.
- 47 Greenman J, Holland K, Cunliffe W. Effects of pH on biomass, maximum specific growth rate and extracellular enzyme production by three species of cutaneous propionibacteria grown in continuous culture. *J Gen Microbiol* 1983; **129**: 1301–1307.
- 48 Lee DY, Yamasaki K, Rudsil J *et al*. Sebocytes express functional cathelicidin antimicrobial peptides and can act to kill propionibacterium acnes. *J Invest Dermatol* 2008; **128**: 1863–1866.
- 49 Gläser R, Harder J, Lange H, Bartels J, Christophers E, Schröder JM. Antimicrobial psoriasin (S100A7) protects human skin from *Escherichia coli* infection. *Nat Immunol* 2005; **6**: 57–64.

- 50 Chronnell CM, Ghali LR, Ali RS *et al.* Human beta defensin-1 and -2 expression in human pilosebaceous units: upregulation in acne vulgaris lesions. *J Invest Dermatol* 2001; **117**: 1120–1125.
- 51 Lee DY, Huang CM, Nakatsuji T *et al.* Histone H4 is a major component of the antimicrobial action of human sebocytes. *J Invest Dermatol* 2009; **129**: 2489–2496.
- 52 Nakatsuji T, Kao MC, Zhang L, Zouboulis CC, Gallo RL, Huang CM. Sebum free fatty acids enhance the innate immune defense of human sebocytes by upregulating beta-defensin-2 expression. *J Invest Dermatol* 2010; **130**: 985–994.
- 53 Nagaoka I, Hirota S, Yomogida S, Ohwada A, Hirata M. Synergistic actions of antibacterial neutrophil defensins and cathelicidins. *Inflamm Res* 2000; **49**: 73–79.
- 54 Wille JJ, Kydonieus A. Palmitoleic acid isomer (C16:1delta6) in human skin sebum is effective against gram-positive bacteria. *Skin Pharmacol Appl Skin Physiol* 2003; **16**: 176–187.
- 55 Georgel P, Crozat K, Lauth X *et al.* A toll-like receptor 2-responsive lipid effector pathway protects mammals against skin infections with gram-positive bacteria. *Infect Immun* 2005; **73**: 4512–4521.
- 56 Kligman A, Wheatley V, Mills O. Comedogenicity of human sebum. *Arch Dermatol* 1970; **102**: 267–275.
- 57 Motoyoshi K. Enhanced comedo formation in rabbit ear skin by squalene and oleic acid peroxides. *Br J Dermatol* 1983; **109**: 191–198.
- 58 Saint-Leger D, Bague A, Cohen E, Chivot M. A possible role for squalene in the pathogenesis of acne. I. *In vitro* study of squalene oxidation. *Br J Dermatol* 1986; **114**: 535–542.
- 59 Graham G, Farrar M, Cruse-Sawyer J, Holland K, Ingham E. Proinflammatory cytokine production by human keratinocytes stimulated with *Propionibacterium acnes* and *P. acnes* GroEL. *Br J Dermatol* 2004; **150**: 421–428.
- 60 Jeremy A, Holland D, Roberts S, Thomson K, Cunliffe W. Inflammatory events are involved in acne lesion initiation. *J Invest Dermatol* 2003; **121**: 20–27.
- 61 Watt F. Role of integrins in regulating epidermal adhesion, growth and differentiation. *EMBO J* 2002; **21**: 3919–3926.
- 62 Jarrousse V, Castex-Rizzi N, Khammari A, Charveron M, Dréno B. Modulation of integrins and filaggrin expression by *Propionibacterium acnes* extracts on keratinocytes. *Arch Dermatol Res* 2007; **299**: 441–447.
- 63 Isard O, Knol AC, Ariès MF *et al.* *Propionibacterium acnes* activates the IGF-1/IGF-1R system in the epidermis and induces keratinocyte proliferation. *J Invest Dermatol* 2011; **131**: 59–66.
- 64 Luger T, Schwarz T. Evidence for an epidermal cytokine network. *J Invest Dermatol* 1990; **95**: 100S–104S.
- 65 Lomri A, Lemonnier J, Delannoy P, Marie P. Increased expression of protein kinase Calpha, interleukin-1alpha, and RhoA guanosine 5'-triphosphatase in osteoblasts expressing the Ser252Trp fibroblast growth factor 2 receptor Apert mutation: identification by analysis of complementary DNA microarray. *J Bone Miner Res* 2001; **16**: 705–712.
- 66 Melnik B, Schmitz G. FGFR2 signaling and the pathogenesis of acne. *J Dtsch Dermatol Ges* 2008; **6**: 721–728.
- 67 Groves R, Ross E, Barker J, Ross J, Camp R, MacDonald D. Effect of *in vivo* interleukin-1 on adhesion molecule expression in normal human skin. *J Invest Dermatol* 1992; **98**: 384–387.
- 68 Lee WJ, Jung HD, Chi SG *et al.* Effect of dihydrotestosterone on the upregulation of inflammatory cytokines in cultured sebocytes. *Arch Dermatol Res* 2010; **302**: 429–433.
- 69 Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med* 1995; **332**: 1351–1362.
- 70 Ganceviciene R, Graziene V, Fimmel S, Zouboulis CC. Involvement of the corticotropin-releasing hormone system in the pathogenesis of acne vulgaris. *Br J Dermatol* 2009; **160**: 345–352.
- 71 Slominski A, Zbytek B, Szczesniowski A *et al.* CRH stimulation of corticosteroids production in melanocytes is mediated by ACTH. *Am J Physiol Endocrinol Metab* 2005; **288**: E701–E706.
- 72 Slominski A, Zbytek B, Semak I, Sweatman T, Wortsman J. CRH stimulates POMC activity and corticosterone production in dermal fibroblasts. *J Neuroimmunol* 2005; **162**: 97–102.
- 73 Ito N, Ito T, Kromminga A *et al.* Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal axis and synthesize cortisol. *FASEB J* 2005; **19**: 1332–1334.
- 74 Kageyama K, Kagaya S, Takayasu S, Hanada K, Iwasaki Y, Suda T. Cytokines induce NF-κB, Nurr1 and corticotropin-releasing factor gene transcription in hypothalamic 4B cells. *NeuroImmunoModulation* 2010; **17**: 305–313.
- 75 Ganceviciene R, Graziene V, Böhm M, Zouboulis CC. Increased *in situ* expression of melanocortin-1 receptor in sebaceous glands of lesional skin of patients with acne vulgaris. *Exp Dermatol* 2007; **16**: 547–552.
- 76 Theoharides TC, Singh LK, Boucher W *et al.* Corticotropin-releasing hormone induces skin mast cell degranulation and increased vascular permeability, a possible explanation for its proinflammatory effects. *Endocrinology* 1998; **139**: 403–413.
- 77 Böhm M, Schiller M, Ständer S *et al.* Evidence for expression of melanocortin-1 receptor in human sebocytes *in vitro* and *in situ*. *J Invest Dermatol* 2002; **118**: 533–539.
- 78 Huang Q, Tatro JB. Alpha-melanocyte stimulating hormone suppresses intracerebral tumor necrosis factor-alpha and interleukin-1beta gene expression following transient cerebral ischemia in mice. *Neurosci Lett* 2002; **334**: 186–190.
- 79 Bhardwaj RS, Schwarz A, Becher E *et al.* Pro-opiomelanocortin-derived peptides induce IL-10 production in human monocytes. *J Immunol* 1996; **156**: 2517–2521.
- 80 Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. *J Immunol* 2008; **180**: 5771–5777.
- 81 Toyoda M, Nakamura M, Morohashi M. Neuropeptides and sebaceous glands. *Eur J Dermatol* 2002; **12**: 422–427.
- 82 Toyoda M, Nakamura M, Makino T, Kagoura M, Morohashi M. Sebaceous glands in acne patients express high levels of neutral endopeptidase. *Exp Dermatol* 2002; **11**: 241–247.
- 83 Skoff AM, Zhao C, Adler JE. Interleukin-1alpha regulates substance P expression and release in adult sensory neurons. *Exp Neurol* 2009; **217**: 395–400.
- 84 Niyonsaba F, Iwabuchi K, Matsuda H, Ogawa H, Nagaoka I. Epithelial cell-derived human beta-defensin-2 acts as a chemotaxin for mast cells through a pertussis toxin-sensitive and phospholipase C-dependent pathway. *Int Immunol* 2002; **14**: 421–426.
- 85 Niyonsaba F, Someya A, Hirata M, Ogawa H, Nagaoka I. Evaluation of the effects of peptide antibiotics human beta-defensins-1/-2 and LL-37 on histamine release and prostaglandin D(2) production from mast cells. *Eur J Immunol* 2001; **31**: 1066–1075.
- 86 Yang D, Chertov O, Bykovskaia SN *et al.* Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. *Science* 1999; **286**: 525–528.
- 87 Harder J, Bartels J, Christophers E, Schröder JM. A peptide antibiotic from human skin. *Nature* 1997; **387**: 861.
- 88 Caillon F, O'Connell M, Eady E *et al.* Interleukin-10 secretion from CD14+ peripheral blood mononuclear cells is downregulated in patients with acne vulgaris. *Br J Dermatol* 2010; **162**: 296–303.
- 89 Holland K, Holland D, Cunliffe W, Cutcliffe A. Detection of *Propionibacterium acnes* polypeptides which have stimulated an immune response in acne patients but not in normal individuals. *Exp Dermatol* 1993; **2**: 12–16.
- 90 Puhvel S, Reisner R. The production of hyaluronidase (hyaluronate lyase) by *Corynebacterium acnes*. *J Invest Dermatol* 1972; **58**: 66–70.
- 91 Hoffer U, Kohler HC, Adam R, Mauff G. Neuraminidase production of *Propionibacterium acnes* and related bacteria. *FEMS Microbiol Lett* 1978; **4**: 177–179.
- 92 Holland C, Mak TN, Zimny-Arndt U *et al.* Proteomic identification of secreted proteins of *Propionibacterium acnes*. *BMC Microbiol* 2010; **10**: 230.

- 93 Webster G, Leyden J, Nilsson U. Complement activation in acne vulgaris: consumption of complement by comedones. *Infect Immun* 1979; **26**: 183–186.
- 94 Webster G, Leyden J, Norman M, Nilsson U. Complement activation in acne vulgaris: *in vitro* studies with *Propionibacterium acnes* and *Propionibacterium granulosum*. *Infect Immun* 1978; **22**: 523–529.
- 95 Russell R, McInroy R, Wilkinson P, White R. A lipid chemotactic factor from anaerobic coryneform bacteria including *Corynebacterium parvum* with activity for macrophages and monocytes. *Immunology* 1976; **30**: 935–949.
- 96 Webster G, Leyden J. Characterization of serum-independent polymorphonuclear leukocyte chemotactic factors produced by *Propionibacterium acnes*. *Inflammation* 1980; **4**: 261–269.
- 97 Nagy I, Pivarcsi A, Koreck A, Széll M, Urbán E, Kemény L. Distinct strains of *Propionibacterium acnes* induce selective human beta-defensin-2 and interleukin-8 expression in human keratinocytes through toll-like receptors. *J Invest Dermatol* 2005; **124**: 931–938.
- 98 Nagy I, Pivarcsi A, Kis K *et al.* *Propionibacterium acnes* and lipopolysaccharide induce the expression of antimicrobial peptides and proinflammatory cytokines/chemokines in human sebocytes. *Microbes Infect* 2006; **8**: 2195–2205.
- 99 Vowels B, Yang S, Leyden J. Induction of proinflammatory cytokines by a soluble factor of *Propionibacterium acnes*: implications for chronic inflammatory acne. *Infect Immun* 1995; **63**: 3158–3165.
- 100 Nakatsuji T, Tang DC, Zhang L, Gallo RL, Huang CM. *Propionibacterium acnes* CAMP factor and host acid sphingomyelinase contribute to bacterial virulence: potential targets for inflammatory acne treatment. *PLoS ONE* 2011; **6**: e14797.
- 101 Grange PA, Chéreau C, Raingeaud J *et al.* Production of superoxide anions by keratinocytes initiates *P. acnes*-induced inflammation of the skin. *PLoS Pathog* 2009; **5**: e1000527.
- 102 Jugeau S, Tenaud I, Knol A *et al.* Induction of toll-like receptors by *Propionibacterium acnes*. *Br J Dermatol* 2005; **153**: 1105–1113.