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New accents in the pathogenesis of acne vulgaris

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Background: Acne is a chronic inflammatory disorder of the pilosebaceous unit (PSU). The PSU is an immunocompetent organ, and the sebaceous gland (SG) exhibits an independent peripheral endocrine function and expresses receptors for neuropeptides (NP). The presence of a complete corticotrophin–releasing hormone (CRH)/CRH receptor (CRHR) system in human sebocytes has also been confirmed. The capability of hypothalamic CRH to induce lipid synthesis, steroidogenesis and to interact with testosterone and growth hormone implicates a possibility of CRH involvement in the clinical development of acne. The purpose of this study was to make cartography of CRH expression in acne, especially in SG in comparison with physiologically normal non-acne-prone skin and skin from persons without acne and to identify a possible CRH involvement in acne pathogenesis.

Materials and Methods: 33 patients with acne and 8 age-matched volunteers without acne participated in the study. Skin biopsies were obtained from the acne-involved face, non-involved thigh skin of the same patients with acne and from normal human skin. Expression of CRH was analyzed by immunohistochemistry.

Results: A very strong positive reaction to CRH was observed in all types of SG cells in acne skin. All control and normal skin SGs demonstrated a weaker staining depending on the differentiation stage of SG cells.

Conclusions: CRH is abundant in acne-involved SG and possibly through the CRH-Rs activated pathway can affect immune and inflammatory processes leading to the development and stress-induced exacerbation of acne.

Key words: sebaceous gland, acne, CRH

INTRODUCTION

The major type of acne, *acne vulgaris*, is a chronic inflammatory skin disorder of the PSU. Sebaceous hyperplasia and increased sebum production, follicular hyperkeratinization that plugs the follicle and induces microcomedo formation, colonization of *Propionibacterium acnes* (*P. acnes*) and perifollicular inflammation are the well-known characteristics of acne. The role of androgen metabolism in SGs function and acne development is also scientifically proved (1). Like the still unknown pathogenesis of acne, also the mechanism of spontaneous comedo formation is yet unclear. Although the major mechanism involved is inflammation, it is still unclear whether bacteria or their products initiate follicular inflammation. *P. acnes* is found both in acne and normal PSU, and, despite initial proposals, free fatty acids in sebum are also unlikely to be bacterial products (2). The nature of the relation between acne and psychical stress remains to be elucidated, too.

In recent years, a dynamic interaction between the nervous, immune and endocrine systems is receiving increasing attention. This communication linked by neuropeptides (NP), hormones and cytokines is essential for biological homeostasis and responses to external and

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internal challenges (3). Activation of the hypothalamic–pituitary–adrenal (HPA) axis is the main adaptive response to chronic systemic stress. This process involves production and release of CRH, followed by production and secretion of the anterior pituitary-derived proopiomelanocortin (POMC) peptides. Adrenocorticotropic hormone (ACTH) induces production and secretion of the powerful anti-inflammatory factor cortisol, which terminates the stress response and buffers tissue damage (4).

NP genes have been detected not only in the central and peripheral nervous systems but also in most peripheral tissues, including skin (5). Participation of NP in response to cutaneous stress and imbalances in the skin stress response system or functional dysregulation of NP is clinically associated with a number of pathologic cutaneous conditions (3, 6, 7). The SG is the organ conferring the skin an independent peripheral endocrine function and, together with sweat glands, accounts for the vast majority of androgen metabolism in skin (6). The PSU is an immunocompetent organ (8) and also seems to be involved in responses to stress. Facial skin of acne patients is characterized by rich innervation, by increased numbers of substance P-containing nerves and mast cells, and by a strong expression of neutral endopeptidase in SG compared with normal skin (9). SGs express receptors for β-endorphin, CRH, urocortin, proopiomelanocortins (POMC), vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP) (7, 9, 10). The presence of a complete CRH/CRH-receptor system in human sebocytes has also been confirmed (7).

The CRH, a 41-amino acid polypeptide, its binding protein (CRH-BP) and receptors (CRH-R) act as a central regulatory system of the HPA axis (10). CRH is an important autocrine hormone in this cell type with a homeostatic pro-differentiation activity. Independently of the HPA axis, it directly induces lipid synthesis and steroidogenesis. Testosterone and growth hormone (GH), which also enhance sebaceous lipid synthesis, were found to antagonize CRH activity and expression of its receptors. These findings implicate involvement of CRH in the clinical development of acne, seborrhea and any other skin disease associated with alterations in the formation of sebaceous lipids (7).

In light of the confirmed facts as outlined above, we set out to explore the expression of CRH in lesional skin from patients with acne vulgaris. The purpose of the current study was to detect changes of the key neuropeptide CRH expression in acne lesions vs normal skin, especially in the SG based on the immunohistochemistry, and to identify a possible CRH involvement in acne pathogenesis.

MATERIALS AND METHODS

Patients and healthy controls. Biopsies (3–5 mm) were obtained from acne lesions of the facial (acne-involved skin) and the thigh (acne-uninvolved skin) of 33 patients with active acne (18 male and 15 female, aged 15–22 years) of the Outpatient Clinics of the "Šeškinės Poliklinika" and the Centre of Dermatovenerology (Santariškių klinikos Hospital, Vilnius, Lithuania), as well as from seven age-matched healthy individuals undergoing routine plastic or post-traumatic surgery. Acne patients did not receive topical treatment for 2 weeks or systemic treatment for 1 month prior to biopsy. Written informed consent was obtained from all subjects enrolled in this study, which was conducted according to the ethical standards of the Lithuanian Bioethics Committee for studies involving human subjects (authorization # 78, protocol # 1, version # 1).

Materials. Primary antibody to CRH was from Santa Cruz Biotechnology Inc., Santa Cruz, CA (CRF (C-20) – goat polyclonal IgG). Secondary rabbit/anti-goat immunoglobulins biotin, the antibody diluent, the alkaline phosphatase anti-alkaline phosphatase complex, the streptavidine enzyme conjugate and the diaminobenzidine tetrachloride reagent (En-Vision System Kit) were from Dako (Hamburg, Germany). Other supplements and reagents were from Biochrom (Berlin, Germany) and Sigma (Deisenhofen, Germany).

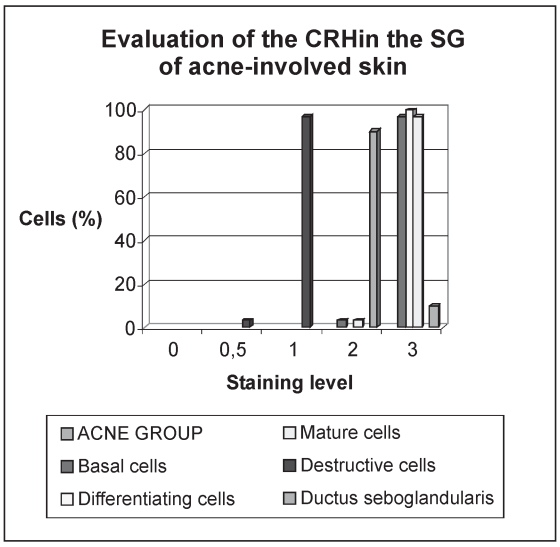
Immunohistochemistry. After deparaffinization the tissue specimens were incubated with diluted 1:50 primary antibodies in a humidified chamber for 30 min at room temperature without retrieval procedure. The optimal concentration of both the primary and secondary antibodies was predetermined by a titration assay. The negative controls consisted of tissues incubated with Antibody Diluent instead of the primary antiserum. Subsequently the specimens were incubated with a biotynilated secondary antibody (diluted $1 \cdot 500$) for 15 min and then with a streptavidine enzyme conjugate for 10 min. Finally, the specimens reacted with a Fuchsin Substrate–Chromogen System for 5–10 min and were counterstained with Mayer's hematoxylin. Immunostaining of SG cells at different stages of differentiation was evaluated semiquantitatively on a scale of 0 to 3: 0, negative; 0.5, barely discernible; 1, moderate intensive; 2, strong staining; 3, very strong uniform immunostaining. For evaluation of specific signals a microscopic view analyzing system (Olympus BX51, Japan) was used. Tissues showing membrane or cytoplasmic staining of any cells were assessed as positive.

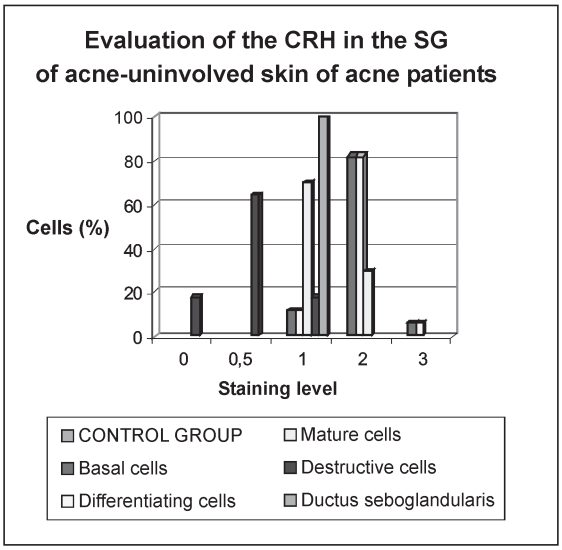
Statistical analysis

Statistical significance of the immunohistological studies was calculated by the Mann–Whitney U test. Mean differences were considered to be significant when $p < 0.05$.

RESULTS

There were many differences between intensity of antibody staining inside skin organoids including SG comparing acne, control and normal skin samples (Fig. 1). Immunohistochemical analysis of acne skin biopsies revealed an intensive expression of the CRH gene in cutaneous adnexa, especially in SG. Very strong positive reaction for





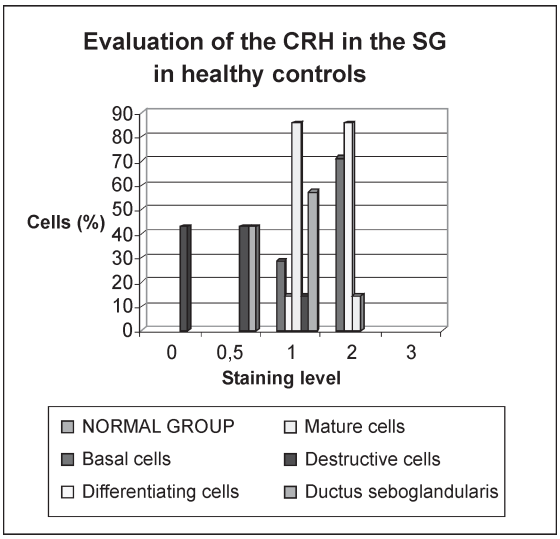


Fig. 1. Semiquantitative expression of IHC signals of the CRH gene in the sebaceous gland of acne-involved and acne-uninvolved skin of acne patients and in healthy controls (scale of 0 to 3: 0, negative; 0.5, barely discernible; 1, moderate intensive; 2, strong staining; 3, very strong uniform immunostaining)

CRH was observed in all types of sebocytes – basal, differentiating and mature cells in acne skin. These structures were the most intense stained structures together with nerves bundles in acne skin samples. Even in apoptotic sebocytes, keratinocytes and epithelium of SG strong positivity for this antibody was observed. Sweat glands and hair follicles demonstrated less intense than SG but sufficiently strong staining for CRH, too. Rather distinct immunostaining was noted in all layers of the epidermis, except the basal membrane and corneal layer that reveal no reactivity for CRH (Figs. 2, 3).

In contrast to the acne skin samples, in all the control and normal skin structures a weak intensity of CRH

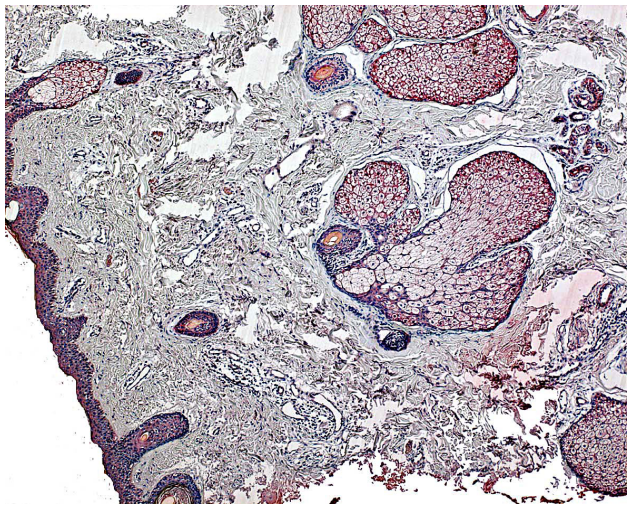


Fig. 2. Localization of IHC signals of the CRH gene in acne skin. Intensive expression of the CRH gene in cutaneous adnexa, especially in sebaceous glands. IHC. Mayer's hematoxylin. × 400

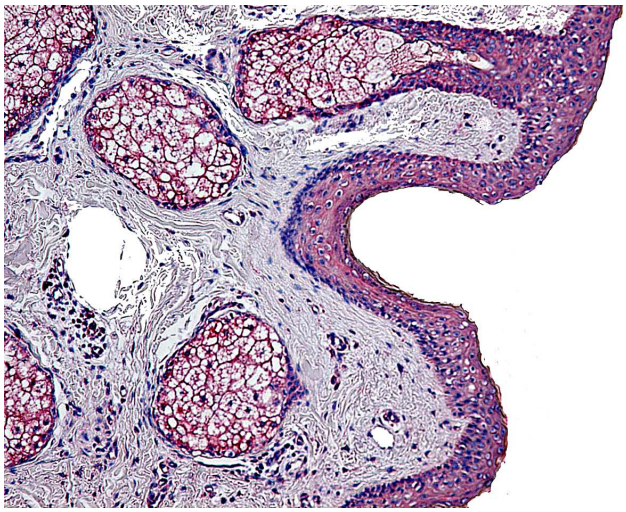


Fig. 3. Localization of IHC signals of the CRH gene in sebaceous gland of acne skin. Very strong positive reaction for CRH in all types of sebocytes – basal, differentiating and mature cells, and in nerve bundles. IHC. Mayer's hematoxylin. × 400

expression was rather significant. The staining within SGs was dependent on the sebocyte differentiating stage. While most of basal and differentiating sebocytes still demonstrated a strong immunoreaction to the CRH gene, mature sebocytes, epithelium and ductal keratinocytes showed only a weak staining and apoptotic sebocytes demonstrated barely discernible staining (Fig. 1). All layers of the epidermis showed a homogeneous weak expression of this antibody, except the basal membrane and stratum corneum where no staining was detected (Fig. 4A, 4B). Most of sweat glands underwent a weak and nerve bundles a strong immunoreaction. Only in the staining of hair follicles negligible differences were noted (not shown).

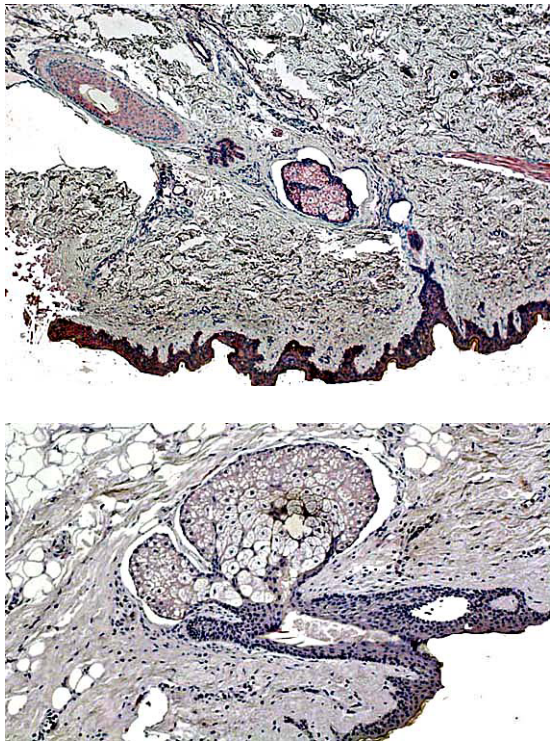


Fig. 4. Localization of IHC signals of the CRH gene in acne-uninvolved skin of acne patients (A) and in healthy controls (B). Significant weaker immunoreaction of sebaceous gland dependent upon sebocyte differentiation stage in control acne-uninvolved skin of acne patients and in normal skin. IHC. Mayer's hematoxylin. × 400

DISCUSSION

In light of the confirmed presence of a complete CRH/CRH-R system in human sebocytes *in vitro* (7) and the potent immunomodulatory actions of these NPs (10, 11), our data, for the first time, suggest that the CRH is involved in the pathogenesis of this inflammatory skin disorder. Data from different experimental models have verified that CRH influences steroidogenesis independently of the HPA axis. It directly induces lipid synthesis and enhances mRNA expression of Δ5-3β-hydroxysteroid dehydrogenase, the enzyme that converts dehydroepiandrosterone to testosterone in human sebocytes (12). Testosterone and the growth hormone (GH), which also enhance sebaceous lipid synthesis (13), were found to antagonize CRH by downregulating or modifying CRH-R expression, respectively. Immunohistochemical analysis of CRH in acne skin in comparison with control, non-involved skin of acne patients and normal skin in the current study has demonstrated that CRH is widely spread throughout the skin. Nevertheless, we found significant diversities and differences between the intensity of antibody staining inside various skin structures comparing acne, non-involved and normal skin samples that indicate participation of the CRH in the pathogenesis of acne. The results of our study showed a very strong positive reaction for CRH in all types of sebocytes. In contrast to the acne skin, SGs of all non-involved and normal skin demonstrated a weaker immunoreaction which was dependent upon the sebocyte differentiation stage. These findings are also important, because CRH activity in skin has been shown to depend on the cellular target. CRH can modify the vascular reaction, act as a local growth factor, or induce cell differentiation. CRH (exogenous and produced locally) can modify the human keratinocyte phenotype through a receptor-mediated pathway (14).

Many data confirmed the ability of CRH to modulate inflammation when secreted in inflammatory sites. Its direct paracrine effects are pro-inflammatory (15). In CNS, cytokines regulate CRH expression; tumor necrosis factor (TNF), interleukin-1 (IL-1), and IL-6 stimulate CRH secretion. IL-1, IL-1α, IL-1β and TNF-α are present at multiple sites in normal skin, including the SGs (16). The detected strong expression of CRH in inflammatory lesions versus normal skin also proposed that pro-inflammatory cytokines stimulate CRH expression in the epidermis (11). However, a stronger expression of CRH in the acne SG and other skin organoids compared with control and normal skin in our study also suggests that CRH may interact with inflammatory mediators and immune factors in acne. This correlation becomes especially evident taking into account that PSU is both an immunocompetent organ and an organ involved in responses to stress, and free fatty acid cascade with metabolites that are critical for numerous biologic processes, including inflammation and immunologic function, also have a special role in acne pathogenesis (17).

In conclusion, the above–mentioned facts and the results of the present investigation provide a novel evidence that CRH, the most proximal element of the HPA axis, which acts as a central coordinator for neuroendocrine and behavioral responses to stress, can affect immune and inflammatory processes and underlies the development and stress-induced exacerbation of acne. Therefore, it is likely that peptidergic systems may be targets for novel therapeutic strategies of acne vulgaris.

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References

1. Zouboulis CC, Eady A, Philpott M, Goldsmith LA, Orfanos C, Cunliffe WC Rosenfield R. What is the pathogenesis of acne? Exp Dermatol 2005; 14: 143–52.
2. 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: 1011–20.
3. Lotti T, Bianchi B, Panconesi E. Neuropeptides and skin disorders. The new frontiers of neuro-endocrine-cutaneous immunology. International J Dermatol 1999; 38: 673–5.
4. Besedovsky HO, Del Rey A. Immune-neuro-endocrine interactions: facts and hypotheses. Endocr Rev 1996; 17: 64–102.
5. Slominski A, Mihm M. Potential mechanism of skin response to stress. Int J Dermatol 1996; 35: 849–851.
6. Zouboulis CC. Human skin: an independent peripheral endocrine organ. Horm Res 2000; 54: 230–42.
7. Zouboulis CC, Seltmann H, Hiroi N et al. Corticotropin-releasing hormone: An autocrine hormone that promotes lipogenesis in human sebocytes. Proc Natl Acad Sci 2002; 99: 10: 7148–53.
8. Zouboulis CC, Böhm M. Neuroendocrine regulation of sebocytes – a pathogenetic link between stress and acne. Exp Dermatol 2004; 13(suppl 4): 31–5.
9. Toyoda M, Nakamura M, Makino T, Kagoura M, Morohashi M. Sebaceous glands in acne patients express high levels of neutral endopeptidase. Experimental Dermatol 2002; 1: 241–7.
10. Slominski A, Wortsman J, Paus R, Luger T, Salomon S. Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress. Physiol Rev 2000; 80: 979–1020.
11. Kono M, Nagata H, Umemura S, Kawana S, Osamura RY. In situ expression of corticotropin-releasing hormone (CRH) and proopiomelanocortin (POMC) genes in human skin. FASEB J 2001; 15: 2297–9.
12. Fritsch M, Orfanos CE, Zouboulis CC. Sebocytes are the key regulators of androgen homeostasis in human skin. J Invest Dermatol 2001; 116: 793–800.
13. Deplewski D, Rosenfield RL. Growth hormone and insulin-like growth factors have different effects on sebaceous cell growth and differentiation. Endocrinology 1999; 140: 4089–94.
14. Slominski A et al. Corticotropin Releasing Hormone and related peptides can act as bioregulatory factors in human keratinocytes. In vitro Cell. Dev Biol – Animal 2000; 36: 211–16.
15. Theoharides TC, Singh LK, Boucher W et al. Corticotropin-releasing hormone induces skin mast cell degeneration and increased vascular permeability, a possible explanation for its proinflammatory effects. Endocrinology 1998; 139: 403–13.
16. Boehm KD, Yun JK, Strohl KP, Elmets CA. Messenger RNAs for the multifunctional cytokines interleukin-1 alpha, interleukin-1 beta and tumor necrosis factor-alpha are present in adnexal tissues and in dermis of normal human skin. Exp Dermatol 1995; 4: 335–41.
17. Alestas T, Ganceviciene R, Fimmel S, Müller-Decker K, Zouboulis CC. Enzymes involved in the biosynthesis of leukotriene B_4 and prostaglandin E_2 are active in sebaceous glands. J Mol Med, 2006; 84(1): 75–87.

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NAUJI *ACNE VULGARIS* PATOGENEZĖS ASPEKTAI

Santrauka

Paprastieji spuogai – *acne vulgaris* – tai lėtinis uždegiminis odos plauko folikulo ir riebalinės liaukos komplekso (PRK) susirgimas. PRK – tai imunokompetentinė odos struktūra, o riebalinė liauka (RL) pasižymi nepriklausomomis periferinėmis endokrininėmis funkcijomis ir ryškiais neuropeptidų receptoriais. Šiuo metu jau moksliškai patvirtinta, kad žmogaus sebocituose funkcionuoja visavertė kortikotropiną išlaisvinančio hormono ir jo receptorių sistema (KIH/KIH-R). Hipotaliamo KIH sugebėjimas indukuoti lipidų sintezę, steroidų genezę ir sąveikauti su testosteronu bei augimo hormonu leidžia daryti išvadą, kad KIH gali dalyvauti atsirandant *acne* klinikai. Šios mokslinės studijos tikslas buvo atlikti KIH raiškos odoje, ypač RL, kartografinį vaizdą, palyginti *acne* pažeistą veido odą, *acne* nepažeistą tų pačių pacientų su paprastaisiais spuogais odą bei sveikų žmonių, nesergančių *acne vulgaris*, odą ir nustatyti galimą KIH dalyvavimą šios ligos patogenezėje.

Medžiagos ir metodai. Studijoje dalyvavo 33 pacientai, sergantys *acne vulgaris*, bei 8 panašaus amžiaus sveiki savanoriai. Odos biopsijos buvo paimtos iš *acne* pažeistos veido odos, *acne* nepažeistos tų pačių pacientų šlaunies odos (kontrolinė) bei normalios žmogaus odos. KIH raiška odoje buvo tyrinėjama imunohistochemijos būdu.

Rezultatai. *Acne* pažeistos odos visose RL ląstelėse pastebėta labai stipri teigiama imuninė reakcija. Kontrolinės bei normalios odos pavyzdžiai imunohistochemiškai nusidažė daug silpniau, o jo intensyvumas priklausė nuo sebocitų diferenciacijos laipsnio.

Išvados. *Acne* pažeistos odos riebalinėje liaukoje gausu KIH, kuris, tikėtina, per KIH receptorių aktyvuotą kelią sugeba paveikti imuninius ir uždegiminius procesus, todėl atsiranda paprastieji spuogai ar paūmėja ši lėtinė odos liga.