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Pathogenic Characteristics of Th17 Cells in the Regulation of Sebaceous Gland Lipoprotein Metabolism in the Acne Rat Model

Yanni He[1], Qiaorong Yang[2], Tong Zhang[1], Yibin Zeng[1], Lingbo Du[1], Chao Zhang[1], Wuqing Wang[1*]

[1]Department of Dermatology, Minhang Hospital Affiliated to Fudan University, Shanghai, 201199, [2]Department of Dermatology, Zhongshan Hospital Fudan University, Shanghai, 200032, China

ABSTRACT

Background: Acne is a common and chronic inflammatory dermatosis of sebaceous gland units of the human hair follicle. Acne is closely related to immune cytokines and cells including T helper 17cells (Th17 cells). Mis-regulated glycolipid metabolism also plays a vital role in the process.

Objective: This investigation aimed to explore the role of IL-17 in signaling pathways controlling sebaceous gland lipoprotein metabolism in a rat model of acne.

Methods: We generated the rat ear acne model, and investigated the pathological changes of acne skin tissue by histological analysis and the changes in the critical factors including DEFB1, GPR65, FADS1, and FADS2 by Western Blot in this model.

Results: There were more Th17 cells in the rat ear acne model than in the control mice. The expression levels of DEFB1, GPR65, FADS1, FADS2 and MOGAT1 were significantly upregulated in serum and tissue from rat acne model, which could be concluded that the Th17 cells play a major role in the pathogenesis of acne based.

Conclusions: Although acne is associated with immune effects and glycolipid metabolism, inhibition of IL-17 signaling pathway might be a novel way for acne therapy. Our findings also suggest a new strategy for targeted therapy of acne.

Keywords: Acne, IL-17 Signal Pathway, Metabolism, Sebaceous Gland Lipoprotein, Th17 Cells

*Corresponding author:
Wuqing Wang,
Department of Dermatology,
Minhang Hospital Fudan University, No.170 Xinsong Road, Minhang District,
Shanghai, 201199, China.
Tel: +86 21 64923400
Email: wwq0711@21cn.com

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INTRODUCTION

Acne is well-known to be a common and chronic inflammatory dermatosis of human hair follicle sebaceous gland units (1). It often occurs in adolescents due to their development and endocrine. Acne also interferes with the adolescent's psychological and social life, to a certain extent. In some cases, acne can lead to acute purulent infection, which is prone to expand into the subcutaneous tissue, resulting in different damages to the skin (2, 3). Notably, acne can be naturally alleviated or cured when one grows up.

The occurrence of acne is closely related to some factors, which are involved in some types of bacterial infections and inflammatory reactions in the skin, blockage of sebaceous ducts in hair follicles, and improper sebum secretion. During one's

puberty, the expression level of androgens, for instance, testosterone, gets upregulated rapidly. Therefore, these androgens promote the development of sebaceous glands which generates large amounts of sebum (4). At the same time, abnormal keratosis of the follicle sebaceous gland duct causes duct blockage, sebum discharge obstacles, and the formation of cutin suppositories, i.e. micro-acne. Microorganisms in hair follicles, especially Propionibacterium acne, proliferate very fast and accumulate in large quantities, which induces and aggravates inflammation by producing lipase, degrading sebum to produce free fatty acids, chemotaxis inflammatory cells, and mediators. Acne is also closely related to immune cytokines including interleukin-17 (IL-17), which is secreted by Th17 cells and can also activate related signaling pathways to aggravate the severity of acne (5-8).

Here, we constructed an animal model of acne, analyzed the pathological changes of acne skin tissue by immunohistochemistry. Animal blood samples were taken out for flow sorting of T cell subtypes (Th17, Treg content) and detecting the expression of RORrt and FOXP 3 in T cells by flow cytometry. By ELISA analysis, expression levels of inflammatory signaling molecules, including CCL20, CXCL9, CXCL10, IL-10, L-17 in blood samples were detected and the expression of GPR65, FADS1, FADS2, and DEFB4A in acne skin tissue was analyzed by WB. Our results demonstrated that these factors were significantly upregulated in serum and tissue from rat acne models, which indicated that Th17 cells based on the IL-17 signal pathway play a critical role in the occurrence of acne.

MATERIALS AND METHODS

Exploration Research

Rat Ear Acne Model

Twelve male Wistar rats, weighing about 200 g, were supplied by Minhang Hospital Fudan University and employed for the rat ear acne model. Propionibacterium acnes were injected intracutaneously into the auricle to simulate the external manifestations of acne and the pathogenesis of inflammatory reaction, and to prepare the rat ear acne model. In the model group, Propionibacterium propionate solution (6×10^7cfu/ml) was injected intracutaneously into the right auricle of each rat once a day for 3 days. On the third day, each rat was given Propionibacterium propionate solution (6×10^7cfu/ml) 1ml intraperitoneally once a day for 7 consecutive days. Each rat received both experimental agents, Propionibacterium propionate solution on the left side and vehicle on the right. This study was approved by the ethics committee of Minhang Hospital Fudan University.

Pathological Analysis

Skin samples were obtained at 12 weeks of treatment which was the experimental end-point. Acne skin tissues were taken to analyze the pathological changes of acne tissues by immunohistochemistry. Hematoxylin and eosin staining was used for routine examination of tissues.

Flow Cytometry Analysis

The eyeball blood was collected, centrifuged at 3000 r/min for 15 min to separate serum, and IL-6 level was detected according to the kit instruction manual. Animal blood samples were taken and T cell subtypes (Th17, Treg content) were sorted by flow cytometry. Animal blood samples were also taken to detect the Retinoic acid receptor-related orphan receptor thymus isomer (RORrt) in T cells by flow cytometry.

ELISA

Cytokines such as TNF-a, IFN-g, IL-1b, IL-10, and IL-12a were investigated by ELISA in treated serum samples from both groups. Samples were assayed in triplicate.

Western Blot

Western blots were carried out on animal

acne tissue extracts. Fifty milligrams of proteins were electrophoresed on 10% SDS-PAGE and transferred onto PVDF membranes. Immunoblotting was performed according to the manufacturer's instructions.

Statistical Analysis

SPSS 13.0 was used for statistical processing. All the data are represented by mean±variance (M±S), and group comparison has adopted the analysis of variance, and the Ridit test has been used to rank the data.

RESULTS

General Condition

Initially, we aimed to construct the rat ear acne model. The color of the auricle epidermis in the normal group was normal and the capillaries were obviously visible and soft. The auricle epidermis of model group rats was rough and dry within the skin. The skin was red and swollen and the tactile sensation of capillaries became hard and thick. According to the experimental acne judgment, it suggested that the experimental acne model of rat auricle be constructed. Acne skin tissues were collected to analyze the pathological changes of acne tissues by immunohistochemistry. HE staining also showed that the rat acne model was constructed successfully (Figure 1).

The Number of Th17 Increased in Acne Model Rats.

To assess the distribution of immune cells related to the initiation of acne, animal blood samples were taken out and T cell subtypes (Th17, Treg content) were sorted and analyzed by flow cytometry. The results showed that there were more Th17 cells in the rat ear acne model in comparison with control mice (Figure 2A). Foxp3 is a specific transcription initiation factor of Treg cells, which is not only a marker of Tregs but also plays a key role in the function of Treg cells. The expression level of Foxp3 indicated the function of Treg cells. Retinoic acid receptor-related orphan receptor thymus isomer (RORrt), is a transcription initiation factor specific for Th17 cells, which can promote the expression of Th17 coding genes and serve as a basis for the differentiation of initial T cells into Th17 cells. Meanwhile, animal blood samples were also taken to detect the expression levels of RORrt and FOXP3 in T cells by q-RT PCR (Figure 2B).

Inflammatory Signaling Molecules Were Activated in Acne Model Rats

To further investigate the correlation between acne occurrence and the immune reaction, the expression levels of inflammatory signaling molecules, including CCL20,

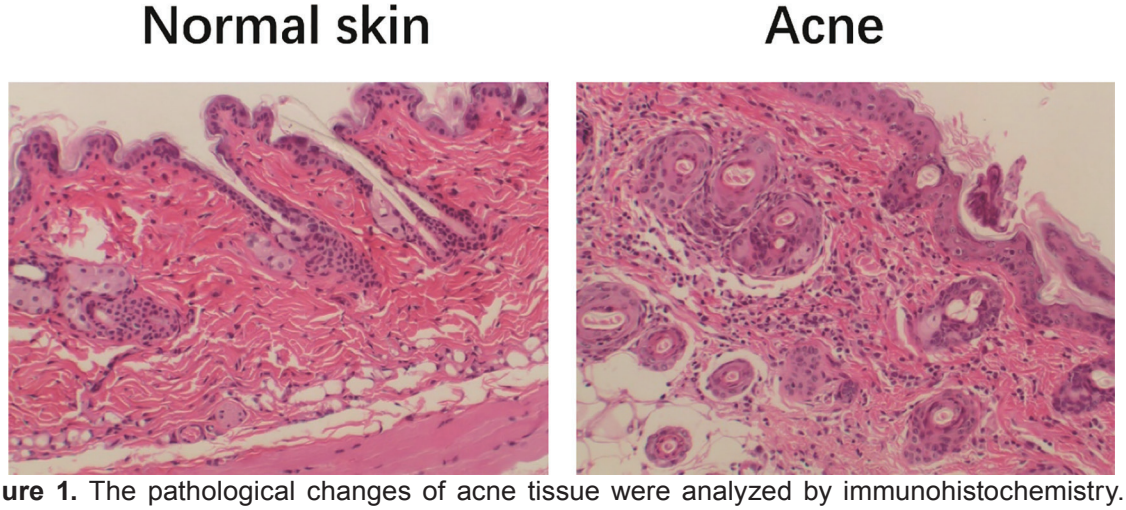

Figure 1. The pathological changes of acne tissue were analyzed by immunohistochemistry. A) Morphology of the pilosebaceous unit and fine structure of the sebaceous gland was shown by a H&E-stained sagittal section.

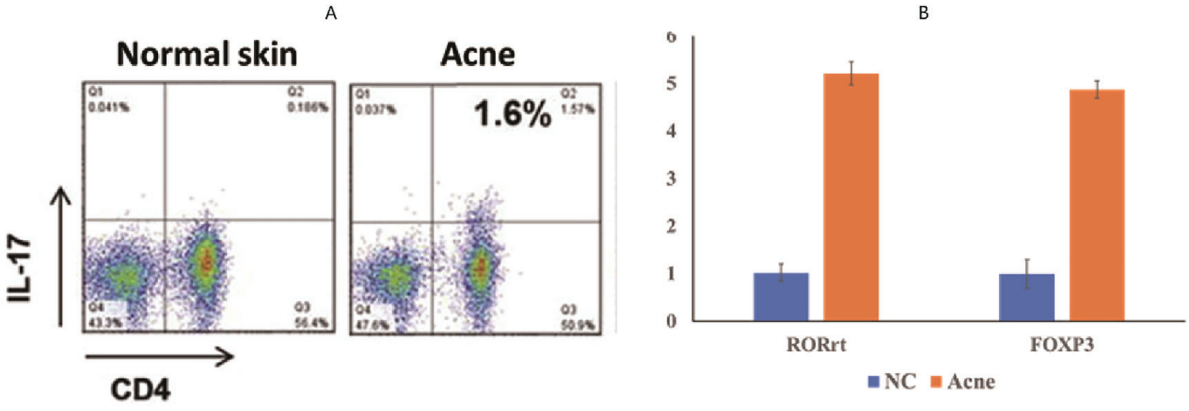

Figure 2. Isolation and identification of Th17 cells from animal blood samples A) T cell subtypes (Th17) were sorted by flow cytometry. B) The expression of RORP3 in T cells was analyzed by q-PCR. NC: Normal control.

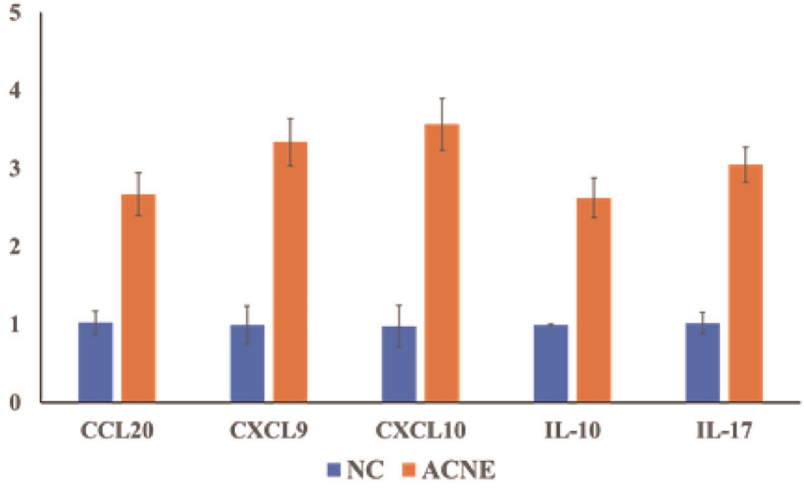

Figure 3. The expression of inflammatory signaling molecules CCL20 and CXCLs in blood samples of animals. The expression of inflammatory signaling molecules CCL20 and CXCL in blood samples of animals was analyzed by ELISA. NC: Normal control.

CXCL9, CXCL10, IL-10, were analyzed by ELISA in blood samples from both acne model rats and control rats. Our results showed that the expression levels of all the cytokines, including IL-17, were significantly upregulated in acne model rats compared with control rats (Figure 3). It implied that the IL-17 signaling pathway was activated in acne model rats.

Analysis of Related Protein Expressions in Acne Skin

To explore the related protein expression levels, we performed Western Blot to analyze the expression of GPR65, FADS1, FADS2 and DEFB4A in rats' acne skin tissues. It has been reported that DEFB1 can interact with CR6 as a ligand together with CC20 to activate inflammatory cascade effects. DEFB1 mediates the production of IL-17 by Th17 cells and regulates the proliferation and differentiation of Treg cells. GPR65 acts as a receptor protein on sebaceous gland cells and participates in immune effects and glycolipid metabolism activities. FADS1 and FADS2 are involved in immune inflammation. The protein expressions of these genes in rat acne skin were evaluated by Western Blot (Figure 4). Our results demonstrated that the expression levels of GPR65, FADS1, FADS2, and DEFB4A were upregulated in acne model rats compared with control rats. These data indicated that the occurrence of acne might be associated with immune effects and glycolipid metabolism.

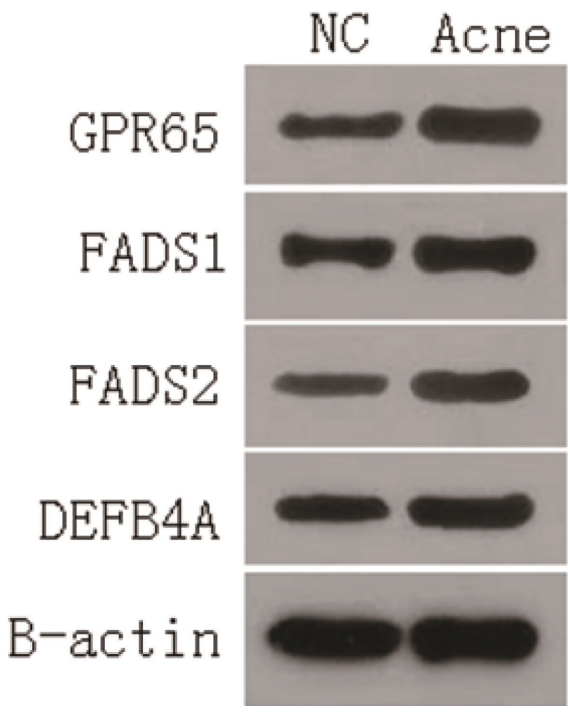

Figure 4. Analysis of ADDEFB4A, GPR65, FADS1, FADS2 and DEFB4A expression in acne skin. The protein expressions of ADDEFB4A GPR65, FADS1, FADS2, DEFB4A in acne skin were detected with Western Blot. ACTB was used as an internal control. NC: Normal control.

DISCUSSION

Acne often appears on the head and face of young people, which is caused by many factors in vivo and in vitro, and the pathogenesis is very complicated (9, 10). Current studies have shown that the pathogenic factors play an important role, including hypersecretion of sebaceous glands and hair follicles, medium microbial overgrowth, dyskeratosis of hair follicle sebaceous ducts, and other remnants transforming factors (11-13).

Acne animal models have been widely used in experimental research and modeling methods. It is simple, operable, and has high repeatability. Rabbits and rats were used as experimental animals for acne. Coal tar, chlorinated hydrocarbons, oleic acid, bis Toluene, and Propionibacterium acne are used to make acne models (14-16). In this study, we employed a rat model, which was generated after only 7 days and can be maintained for 14 days. Based on the observation and further analysis, our results showed that the acne model had been constructed successfully and could be employed for further studies.

Human β-defensins (hBDs) are a group of small peptides which has a broad spectrum of antimicrobial activity against bacteria, fungi, and viruses (17, 18). A total of 28 hBDs have been reported, but the expressions of only four of them have been demonstrated. Among them, Humanβ-defensin-1 (DEFB1) has been reported to be expressed in the oral mucosa (19, 20). DEFB1 is an important component of antimicrobial peptides. Current studies confirmed the polymorphism of the DEFB1 gene more than 20 diseases are associated, including caries, candidiasis, periodontal disease, tuberculosis, chronic obstructive pulmonary disease, sepsis, and Crohn's disease (17). It has been reported for the involvement of CatSper in a complex with CCR6 that mediates the CCR6 ligands (DEFB1-CCL20)-induced Ca^{2+} influx as well as DEFB1/CCL20-induced sperm motility. The pathogenic gene DEFB1 of one sore can bind to CR6 as a ligand together with CC20 to mediate inflammatory cascade effects induced by Propionibacterium spasticum. For example, it mediates the production of IL-17 by Th17 cells and regulates the proliferation and differentiation of Treg cells (21). GPR65 acts as a receptor protein on sebaceous gland cells and participates in immune effects and glycolipid metabolism activities. Fatty acid desaturase 1 (FADS1) and fatty acid desaturase 2 (FADS2) are fatty acid desaturase groups. FADS1 and its homologous gene FADS2 encode fatty acid desaturase, which is responsible for polyunsaturated lipids, respectively (22-24).

In this study, we provided the first evidence to show that, apart from inflammatory signaling molecules, the protein levels of GPR65, FADS1, FADS2, and DEFB4A were significantly upregulated in serum and tissue from rat acne models, which indicated that Th17 cells based on IL-17 signaling pathway play a critical role in the sebaceous gland lipoprotein metabolism and the occurrence

of acne. It is an update on the relationship of the Th17 cells, sebaceous gland metabolism, and innate immunity. Although acne is associated with immune effects and glycolipid metabolism, inhibition of the IL-17 signaling pathway might be a novel way for acne therapy. Our study also provides new ideas for the treatment of acne. However, the role of each protein during sebaceous gland metabolism and innate immunity remains unclear. Therefore, whether and how Th17 cells based on the IL-17 signaling pathway directly regulates the lipoprotein metabolism in the sebaceous gland and the occurrence of acne need to be further studied.

DATA AVAILABILITY STATEMENT

The data in the research can be obtained if necessary.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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