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Increased interferon-γ, interleukin-12p40 and IL-8 production in *Propionibacterium acnes*-treated peripheral blood mononuclear cells from patient with acne vulgaris
Host response but not bacterial species is the determinant factor of the disease

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ABSTRACT

Background: Acne vulgaris is a multifactorial inflammatory disease of the sebaceous follicles of the face and torso that frequently occurs in adolescence. Initially, acne starts as a non-inflammatory comedo. Subsequently, inflammatory reactions evolve to pustules, granulomas and cystic lesions. Many pathogenic mechanisms have been proposed including sebum excretion, obstruction of hair follicles, impaired keratinization of hair epithelium, bacterial overgrowth and immunological mechanisms; the role of *Propionibacterium acnes* (*P. acnes*) is particularly important. Facultative anaerobic gram-positive rods have been implicated in acne pathogenesis. However, the host immune response to *P. acnes* has not been as yet elucidated.
Objectives: The aim of the present study is to evaluate the importance of the immune response to *P. acnes* and the bacteriological factor in the pathogenesis of acne.
Methods: *P. acnes* isolated from acne lesions and healthy volunteers skin were cultured. The peripheral blood mononuclear cells (PBMC) from acne patients or healthy volunteers were stimulated with viable *P. acnes*, and cytokine production was evaluated using RT–PCR and ELISA.
Results: IFN-γ, IL-12p40, and IL-8 mRNA and protein production were significantly increased in PBMC from acne patients compared to that from normal donors. However, different *P. acnes* species isolated from acne lesions or normal subjects showed no difference in cytokines production from acne patients and normal subjects PBMC.
Conclusions: The inflammatory response of acne appears to be attributable to *P. acnes*-induced host immune response rather than *P. acnes* strains from normal skin or acne lesions.

1. Introduction

Acne vulgaris is a multifactorial inflammatory disease of the sebaceous follicles of the face, breast and upper region of the back trunk, that affects commonly adolescents [1]. It begins as micro-comedo that then develops to non-inflammatory open comedo or closed comedo. In many cases, an inflammatory reaction occurs leading to the formation of red papules, pustules and subcutaneous abscess. Increased sebum excretion, elevated androgenic hormones, abnormal hyperkeratinization of the follicular infundibulum, increased proliferation of *Propionibacterium acnes* (*P. acnes*) and exacerbated immune response [2,3] have been proposed to be involved in the pathogenesis of acne vulgaris. During the initial changes of acne, there are interleukin (IL)-1α-containing micro-comedo [4], disturbed terminal differentiation of keratinocytes [5], and dihydrotestosterone in the infundibulum [6]. *P. acnes*, a facultative anaerobic gram-positive rod, has been particularly implicated in the pathogenesis of acne. However, the mechanism of *P. acnes* participation in the disease is not well understood [7]. Recently, the innate immunity has been involved in the pathogenesis of acne, suggesting that *P. acnes* triggers cytokine production via Toll-like receptor (TLR) 2 [8]. The immune response precedes

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hyperkeratinization [9]. In the early stages of the inflammatory lesions such as microcomedo and comedo, many CD4+ T cells are detected in the lesions [10]. In addition, *P. acnes* may contribute to the inflammatory response by stimulating the secretion of tumor necrosis factor (TNF)-α, IL-1β and IL-8 from monocytes [11]. IL-8, a neutrophil chemotactic factor, may also contribute to inflammatory lesions of acne. Besides IL-8, *P. acnes* induces the production of IL-12 by via TLR-2 from monocytes and keratinocytes [11]. Under the influence of IL-12 and IL-18, naïve T cells differentiate to Th1 cells that secrete interferon (IFN)-γ [12]. We previously reported heat-killed *P. acnes* induced Th1 response in mice, producing IL-12, IL-18, and IFN-γ [13]. Both antigen-dependent and antigen-independent mechanisms involving monocytes and keratinocytes have been implicated in the pathogenesis of *P. acnes*-associated inflammatory acne.

In this study, we isolated peripheral blood mononuclear cells (PBMC) and clinical isolates of viable *P. acnes* from acne patients and healthy volunteers; they were co-cultured and cytokine production was measured using RT–PCR and ELISA. The aim of our study is to clarify whether *P. acnes* strains or the host immune response against the bacterium is the main factor involved in the pathogenesis of acne.

2. Materials and methods

2.1. Reagents

Lymphocyte separation medium (LSM) was purchased from MP Biomedicals (Irvine, CA). RPMI-1640 and Dulbecco's modified Eagle's medium were purchased from Nikken Biomedical Laboratories (Kyoto, Japan). Fetal bovine serum (FBS) was from American Type Culture Collection (Manassas, VA). ELISA kits for human IL-8, IL-12p40 and IFN-γ were from Bio Source International (San Jose, CA), and IL-18 was from MBL International (Nagoya, Japan).

2.2. P. acnes and blood samples

P. acnes were isolated from 18 healthy adults (2 males and 16 females, mean age of 19.8 (±4.12)) and 18 acne patients (5 males and 13 females, mean age of 18.8 (±6.51)) after obtaining written informed consent from the subjects. PBMC were prepared from two healthy adults (1 male and 1 female, with 47 and 46 years old), and three acne patients (1 male and 2 females, 30, 26 and 26 years old of age). The investigational protocol including the use of *P. acnes* and blood samples was approved by the Institutional Review Board of Mie University (No. 575).

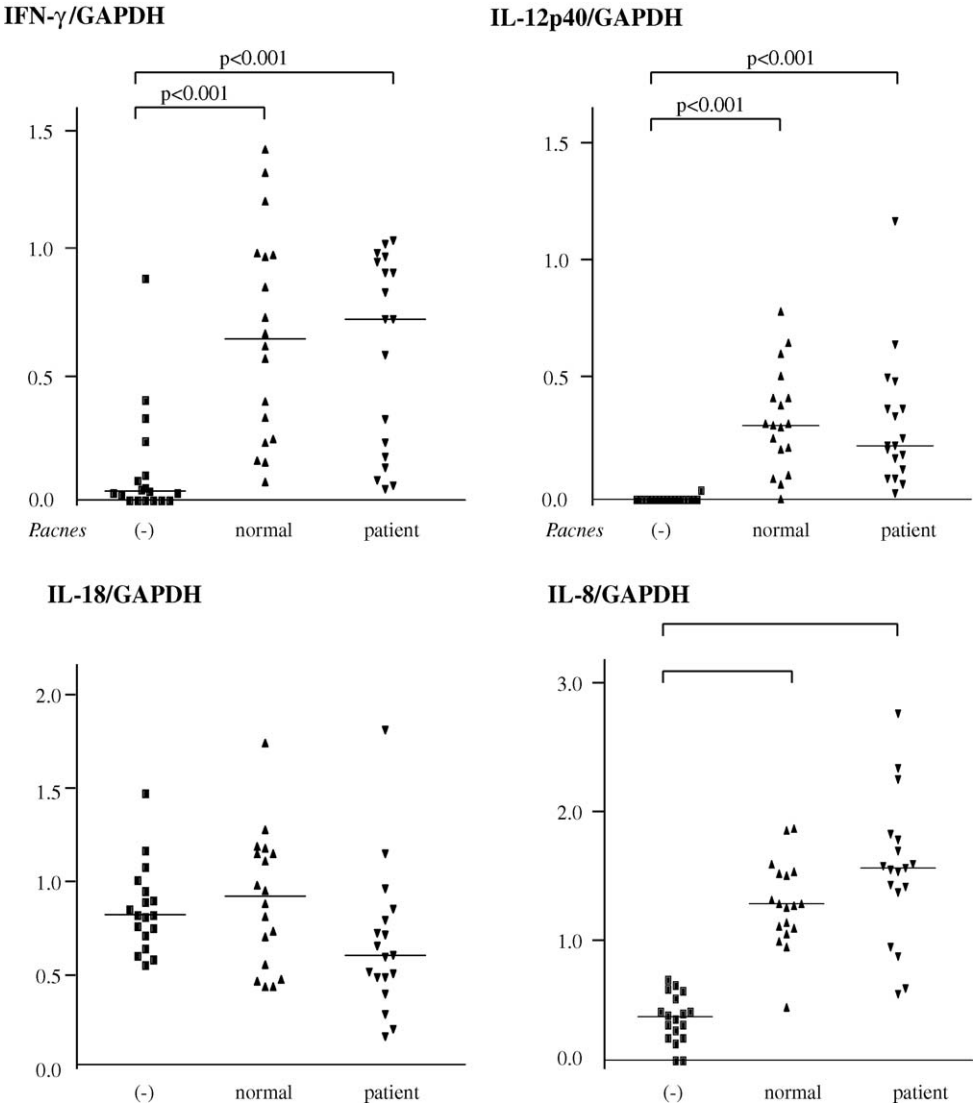

Fig. 1. RT-PCR analysis using PBMC from normal donors; PBMC (1×10^6 cells/well) was cultured with or without *P. acnes*. Viable *P. acnes* were from 18 normal donors and 18 acne patients. mRNA levels for IFN-γ, IL12p40, IL-18, and IL-8 were measured by semiquantitative RT-PCR. The levels of their mRNA were expressed as the ratio of cytokine mRNA to GAPDH mRNA. The difference between *P. acnes*-free and *P. acnes*-treated groups was statistically significance for IFN-γ, IL-12p40, and IL-8 ($P < 0.001$).