The detection of *Propionibacterium acnes* signatures in granulomas of lupus miliaris disseminatus faciei

Running title: *Propionibacterium acnes* in LMDF

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ABSTRACT

Lupus miliaris disseminatus faciei is a papular eruption of the face in adults, which occurs predominantly on the lower eyelids. Histologically, the granulomatous lesions are primarily situated around the hair follicles, particularly the superficial region/infundibula. Its etiology remains to be elucidated. *Propionibacterium acnes* (*P. acnes*) has been recently suspected as a cause of sarcoidosis. In light of the sarcoid-like reactions that are present in lupus miliaris disseminatus faciei, we hypothesized that *P. acnes* may also be implicated in the granulomas associated with the disease. We evaluated nine DNA samples from the granulomatous lesions of the skin of the patients with lupus miliaris disseminatus faciei. We used laser capture microdissection to extract DNA from these regions. Polymerase chain reaction was performed to amplify segments of the 16S ribosomal RNA of *P. acnes*; the *P. acnes* gene was clearly detectable in all nine DNA samples. The gene was also detected in the samples from normal-appearing skin; however, these bands were faint in all samples. The results of the present study suggest that *P. acnes* plays one of the pathogenetic roles in lupus miliaris disseminatus faciei.

Keywords: epithelioid cell granuloma, laser capture microdissection, lupus miliaris disseminatus faciei, *Propionibacterium acnes*, sarcoid-like reaction

INTRODUCTION

The histopathological hallmark of LMDF is an area of dermal necrosis—caseous necrosis—surrounded by epithelioid histiocytes, multinucleate giant cells, and lymphocytes. However, this feature is not consistent. Darouti and Zaher[1] reported that all the 25 patients with LMDF that had been examined had sarcoidal granulomas (without caseous necrosis) rather than tuberculoid granulomas. Similar results were reported by Shitara[2] and Sehgal et al.[3]

The accumulation of *P. acnes* genomes in the sarcoidal granulomas in the sarcoid lymph nodes has been recently detected using *in situ* hybridization by catalyzed reporter deposition.[4] Therefore, considering that LMDF presents with sarcoidal granulomas (epithelioid cell granulomas), we hypothesized that *P. acnes* may be implicated as a pathogenetic factor for the granulomas of LMDF.

In the present study, we obtained LMDF tissue samples using laser capture microdissection (LCM). LCM is a reliable method for the procurement of pure populations of targeted cells from specific microscopic regions of tissue sections for subsequent analysis.[5] After LCM, PCR amplification was performed to search for the *P. acnes* genes in the epithelioid cell granulomas from these patients.

METHODS

Tissue samples

Biopsy samples were obtained from nine patients with LMDF (Male/Female = 3/6; age range, 9–69 years; median age, 30 years; Table 1). The diagnosis of LMDF was made on the basis of established clinicopathological features [Fig. 1(a)]. [6, 7]

Samples were obtained with informed consent and provision of an opt-out method. The study was approved by the ethical review board of Miyazaki University in accordance with the tenets of the Declaration of Helsinki.

Positive control sample

We used DNA extracted from a pure culture of *P. acnes* as a positive control. The *P. acnes* strain (Japan collection of microorganisms no. 6425) was provided by the RIKEN Bio Resource Centre through the National BioResource Project of the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Negative control sample

An area of the section not containing tissue (i.e., wax block only) was dissected using the LCM system; DNA was extracted from the dissected sample and tested as a negative control sample to assess contamination in the block.

Specimens and Microdissection

The tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Ten-micrometer-thick sections were cut from the paraffin block and mounted on membrane slides (Matsunami Glass, Osaka, Japan and Leica Microsystems GmbH, Wetzlar, Germany). To prevent contamination, the cutting knives were changed for each sample, and the cuts were made vertically from the epidermis to dermis. The membrane slides were coated with bovine serum albumin before mounting. After staining with hematoxylin and eosin, the granulomatous lesions and normal-appearing regions as internal control in dermis were selectively and separately microdissected from the paraffin-embedded tissue sections using the LCM system [PALM MicroBeam, Carl Zeiss MicroImaging, Oberkochen, Germany; Fig. 1(b), (c)]**.** The normal-appearing regions were the portions similar to the area of the granuloma of the LMDF identified, but these portions had little inflammatory cell infiltration.

DNA extraction

DNA was extracted from the microdissected segments using a QIAamp® DNA Micro Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions.

Detection of *P. acnes*

We performed PCR to amplify the 16S ribosomal RNA (rRNA) gene fragments of *P. acnes*. The forward (5′-AAGAAGCACCGGCTAACTACGT-3′) and reverse (5′-TACGAGCCCTTTACGCCCAAT-3′) primers (Life Technologies, Carlsbad, CA, USA) were designed to amplify an 89-base pair (bp) portion of the *P. acnes* 16S rRNA gene (NCBI RefSeq accession number: NC_006085; this reference sequence was derived from AE017283).

RESULTS

Detection of the *P. acnes* 16S rRNA gene

All samples contained the same amount of PCR products of albumin gene, used as an internal control, with a predicted size as 69 bp. The *P. acnes*-specific PCR and electrophoresis revealed that the amplified products of the predicted size (89 bp) were clearly detectable in diverse intensity in all the nine DNA samples from the LMDF granulomas. In addition, the *P. acnes* gene was also detected in the normal-appearing regions, but these bands were fainter than those in the granulomatous lesions in each case (Fig. 2).

DISCUSSION

The aim of this study was to elucidate the pathogenetic factor(s) of LMDF. Histological evaluation of the LMDF lesions showed epithelioid cells/sarcoidal granulomas around the pilosebaceous complex. Several investigators have also reported a correlation between the pilosebaceous units and the lesions of LMDF.[2]

P. acnes, generally present in the hair follicle as a commensal bacterium, is a strong adjuvant that

causes granulomas when injected experimentally into sensitized mice[8] and rabbits.[9] However, Furukawa et al.[10] indicated that no difference was detected between the *P. acnes* isolates from the sarcoid and non-sarcoid tissue from the lymph nodes, lungs, skin, conjunctiva, prostate, and intestine; therefore, the host factors are more critical in sarcoid granuloma formation than the agent factors. Hence, we also postulated that the host factors in case of LMDF may critical as in case of sarcoidosis.

In this study, the *P. acnes* 16S rRNA gene was detected in all samples from the granulomatous lesions. Electrophoresis revealed high-intensity bands in the granulomatous lesions. Moreover, the *P. acnes* gene was also detected in the normal-appearing regions. These bands were weaker or fainter than those in the granulomatous lesions in all individual cases, although a diversity of band density was present between cases. This result may indicate that *P. acnes* also resides in the normal-appearing regions as a latent infection, a result that is similar to that of Eishi.[11] *P. acnes* is present as a commensal in hair follicles. If the hair follicle is destroyed by various agents (e.g., cytokines and chemokines), *P. acnes* is spread out into the dermis, resulting in suppuration, followed by granuloma and/or latent infection.

The present study showed that cell-mediated immunity to *P. acnes* as an antigen may participate in granuloma formation. We postulated that cell-mediated immunity transpired when a hair follicle was destroyed, and *P. acnes* subsequently entered into the dermis.

For several years, LMDF has been regarded as a manifestation of the papular type of rosacea [12]or as a variant of rosacea.[2] Indeed, the *P. acnes* 16S rRNA gene was also detected in tissue samples from granulomas in our all nine cases of acne rosacea (data not shown). Therefore, we hypothesized that the molecular mechanisms in the onset of LMDF were similar to those of granuloma formation in the papular type of rosacea. Recently, cytokines and chemokines are believed to be participating in the pathogenesis of rosacea.[13,14] The pilosebaceous units in LMDF may also be destroyed by various agents including the aforementioned cytokines and chemokines as in case of rosacea. This

event may cause diffusion of *P. acnes* into the dermis and induce subsequent reactions, such as cell-mediated immunity to *P. acnes*. Then, sarcoidal granuloma may develop around the pilosebaceous complex as a result.

In our study, the intensity of the PCR product band showed diversity among the cases studied, even within each case. These results may correspond to the different degrees of individual host immune response to *P. acnes*. Finally, we postulated that there was a hereditary or acquired abnormal cellular immune response to *P. acnes* in some patients with LMDF, similar to patients with sarcoidosis.[15] However, further studies are required to analyze the sensitivity of the host response to *P. acnes.*

In conclusion, *P. acnes* plays a potential role in the pathogenesis of LMDF

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CONFLICT OF INTEREST: The authors have no conflicts of interest to declare.

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Figure Legends

Figure 1. In the LMDF samples, granulomatous lesions were selectively microdissected using laser capture microdissection.

(a): Histopathological examination showed a sarcoidal granuloma in the perifollicular area extending to the lower dermis. The granuloma is composed of histiocytes, epithelioid cells, giant cells, and a few lymphocytes (Case 1). (b) and (c): Before and after microdissection. The isolated granuloma is inserted in (c) (Case 5).

Figure 2. Electrophoresis of polymerase chain reaction (PCR) amplification products of the *Propionibacterium acnes* 16S rRNA gene, and a comparison between the granulomatous lesions and non-granulomatous regions.

The *P. acnes*-specific PCR and electrophoresis reveal**s** that the amplified products of the predicted size [89 base pairs (bp)] are clearly detectable with diverse intensity in all nine DNA samples from the LMDF granulomas. The *P. acnes* gene is also detected in the normal-appearing regions, but their bands are weaker or fainter than those in granulomatous lesions in each case. Moreover, PCR amplification of the albumin gene produced amplicons of the predicted size (69 bp) in all extracted DNA samples. M: size markers (GeneRuler Ultra Low Range DNA Ladder); N: normal-appearing regions; L: granulomatous lesions; NC: negative control (wax block only); PC: positive control (genomic DNA of *P. acnes*); AC: albumin control (human genomic DNA).

Table

Table 1. Cases of LMDF studied

Case	Age (years)	Gender (Male/Female)	Region of the lesion
1	12	M	Cheek
2	57	F	Cheek
3	15	F	Lower eyelid
4	69	F	Cheek
5	9	F	Cheek
6	47	F	Lower eyelid
7	25	M	Lower eyelid
8	30	M	Lower eyelid
9	40	F	Cheek

Fig 1.

(a) (b) (c)

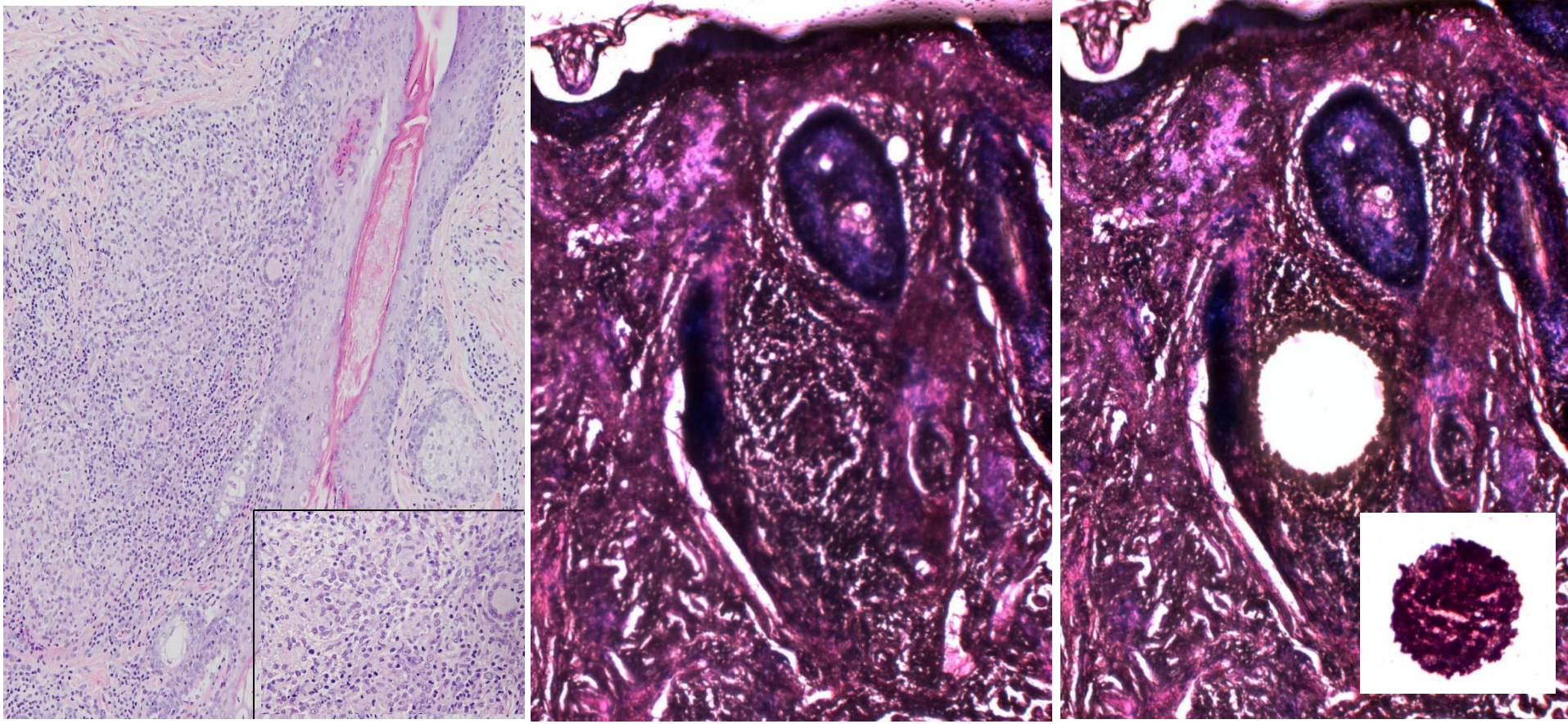
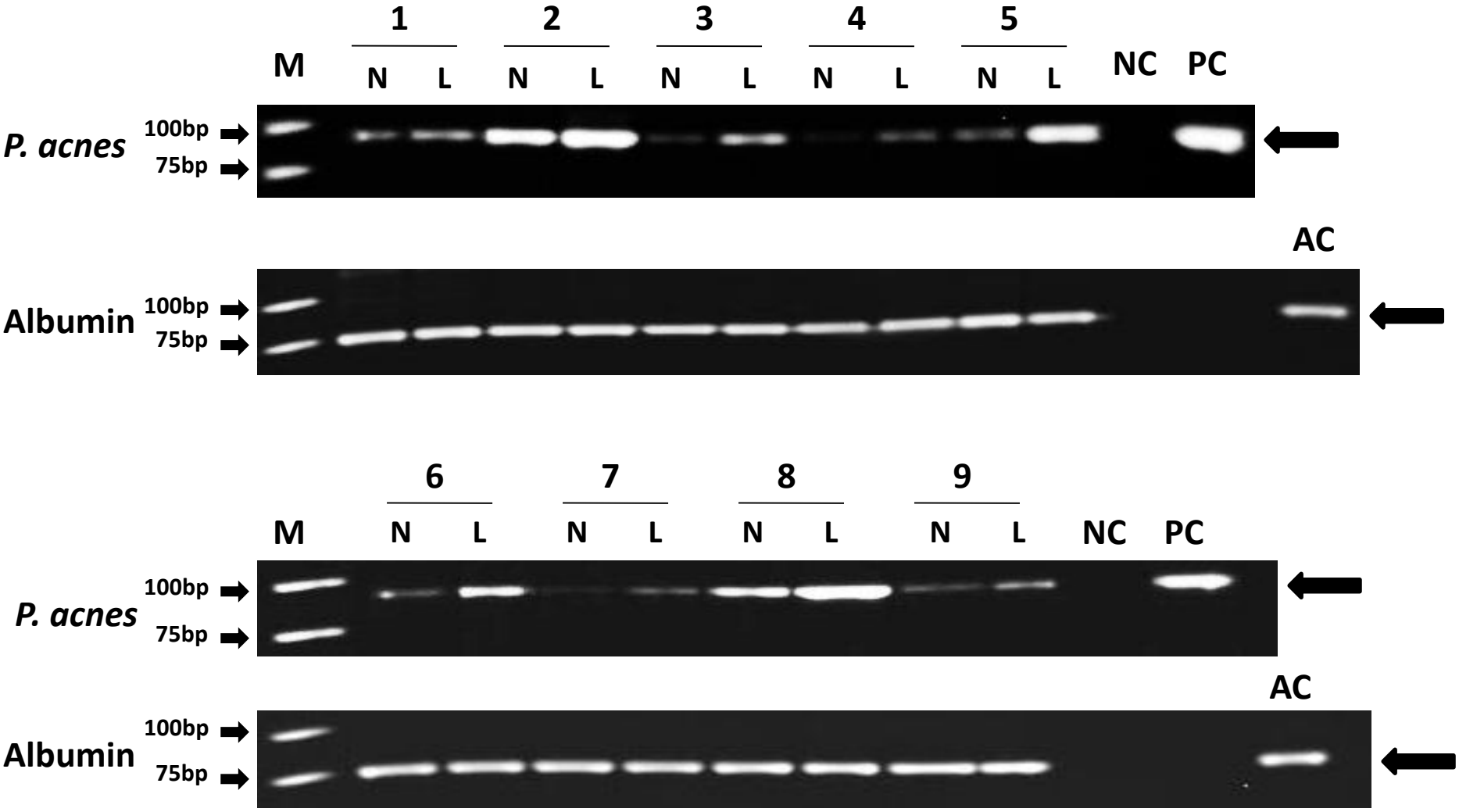


Fig 2.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