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Characterization of *Propionibacterium acnes* isolates from sarcoid and non-sarcoid tissues with special reference to cell invasiveness, serotype, and trigger factor gene polymorphism

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ABSTRACT

Sarcoidosis is a systemic granulomatous disease of unknown etiology. *Propionibacterium acnes* is the only microorganism so far isolated from sarcoid lesions. To examine whether *P. acnes* isolates from sarcoid tissues differ from those obtained from non-sarcoid tissues, we studied cell invasiveness, serotype, and polymorphisms of the *P. acnes* trigger factor protein and the two invasion-associated proteins (named PAmce and PAp60) in 35 *P. acnes* isolates from sarcoid lymph nodes and 127 isolates from non-sarcoid tissues. Most of the serotype I isolates (79/112; 71%), but none of the serotype II isolates (0/50) were cell-invasive. Two prominent types of trigger factors, one with and one without a 15 amino acid-residue deletion, corresponded to serotype II and serotype I, respectively. Non-invasive isolates had genomic mutations that caused more than one amino acid change in either the *PAmce* or *PAp60* gene, with four exceptional isolates. *P. acnes* was finally classified into nine isotypes, and isolates obtained from sarcoid and non-sarcoid tissue did not differ. Although the finding did not link *P. acnes* to sarcoidosis, the present study clarified the cell invasiveness of *P. acnes* and the close correlation of cell invasiveness to the serotype and genotype of the two invasion-associated *P. acnes* genes.

1. Introduction

Propionibacterium acnes is a Gram-positive, non-spore-forming, anaerobic bacillus, found predominantly in the sebaceous gland-rich areas of the skin in adults [1]. It can also be isolated from the conjunctiva, external ear canal, mouth, upper respiratory tract, and intestine [2]. Historically, *P. acnes* has been thought to be of low virulence but was recently found to be the causative agent in various pathologies. *P. acnes* is most notably implicated in acne vulgaris [3], but it is also associated with endophthalmitis [4], endocarditis [5], osteomyelitis [6], prosthetic hip infection [7], severe sciatica [8], and prostatic inflammation [9]. Sarcoidosis, a systemic granulomatous disease of unknown etiology, seems to result from an antigen-driven immune response of a genetically predisposed subject to an environmental agent, possibly an infectious agent [10]. *P. acnes* is the only microorganism that has been isolated from sarcoid lesions [11]. Many *P. acnes* genomes have been detected in sarcoid lymph nodes using quantitative PCR [12,13] and in sarcoid granulomas by *in situ* hybridization [14], thus suggesting an etiologic link between *P. acnes* and some cases of sarcoidosis.

Evaluation of the possible connection of *P. acnes* to the etiology of sarcoidosis is difficult because the bacterium has been cultured from the lungs and mediastinal lymph nodes of some patients with diseases other than sarcoidosis serving as controls [11], and a few *P. acnes* cells have been detected in some superficial, gastric, and mediastinal lymph nodes of other such patients [12,13]. *P. acnes* might also cause latent infection in peripheral lung tissue and mediastinal lymph nodes [15]. As these organs are frequently involved in sarcoidosis, local proliferation of *P. acnes* at the sites of

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latent infection may trigger granulomatous inflammation in sarcoidosis patients.

Although *P. acnes* is a strong candidate among the suspected causative agents of sarcoidosis, it is unclear why it would cause granulomas only in sarcoidosis patients. One possible explanation is that this indigenous bacterium causes granulomatous inflammation only in genetically susceptible subjects who are hypersensitive to certain antigens from indigenous *P. acnes*. Another possibility is that there is a disease-specific strain; that is, the characteristics of *P. acnes* isolates from sarcoid tissues may differ from those of *P. acnes* isolates obtained from non-sarcoid tissues.

Analysis of serotypes and trigger factor gene polymorphisms of *P. acnes* may help to clarify the immune response involved in the etiology. Differences in the antigenic determinants of an immunogen from the indigenous bacterium may contribute to an altered immune response to the bacterium in susceptible hosts. Differences in serotype are indicative of differences in the bacterial antigens that stimulate the host immune response. The *P. acnes* trigger factor protein causes a sarcoidosis-specific immune response [16], and hypersensitivity to the protein results in pulmonary granulomatosis in mice [17].

Characterization of the invasion capacity of the bacterium is another approach that may elucidate the etiology of sarcoidosis. Tanabe et al. [18] reported that intracellular *P. acnes* activated nuclear factor-κB in both a NOD1- and NOD2-dependent manner, and that impaired recognition of intracellular *P. acnes* through NOD1 affects the susceptibility to sarcoidosis in the Japanese population. These results seem to suggest that *P. acnes* strains that invade the epithelial cells are more closely linked to the cause of sarcoidosis, because nonphagocytic epithelial cells express the cytosolic protein NOD1, but not NOD2, whereas macrophages express both NOD1 and NOD2.

Several bacterial proteins are associated with cell invasion. The mammalian cell entry protein is a surface protein of *Mycobacterium tuberculosis* that mediates the uptake of nonpathogenic *Escherichia coli* and latex beads by nonphagocytic mammalian cells [19]. Invasion-associated protein p60 is a surface protein of *Listeria monocytogenes* that is involved in listerial cell attachment [20]. A p60 mutant of the bacterium exhibits diminished ability to invade and multiply within intestinal epithelial cells [21].

The aim of the present study was to clarify whether *P. acnes* isolates from sarcoid tissues differ from those obtained from non-sarcoid tissues. We examined the cell invasiveness and serotype of *P. acnes* isolates from lymph nodes affected by sarcoidosis, together with isolates from non-sarcoid tissue from the lymph nodes, lungs, prostate, skin, conjunctiva, and intestine. Polymorphisms of the *P. acnes* trigger factor (PAtig) protein and the two invasion-associated proteins were also examined by direct DNA sequencing of the genes.

2. Results

2.1. Cell-invasion assay

To determine the cell invasiveness of *P. acnes* isolates, a cut-off value for the invasion assay was determined using three isolates from sarcoid lymph nodes with cell invasiveness and three isolates without cell invasiveness. The invasiveness of these *P. acnes* isolates into Human embryonic kidney cells (HEK293 T cells) was examined by immunohistochemistry with the serotype-specific antibody and the intracellular localization of the invasive isolates was confirmed by electron microscopy (Fig. 1). Each of the six isolates was tested in five separate assays on different days, and a cut-off value for the invasion assay was determined (Fig. 2). *P. acnes* isolates with more than 1×10^5 CFU/well were determined to be invasive and isolates with fewer than 1×10^5 CFU/well were determined to be non-invasive. Fourteen (40%) of the 35 sarcoid isolates and 65 (51%) of the 127 non-sarcoid isolates invaded the cells. The proportion of cell-invasive isolates did not differ between the sarcoid and non-sarcoid tissues ($P = 0.241$).

2.2. Serotyping

Whole-bacterium ELISA with polyclonal antisera and monoclonal antibodies was used to determine the serotype of all 162 isolates (112 serotype I and 50 serotype II strains) with complete concordance of the results between the two assays. The proportions of the two serotypes did not differ between sarcoid and non-sarcoid tissues ($P = 0.364$).

2.3. P. acnes trigger factor gene

DNA sequence analysis of a *trigger factor* gene of 162 isolates revealed a major polymorphism in the *P. acnes*-specific amino acid sequence called the lysine-rich region (lysine-462 to lysine-530), which includes a repetitive 45-bp sequence at two sites (1441–1455 and 1486–1500; Fig. 3). A short 15-amino acid residue (lysine-486 to lysine-500) was deleted in 50 isolates (deletion-type), whereas the fragment was conserved in the remaining 112 isolates (wild-type). Referring to the database sequence (AAT83318), the wild-type isolates were further classified into four subtypes according to the number of amino acid changes as follows: tigWa, tigWb, tigWc, and tigWd with 0, 1, 3, and 13 changes, respectively. The deletion-type isolates were subclassified into two types according to the number of amino acid changes as follows: tigDa and tigDb with 8 and 40 changes, respectively. Many of the amino acid replacements were conservative changes. The proportions of the two most prominent types or of the six subtypes did not differ between the isolates from sarcoid and non-sarcoid tissue ($P = 0.339$).

2.4. P. acnes mce gene

Based on the sequences of the cell-invasive isolates, *P. acnes* isolates were classified into five types according to the number of amino acid changes in the *PAmce* gene as follows: mceA, mceB, mceC, mceD, and mceE with 0, 1, 5, 8, and 27 changes, respectively. Isolates of the mceA and mceB type were similar, with only one change. The three others, mceC, mceD, and mce E, had five amino acid changes in common. There was no significant difference in the proportions of each *PAmce* gene type between sarcoid and non-sarcoid tissue isolates ($P = 0.627$).

2.5. P. acnes P60 gene

Based on the sequences of cell-invasive isolates, *P. acnes* isolates were classified into five types according to the number of amino acid changes in the *PAp60* gene as follows: p60A, p60B, p60C, p60D, and p60E with 0, 5, 8, 8, and 9 changes, respectively. Isolates of the p60C and p60D type had the same number, but different kinds of amino acid changes. Four types, p60B, p60C, p60D, and p60E, had five amino acid changes in common. There was no significant difference in the proportion of each *PAp60* gene type between sarcoid and non-sarcoid tissue isolates ($P = 0.070$).

2.6. Correlation between cell invasiveness and serotype

Cell invasiveness was found in 79 (71%) of the 112 serotype I isolates and in none of the 50 serotype II isolates (Table 1).