Is *Propionibacterium acnes* a probable causative infectious agent in the pathogenesis of sarcoidosis?

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Abstract

More than 120 years have passed since sarcoidosis was recognized for the first time, the exact cause of this systemic granulomatous disease is still unknown. Sarcoidosis is generally considered to be caused by exposure to specific environmental substances to genetically susceptible hosts. *Propionibacterium acnes* (*P. acnes*) has been regarded as one of the most probable causative agents of sarcoidosis, since this ubiquitous bacteria is isolated in culture from biopsy samples of lymph nodes from patients with sarcoidosis. Evidences suggesting that *P. acnes* is involved in the pathogenesis of sarcoidosis are accumulating. These days, intracellular persistence of *P. acnes* is thought to be implicated in not only sarcoidosis but also diseases of the prostate gland and possibly Parkinson's disease. The importance of *P. acnes* as a pathogenic substance is increasing. In this review we would like to summarize researches about *P. acnes* and sarcoidosis.

Key words: sarcoidosis, *Propionibacterium acnes*（*P. acnes*）, pathogenesis

Introduction

Sarcoidosis is a systemic disease that is characterized by the formation of immune granulomas in various organs, mainly the lungs and the lymphatic system. The causes of this disease are still elusive (1). It is widely accepted that sarcoidosis results from exposure of genetically susceptible hosts to specific environmental agents (2). Etiology of this enigmatic disease has been recognized to be ubiquitous around the world, because sarcoidosis affects people of all racial and ethnic groups, affecting both sexes and ages. Environmental studies have shown the association of sarcoidosis susceptibility and selected microbial-rich environments (3, 4, 5). In regard to the role of causative environmental agents, many reports have been demonstrated (2, 6). Molecular and immunological studies have strengthened etiologic links between sarcoidosis and infectious agents. Two agents, mycobacteria and propionibacteria, have been considered as probable causative agents (7, 8). Recent meta-analysis reported a strong relationship between sarcoidosis and these two microbes. In this meta-analysis, the odds ratios (OR) derived from 11 studies about the association of *P. acnes* and sarcoidosis was 18.8 (95% CI: 12.62-28.01), and the OR about mycobacteria was 6.8 (95% CI: 3.73-12.39) (9). Other pathogens including mycoplasma, borrelia, fungi, viruses, aluminum, zirconium, talc, pine tree pollen have lacked wide confirmation by many research groups, because there is little clinical or microbiological evidence that these pathogens are related to the etiology of sarcoidosis. In Japan, since *P. acnes* was isolated in culture from biopsy samples of lymph nodes from patients with sarcoidosis (10, 11), this bacteria has been thought to be one of the most causative agent in the pathogenesis of sarcoidosis.

Granulomatous reactions are the immunological products of continuing presence of a poorly degradable antigen (3). Eishi and colleagues suggest that *P. acnes* can cause latent infection in the lungs and lymph nodes and exist in a cell wall-deficient form which can endogenously activate triggering granulomatous inflammation under certain environmental conditions (12). There are increasing evidences that intracellular persistence of *P. acnes* is associated with benign prostate hypertrophy (BPH) and prostatic cancer and may be implicated in Parkinson's disease (13). It was also reported that *P. acnes* has been associated with chronic recurrent multifocal osteomyelitis (CRMO), synovitis-pustulosis-hyperostosis and osteitis (SAPHO) syndrome and sciatica (14, 15). The relationship between *P. acnes* and sarcoidosis attracts more attention these days.

Propionibacterium acnes

P. acnes is a slow growing, pleiomorphic rod-shaped, anaerobic-aerotolerant Gram-positive bacillus that resides in pilosebaceous follicles of the skin. Although this bacteria was named *Bacillus acnes* or *Corynebacterium parvum* (*acnes*), it was reclassified to propionibacteria genus in 1974 because it was an obligate anaerobe bacterium having propionate synthetic

ability and could not compose formic acid (16). *P. acnes* is found in the oral cavity, conjunctiva (17), external ear canal (18), and intestinal tract (19). It is well accepted that this microorganism is involved with acne vulgaris, however the exact mechanisms have not been fully unraveled (20). This bacteria, that has the ability to initiate an inflammatory response in the pilosebaceous follicle, is recognized to be involved in the inflammatory phase of acne because it has been shown that this microorganism induces the mononuclear cells and keratinocytes to release proinflammatory cytokines including IL-1β, IL-8, IL-12 and TNF-α and activate and upregulate Toll-like receptors 2 and 4 (21, 22, 23). *P. acnes* has been also thought to be an opportunistic pathogen that causes post-operative and device-related infections (24, 25) such as ocular implants (26), neurosurgical shunts (27), cardiovascular devices (28), breast implants (29, 30), and prosthetic joints (31). *Propionibacteria* and *mycobacteria* are intracellular pathogens and, it is known that they can persist in macrophages because of the high lipid content of their cell walls (32). Evidences that indicate the etiological links between *P. acnes* and sarcoidosis can be divided as follows: 1. detection of *P. acnes* by tissue culture, 2. isolation of microbial components in sarcoidosis lesions, 3. hypersensitivity to this microorganism, 4. cytokine responses induced by components of this bacteria, 5.immunohistochemistry, and 6.experimental models.

Detection of *P. acnes* by tissue culture

In 1970, Homma et al. started research to find the etiological agent of sarcoidosis, only *P. acnes* was isolated from the large number of samples (10). Abe et al. reported that 31 (78%) of 40 sarcoidosis lymph nodes from 40 patients showed positive culture of *P. acnes*, whereas this microorganism was isolated from 38 (21%) of 180 lymph nodes from non-sarcoidosis patients, the difference being significant ($P<0.01$) (11). These days, it was reported by de Brouwer that *P. acnes* was cultured from mediastinal lymph nodes from two non-Japanese patients with sarcoidosis (33). *P. acnes* is so far the only bacterium to be isolated in culture from biopsy samples of lymph nodes from patients with sarcoidosis.

The lungs are constantly exposed to the substance including microorganisms in the air. Although it has been believed for a long time that the lower respiratory tract is germ free, *P. acnes* was cultured from the lungs of 8 (33%) of 24 untreated mice (34). Ishige et al. revealed the existence of *P. acnes* in the peripheral lung and mediastinal lymph nodes (35). They cultured peripheral lung tissues and mediastinal lymph nodes from patients with diseases other than sarcoidosis. *P. acnes* was detected in 24 (56%) of 43 lung tissues and 8 (73%) of 11 mediastinal lymph nodes, which may suggest that this microorganism usually resides in the lower respiratory tract.

Isolation of microbial components in sarcoidosis lesions

Ishige et al. used quantitative PCR to detect bacterial genomes in lymph nodes from patients with sarcoidosis, tuberculosis, and gastric cancer as controls (36). Genomes of *P. acnes* were found in 12 (80%) of 15 patients with sarcoidosis, two (13%) of 15 tuberculosis patients, and three (20%) of 15 controls. Many *P. granulosum* genomes were detected from biopsy samples from three patients with sarcoidosis without *P. acnes* genomes.

An international collaborative study was performed (37). Using quantitative real-time PCR (QPCR), the numbers of bacterial genomes were measured. Either *P. acnes* or *P. granulosum* was found in 106 (98%) of the 108 sarcoid samples. *M. tuberculosis* was found in 0 to 9% of the sarcoid samples but in 65 to 100% of the tuberculosis samples.

Zhou et al. measured the ribosomal RNA (rRNA) of *P. acnes* and *P. granulosum* of lymph node biopsy samples from Chinese patients. *P. acnes* or *P. granulosum* rRNA was detected in 48 (74%) of the 65 sarcoidosis samples but only in four (9%) of the 45 tuberculosis samples and three (6%) of the 50 control samples (38). They also reported high amounts of *P. acnes* 16SrRNA in all the 17 lymph node biopsy samples of patients with sarcoidosis, whereas it was not detected in the 8 patients with tuberculosis and 11 patients with non-infectious lung diseases (control group) (39).

Sarcoidosis involved the lungs and mediastinal lymph nodes most frequently (40), and the lungs are the first target organ (41). Hiramatsu et al. reported that, using nested PCR for 16S rRNA of *P. acnes*, bacterial genomes were detected in bronchoalveolar lavage (BAL) cells from 21(70%) of 30 patients with sarcoidosis and 7(23%) of 30 control patients with other lung diseases (42). Ichikawa et al. showed that, using QPCR, the numbers of *P. acnes* DNA extracted from BAL cells from patients with sarcoidosis were significantly higher than those from control patients (43) (Fig. 1).

Figure 1

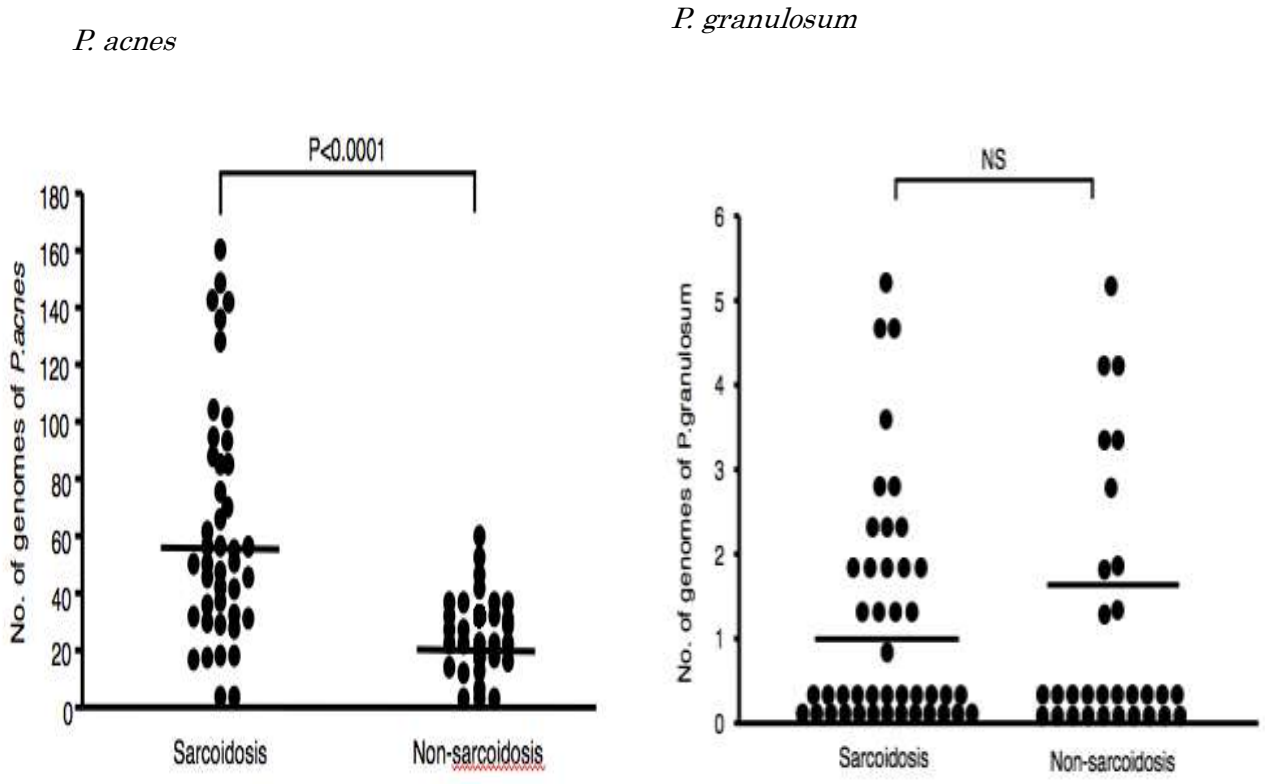


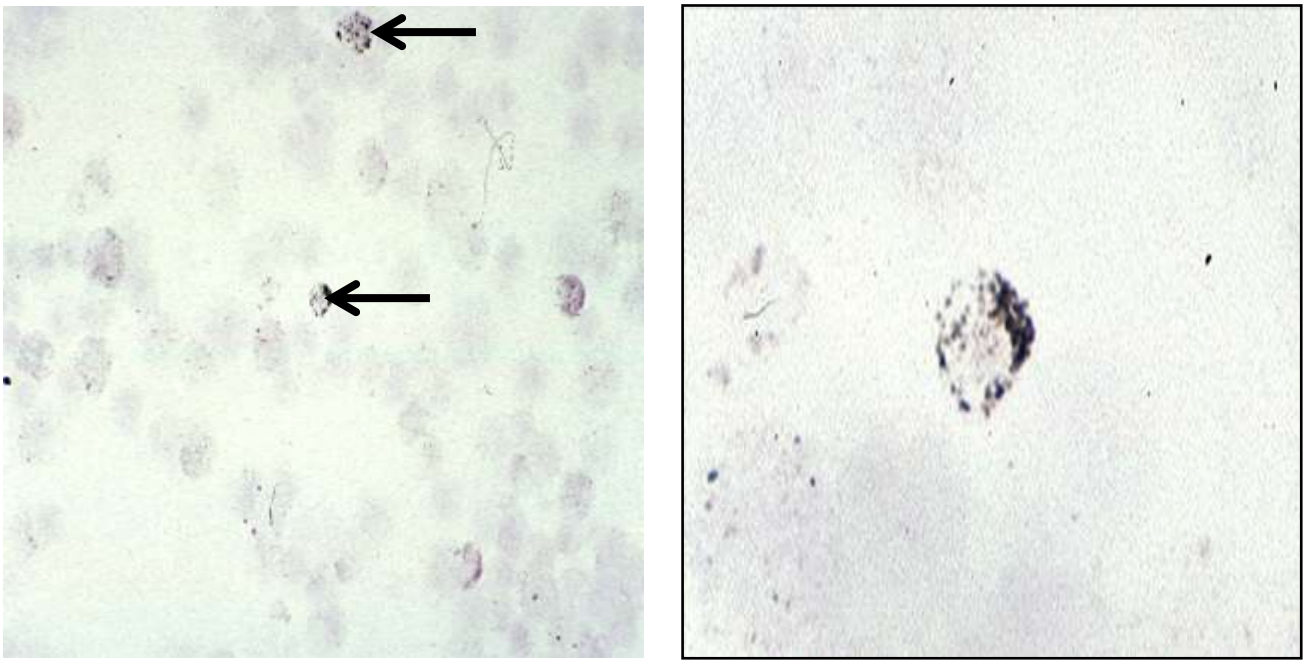
Fig.1. Real-time PCR measurement of *P. acnes* and *P. granulosum* DNA extracted from BAL cells in 42 patients with sarcoidosis and 30 patients with non-sarcoidosis lung diseases. The y axis shows the number of genomes of *P. acnes* and *P. granulosum* in 500 ng of total DNA from BAL cells. The horizontal line in each group represents the mean expression. A significant difference was found in *P. acnes* measurement.

In situ hybridization and PCR

To detect the genomes of *P. acnes* in sarcoid lymph nodes and alveolar macrophages by in situ hybridization (ISH) and in situ PCR may help to explain the etiologic link between sarcoidosis and this microorganism.

Yamada et al. examined the existence of 16 S rRNA of *P. acnes* in biopsy samples of lymph nodes from patients with sarcoidosis, tuberculosis and nonspecific lymphadenitis by ISH using catalyzed reporter deposition (CARD) for signal amplification with digoxigenin-labeled oligonucleotide probes (44). In sarcoid samples, many signals were detected in the cytoplasm of some epithelioid cells in granulomas and of many mononuclear cells around granulomas. Hiramatsu et al. showed in situ signals of *P. acnes* DNA in the cytoplasm of 0.2% to 2.8% of alveolar macrophages among the BAL cells from patients with sarcoidosis, but no signals from patients with other lung diseases (42) (Fig. 2).

Figure 2



(A: ×400) (B: ×1000)

Fig. 2. BAL macrophages from a patient with sarcoidosis with signals after in situ PCR for *P. acnes* DNA. A: Signals were found in the cytoplasm of the two small macrophage from BAL (×400). B: High magnification (×1000) of one macrophage

Nakata et al. showed the hypersensitivity to *P. acnes* in BAL cells from patients with sarcoidosis (45). The proliferation of BAL lymphocytes evoked by crude extract of *P. acnes* with pyridine was significantly higher in untreated sarcoidosis patients than in treated sarcoidosis patients and controls.

Ebe and colleagues searched for *propionibacterial* antigens that induced cellular immune responses only in patients with sarcoidosis (46). They found RP35 that is a recombinant protein obtained from a λ gt-11 genomic DNA expression library from *P. acnes* and the C-terminal region of *P. acnes* trigger factor, and showed that RP35 induced sarcoidosis specific proliferation of peripheral blood mononuclear cells (PBMCs) in 9 (18%) of 50 patient with sarcoidosis.

Cytokine responses induced by components of *P. acnes*

Sarcoidosis results from exposure of genetically susceptible hosts to specific environmental agent(s), and is a chronic granulomatous disorder characterized by an accumulation of lymphocytes and macrophages in the alveoli (1, 2, 45). Increased expression of several proinflammatory

cytokine in BAL cells from patients with sarcoidosis has been shown (47). The exposure to causative antigen (*P. acnes* and so on) leads to an activation of macrophages, a helper T cell type 1 (Th1) immune response against the antigen mediated by antigen processing and presentation by macrophages, and finally to induction of granuloma formation (Figure 3).

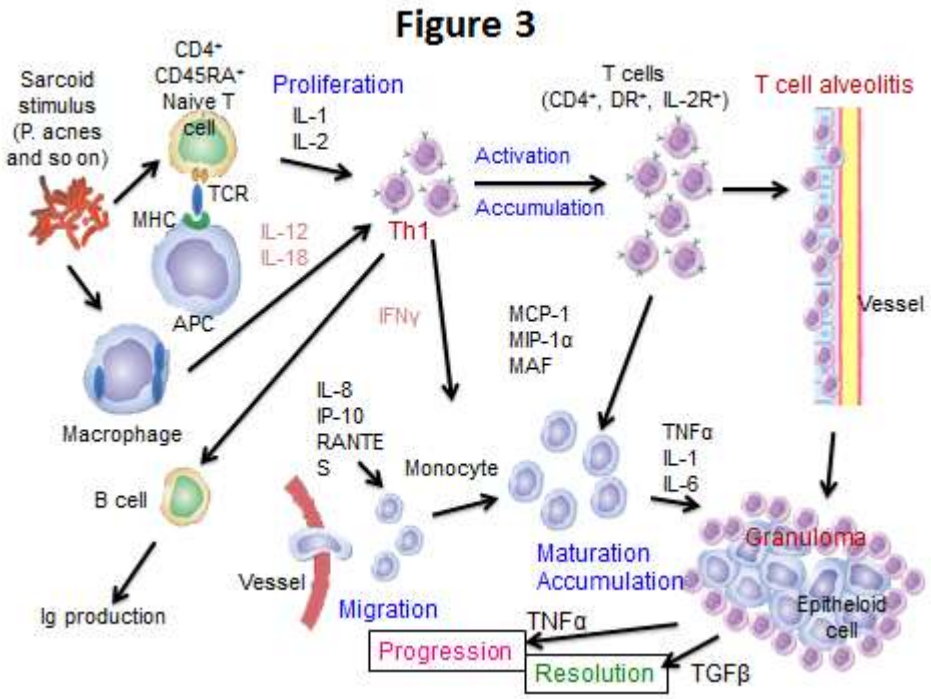


Fig. 3. Immunopathogenesis of sarcoidosis.

If some sarcoidosis cases were caused by *P. acnes*, an antigen from this indigenous bacterium may provoke a Th1 immune response in the lesions.

Mori et al. showed Interleukin-2 (IL-2) production and IL-2 receptor expression of alveolar lymphocytes, stimulated by the *P. acnes* antigen, obtained from patients with and without sarcoidosis, was significantly higher in untreated sarcoidosis patients than in control subjects and treated sarcoidosis (48) (Figure 4).

Figure 4

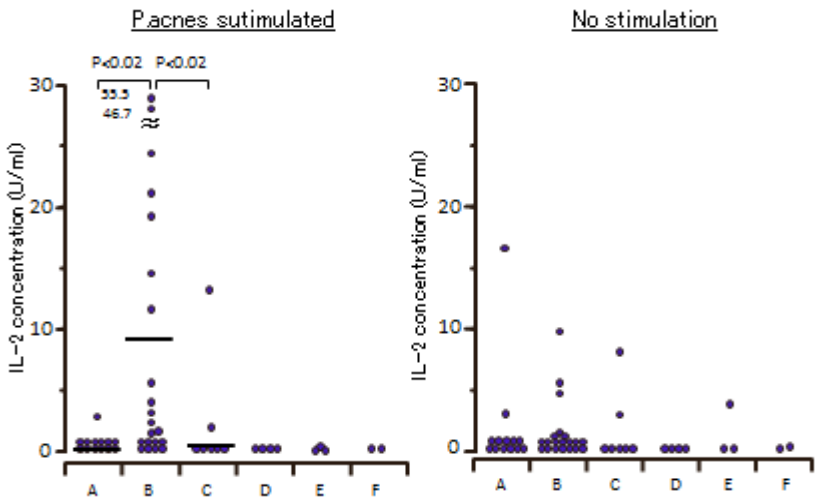


Fig. 4. Interleukin-2(IL-2) activities of a fluid released from cultured alveolar lymphocytes stimulated by *P. acnes*. The y axis shows the number of IL-2 concentration. IL-2 activities of untreated sarcoidosis patients were significantly higher than those of control and treated sarcoidosis patients. A: controls, B: untreated sarcoidosis, C: treated sarcoidosis, D: pneumoconiosis, E: hypersensitivity pneumonitis, F: idiopathic interstitial pneumonia.

Furusawa and colleagues reported that IL-2 secretion from PBMCs obtained from patients with sarcoidosis after stimulation with viable *P. acnes* is significantly higher than from healthy volunteers (49). They also showed IL-2 and IL-12 mRNA expression in PBMCs after stimulated with *P. acnes* was significantly higher from patients with sarcoidosis than from controls, and IL-17 mRNA expression was significantly lower in PBMCs from patients with sarcoidosis than in PBMCs from controls.

TNF-α and GM-CSF production of BAL cells from patient with sarcoidosis after stimulation with heat-killed *P. acnes* were significantly higher than from healthy volunteers (50).

Interferon (IFN)-γ secretion from PBMCs obtained from patients with sarcoidosis after stimulation with *P. acnes* catalase is significantly higher than from healthy volunteers and other pneumonitis patients (51).

Stimulation with *P. acnes* can cause the Th1 immune responses in sarcoidosis patients.

Immunohistochemistry

In the late 1980s, using immunofluorescence and immunoperoxydase staining techniques, *P. acnes* was detected in biopsy samples of sarcoid lymph nodes. Clusters of *P. acnes* were located in epithelioid cell granulomas, lymphocyte aggregated areas and in the lumens of small

vessels (52).

To evaluate the pathogenic role of *P. acnes*, Negi et al. produced PAB antibody that was obtained by immunization of the bacterial lysate, and TIG antibody obtained from immunization of the recombinant trigger-factor protein (53). They reported that small round bodies were detected within sarcoid granulomas by immunohistochemistry with PAB antibody in 20/27 (74%) video-assisted thoracic surgery lung samples, 24/50 (48%) transbronchial lung biopsy samples, 71/81 (88%) Japanese lymph node samples, and 34/38 (89%) German lymph node samples.

There were reports in which, using specific monoclonal antibodies, *P. acnes* was detected in tentorial sarcoidosis (54), neurosarcoidosis (55), ocular sarcoidosis (56), and cardiac sarcoidosis (57).

Experimental models

Many experimental animal models have contributed to understand the pathology of sarcoidosis. Intravenous injection of heat-killed *P. acnes* to mice induced granulomatous formation in the liver (58, 59, 60), but not in the lung. Intratracheal challenge with heat-killed *P. acnes* to nonsensitized C57BL/6 mice did not induce the development of granulomatous changes (61, 62), but challenging with viable bacteria could cause pulmonary granulomas (63). Challenging with *P. acnes*-primed helper T cells intravenously to immunized mice induced pulmonary granulomatous changes (61). Murine model of sensitizing and challenging with heat-killed *P. acnes* led to peribronchovascular granulomatous inflammation (64) and fibrosis (65). Iio et al. reported that subcutaneously sensitized mice with heat-killed *P. acnes* and complete Freund's adjuvant (CFA) developed epitheloid cell granulomas in the lungs, and these mice showed an increased number of T-lymphocytes, especially CD4+ cells, and elevation of the ratio of CD4+/CD8+ in BAL fluid (66). Experimentally made granuloma formation was aggravated in mice deficient of intercellular adhesion molecule (ICAM)-1 (67). Priming with *P. acnes* to mice sensitized with recombinant -protein RP35 and CFA could induce pulmonary granulomas (68). It was also reported challenging with *P. acnes* produced pulmonary granulomatous inflammation in the lungs of sensitized rabbits and rats (69, 70).

These days, it is generally accepted that the pathogenesis of sarcoidosis is mediated by a panel of immune reactions mediated by both the innate and the adoptive immune system (71, 72). *P. acnes* may induce a direct inflammatory response via TRL1 (64) and TLR2 (73). TLR9 is essential for the induction of granuloma formation and the production of INF-γ by *P. acnes* (74).

Nucleotide binding and oligomerization domain (NOD)-like receptors (NLRs) are component of the innate immune system that recognize pathogens in the cytosol. One study revealed enhanced expression of TNF and IL-12/IL-23p40 via NOD1 pathway in a subset of sarcoidosis patient (75). Tanabe et al. showed that the impaired recognition of intracellular *P. acnes*

through NOD1 reduced NF-κB activation, causing the increased susceptibility of sarcoidosis (76).

It is recently recognized that autophagy dependent mechanisms are related to the pathogenesis of several inflammatory diseases, such as infectious diseases, Crohn's disease, cystic fibrosis, pulmonary hypertension and chronic obstructive disease (77). Intracellular persistency of *P. acnes* in macrophages (78) and autophagy induced by intracellular infection of this microorganism (79) were reported. These date suggested autophagy may contribute to the pathogenesis of sarcoidosis.

Treatment

Although systemic corticosteroids remain the standard treatment for symptomatic sarcoidosis because of the short-term benefits, there is little evidence for a long-term effect (1). Owing to the concern over side-effects of long term use of corticosteroids, steroid-sparing agents have been searched.

As latent infection of *P. acnes* may endogenously activate triggering granulomatous inflammation under certain environmental conditions (12), antibiotic treatment strategy to eradicate the bacterium is considered. Bachelez et al. reported tetracycline was effective in 10 of 12 patients with cutaneous sarcoidosis (80). Case reports showed treatment with minocycline (MINO) was effective to ocular and ocular adnexal sarcoidosis (81), and muscular sarcoidosis (82). The efficacy of clarithromycin (CAM) to a Japanese female suffering from systemic sarcoidosis was reported (83). It was reported that combination therapies of MINO and CAM improved the multiple endobronchial sarcoidosis (84). The effect of these antibiotics may be not only due to bacterial eradication, but also due to their anti-inflammatory or immunomodulatory effects.

Because all of these reports are studies in a small number of cases, and the effective rate of antibiotic therapy in clinical practice seems to be low (85), a new scheme of antibiotic treatment may be needed. In Japan, J-ACNES trial (Japanese antibacterial drug management for cardiac sarcoidosis trial) which is designed to evaluate the efficacy of add on combination therapy of MINO and CAM to the cardiac sarcoidosis patients treated with standard glucocorticoid therapy, is now ongoing. The results of this trial are expected.

Conclusion

Molecular, genetic, and immunologic researches these days strengthen the hypothesis that infectious agents, particularly propionibacteria or mycobacteria, are related to the etiology of sarcoidosis. Intracellular persistence of *P. acnes* may cause immune reactions mediated by the innate and the adoptive immune system, and induce the granuloma formation, in genetically susceptible patients under certain conditions. It is expected that new therapeutic strategies will be developed through accumulation of investigations about the pathogenesis of sarcoidosis and result in satisfactory improvement

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