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ORIGINAL ARTICLE

Catalase expression of *Propionibacterium acnes* may contribute to intracellular persistence of the bacterium in sinus macrophages of lymph nodes affected by sarcoidosis

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Abstract

Bacterial catalase is important for intracellular survival of the bacteria. This protein of *Propionibacterium acnes*, one of possible causes of sarcoidosis, induces hypersensitive Th1 immune responses in sarcoidosis patients. We examined catalase expression in cultured *P. acnes* isolated from 19 sarcoid and 18 control lymph nodes and immunohistochemical localization of the protein in lymph nodes from 43 sarcoidosis and 102 control patients using a novel *P. acnes*-specific antibody (PAC) that reacts with the catalase protein, together with the previously reported *P. acnes*-specific PAB and TIG antibodies. High catalase expression of *P. acnes* cells was found during stationary phase in more isolates from sarcoid than from non-sarcoid lymph nodes and was associated with bacterial survival under H_2O_2-induced oxidative stress. In many sarcoid and some control lymph nodes, catalase expression was detected at the outer margins of PAB-reactive Hamazaki-Wesenberg (HW) bodies in sinus macrophages, the same location as catalase expression on the surface of cultured *P. acnes* and the same distribution as bacterial cell membrane-bound lipoteichoic acid in HW bodies. Some or no catalase expression was detected in sarcoid granulomas with PAB reactivity or in clustered paracortical macrophages packed with many PAB-reactive small-round bodies. HW bodies expressing catalase may be persistent *P. acnes* in sinus macrophages whereas PAB-reactive small-round bodies with undetectable catalase may be activated *P. acnes* proliferating in paracortical macrophages. Intracellular proliferation of *P. acnes* in paracortical macrophages may lead to granuloma formation by this commensal bacterium in sarcoidosis patients with Th1 hypersensitivity to certain *P. acnes* antigens, including catalase.

Keywords Sarcoidosis · *Propionibacterium acnes* · Catalase · Monoclonal antibody

Introduction

Sarcoidosis is an idiopathic systemic granulomatous disease that may be due to exposure of a genetically susceptible individual to an environmental antigen [1]. The most commonly implicated etiologic agents of sarcoidosis are mycobacterial and propionibacterial organisms [2–4].

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The only microorganism that has been isolated from sarcoid lymph nodes by bacterial culture is *Propionibacterium acnes*, although isolation of this bacterium is not specific to the disease [5, 6]. The amount of *P. acnes* DNA detected by quantitative real-time polymerase chain reaction in sarcoid lymph nodes suggests that this bacterium proliferates at the site of latent infection in sarcoidosis patients [7, 8]. Histologic localization of *P. acnes* within sarcoid granulomas by in situ hybridization [9] and immunohistochemistry (IHC) [10] suggests that this bacterium is a cause of granuloma formation. Eishi hypothesized that sarcoid granuloma is caused by this commensal bacterium only in susceptible subjects with Th1 hypersensitivity to certain *P. acnes*-specific antigens [11, 12].

Th1 hypersensitivity to viable *P. acnes* is reported in patients with sarcoidosis [13], and *P. acnes* antigens responsible for the allergic reaction have been explored by evaluating the immunoreactivity of patient sera on Western blot membranes with a *P. acnes* genomic DNA expression library [14] or bacterial whole-cell lysate of *P. acnes* [15]. The antigenic protein identified by screening a *P. acnes* DNA expression library is a ribosome-bound trigger-factor protein (TIG) that induces disease-specific mitogenic responses in 18% of sarcoidosis patients [14]. Another antigen identified by screening *P. acnes* whole-cell lysate is a catalase protein that induces hypersensitive Th1 immune responses in 58% of sarcoidosis patients [15].

Bacterial catalase is an enzymatic antioxidant important for intracellular survival of the bacterium in macrophages by inducing resistance to phagocyte-mediated killing [16]. *P. acnes* catalase may contribute not only to Th1 hypersensitivity of sarcoidosis patients but also to the bacterial competency that allows this commensal bacterium to persist in macrophages at the sites of latent infection, eventually followed by endogenous activation of the latent *P. acnes*, resulting in granuloma formation in susceptible subjects with Th1 hypersensitivity to certain *P. acnes* antigens, such as trigger-factor and catalase proteins [11, 12].

Here, we developed a novel *P. acnes*-specific monoclonal antibody (PAC antibody) targeting the *P. acnes* catalase protein. Using this novel antibody, we examined catalase expression in *P. acnes* isolated from the lymph nodes of patients with or without sarcoidosis. Immunohistochemistry with the PAC antibody was performed to locate the *P. acnes* catalase protein in these lymph node samples. The results from the culture experiments of *P. acnes* clinical isolates and histologic analysis with *P. acnes*-specific antibodies, including the novel PAC antibody, are discussed with regard to the etiology of sarcoidosis as an allergic endogenous infection caused by this commensal bacterium.

Materials and methods

Clinical samples

Lymph node samples that were formalin-fixed and paraffin-embedded (FFPE) were obtained from 43 sarcoidosis patients and 102 control patients (35 with gastric cancer, 29 with necrotizing lymphadenitis, and 38 with reactive lymphadenitis) between April 1995 and March 2017, at the Tokyo Medical and Dental University Hospital, Japanese Red Cross Medical Center, National Hospital Organization Tokyo National Hospital, and Tokyo Kita Medical Center.

Sarcoidosis was diagnosed on the basis of both histologic and clinical findings following the recommendations of the American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders on Sarcoidosis [1]. Necrotizing and reactive lymphadenitis was used as a control and was diagnosed and histologically confirmed at each hospital before the study. The samples obtained from cancer patients were all metastasis-free draining lymph nodes of the primary cancer tissue. The clinical characteristics of the sarcoidosis and control patients from whom lymph node samples were obtained are provided in Table 1.

The study was approved by the ethics committees of each participating hospital: Tokyo Medical and Dental University Hospital (reference M2017-336, April 17, 2018), Japanese Red Cross Medical Center (reference 625, June 1, 2018), National Hospital Organization National Hospital (reference 407, September 26, 2018), and Tokyo Kita Medical Center (reference 221, May 30, 2018). All of the ethics committees waived the requirement for informed consent.

P. acnes strains and bacteria culture

All of the clinical isolates of *P. acnes* used for the study were collected previously [17–19]. A representative sarcoid isolate of phylotype I *P. acnes* (C1 strain) was previously used for complete genome sequencing [20] and for the identification of *P. acnes* catalase protein by matrix-assisted laser desorption/ionization time-of-flight/mass spectrometry analysis [15]. Reference strains of *P. acnes* used in the experiments were phylotype I (ATCC 6919) and phylotype II (ATCC 11828) from the American Type Culture Collection (Manassas, VA, USA), similar to our previous report [18]. Of the 37 *P. acnes* isolates evaluated, 19 were isolated from 19 lymph nodes of sarcoidosis patients and 18 were isolated from 18 lymph nodes draining from the lung, stomach, or colon with primary cancer (7, 8, and 3 strains, respectively). Stored *P. acnes* isolates were cultured in Gifu anaerobic medium (GAM) broth (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) at 37 °C under anaerobic conditions (10% H_2, 10% CO_2, 80% N_2) for 2 days.

Table 1 Comparison between sarcoidosis and control patients on the basis of available clinical profiles

			Control disease		
	Patients with sarcoidosis ($n = 43$)	Patients with control disease ($n = 102$)	Gastric cancer ($n = 35$)	Necrotizing lymphadenitis ($n = 29$)	Reactive lymphadenitis ($n = 38$)
Subjects (men/women)	18/24	52/50	24/11	10/19	18/20
Age, years	56.9 ± 16.0	48.4 ± 21.1[*1]	67.3 ± 12.0[*2]	31.4 ± 12.0[*3]	44.8 ± 19.5[*4]
Location of lymph node biopsy					
Hilum	10	1			1
Mediastinal	7				
Tracheal	2				
Abdominal	3	37	35		2
Para arterial	1				
Cervical	9	49		27	22
Inguinal	4	7			7
Supraclavicular	4	1			1
Submandibular		1			1
Axilla		6		2	4
Anterior scalen	3				

[*1] $P = 0.017$, [*2] $P = 0.003$, [*3] $P < 0.001$, [*4] $P = 0.004$, compared with sarcoidosis patients (*t* test)

Production of anti-*P. acnes* catalase monoclonal antibody

We developed a novel *P. acnes*-specific monoclonal antibody targeting the *P. acnes* catalase protein using previously described methods [21] with modifications. A full-length recombinant catalase protein from *P. acnes* was constructed using the previously published database and used to immunize BALB/c mice (CLEA Japan, Tokyo, Japan) [20]. Anti-catalase antibodies produced by hybridoma cell lines were evaluated using an enzyme-linked immunosorbent assay (ELISA) with the catalase protein as the immunogen. The positive hybridoma cell lines were screened by IHC of FFPE lymph node tissue sections from a patient with sarcoidosis. The hybridoma clone that produced the most intense positive reaction by IHC was implanted into the intraperitoneal space of mice with severe combined immunodeficiency (CLEA Japan). Two weeks after implantation, ascites was collected and used as an undiluted antibody without further purification. This antibody (IgG2b, kappa) was named PAC antibody and used in the present study. The specificity of the PAC antibody was evaluated by IHC using FFPE bacterial cell pellets from each strain described in Table 2. The Institutional Animal Care and Use Committee of Tokyo Medical and Dental University approved all the animal experimental protocols.

Western blotting

The specificity of the PAC antibody was also examined by Western blotting using whole-cell lysates from either *P. acnes* (C1 and ATCC strains), other cutaneous propionibacteria (*Propionibacterium granulosum* and *Propionibacterium avidum*), dairy propionibacteria (*Propionibacterium jensenii*, *Propionibacterium thoenii*, and *Propionibacterium acidipropionici*), *Mycobacterium tuberculosis*, other catalase-positive bacteria and fungi, or mammalian cells (Table 2). Catalase expression of a sarcoid isolate of *P. acnes* C1 strain was also examined by Western blotting using whole-cell lysate of the bacterium harvested at each timepoint of culture. Western blotting was performed according to the previously described methods [22].

Measurement of *P. acnes* catalase expression

Sandwich ELISA for measuring *P. acnes* catalase expression was performed using the PAC antibody (1 μg/well) as a capture antibody, *P. acnes* lysate samples (12 mg wet weight of bacteria/well) and standard samples of recombinant catalase protein (0.12–250 ng/mL), and rabbit anti-*P. acnes* catalase polyclonal antibody (produced by Eurofins Genomics, Ebersberg, Germany), followed by biotinylated swine anti-rabbit immunoglobulins (DAKO; Glostrup, Denmark) as detection antibodies. The ELISA procedure was carried out according to the previously described methods [23].

Bacterial oxidative stress experiment

The resistance of *P. acnes* against oxidative stress was examined by bacterial culture using GAM broth and GAM broth supplemented with 0.04% hydrogen peroxide (H_2O_2) every

Table 2 Specificity of PAC antibody examined by Western blotting and IHC

Bacterial species	Strain	Reactivity of PAC antibody by:	
		Western blotting	IHC
Propionibacteria sp.			
Propionibacterium acnes	C1	+	+
Propionibacterium acnes	ATCC 6919	+	+
Propionibacterium acnes	ATCC 11828	+	+
Propionibacterium acidipropionicum	ATCC 25562	–	–
Propionibacterium avidum	ATCC 25577	–	–
Propionibacterium granulosum	ATCC 25564	–	–
Propionibacterium jensenii	ATCC 4868	–	–
Propionibacterium thoenii	ATCC 4874	–	–
Mycobacteria sp.			
Mycobacterium tuberculosis	ATCC 25177	–	–
Catalase-positive bacteria			
Escherichia coli	ATCC 25922	–	–
Helicobacter pylori	ATCC 43504	–	–
Nocardia asteroids	ATCC 19247	–	–
Staphylococcus aureus	ATCC 25923	–	–
Staphylococcus epidermidis	ATCC 14990	–	–
Staphylococcus warneri	ATCC 27836	–	–
Catalase-positive fungi			
Aspergillus fumigatus	ATCC 1022	–	–
Candida albicans	ATCC 20308	–	–
Mammalian cells			
A549		–	–
HeLa		–	–
Raw264.7		–	–
THP-1		–	–

IHC, immunohistochemistry

24 h. In the experiment, two representative isolates with either high or low catalase expression were selected from sarcoidosis or control lymph nodes, respectively, according to the results obtained by sandwich ELISA. Bacterial density was determined by measuring cell optical density at 600 nm (OD_{600}). *P. acnes* cell suspension after culture for 2 days was adjusted to $OD_{600} = 0.01$, and the bacterial density was then measured every day for the following 4 days.

Immunohistochemistry

Enzyme IHC with the PAC antibody (diluted 1:4000 as the primary antibody) was performed according to the methods described in a previous study [10] that examined sarcoid and non-sarcoid lymph nodes by IHC with other two *P. acnes*-specific monoclonal antibodies (PAB and TIG antibodies that react with *P. acnes* lipoteichoic acid and trigger-factor protein, respectively).

Double-fluorescence IHC and enzyme IHC for immunoelectron microscopy were performed to further clarify the localization of the catalase protein and the other two *P. acnes* cell components using a representative sarcoid lymph node, according to the previously described methods [23]. To locate catalase expression of cultured *P. acnes* cells, immunoelectron microscopy was also performed using FFPE sections of the cell pellets.

Statistical analysis

Statistical analyses were performed using GraphPad PRISM ver. 6 (GraphPad Software, Inc., San Diego, CA, USA). Differences in the clinical profiles between the patients with sarcoidosis and those with control diseases were evaluated by *t* test. Comparison of the *P. acnes* catalase expression levels between isolates from sarcoid and control lymph nodes was performed using the Mann–Whitney *U* test. Evaluation of the difference of *P. acnes* growth curves in culture medium with or without H_2O_2 was performed by two-way analysis of variance. Differences in the detection frequency of *P. acnes* by IHC with the PAC, PAB, and TIG antibodies between sarcoid

lymph nodes and each control group were evaluated by Fisher's exact test. A *P* value of less than 0.05 was considered statistically significant.

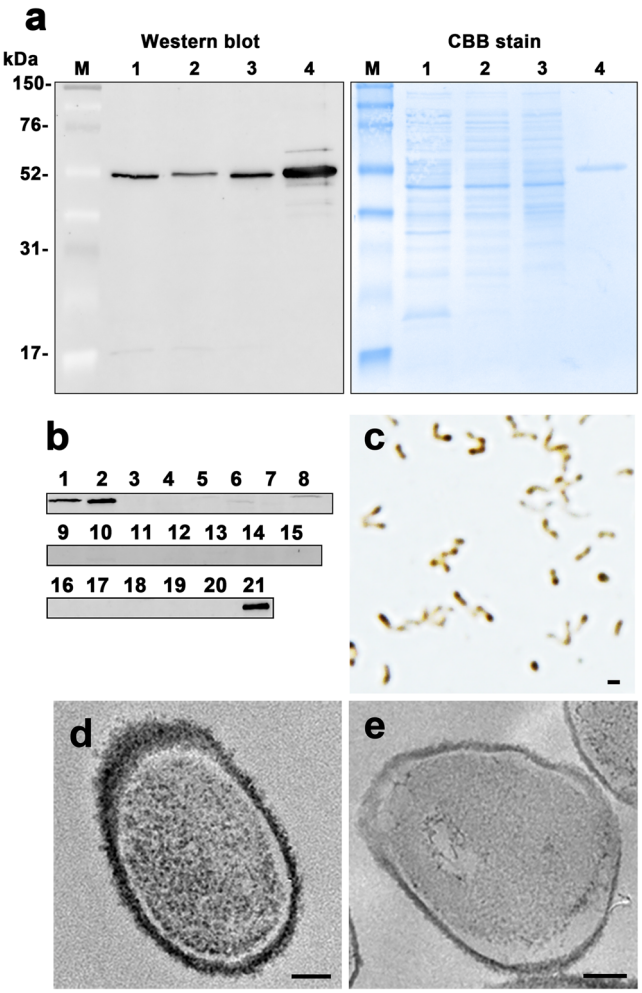

Fig. 1 Reactivity and specificity of the novel anti-*P. acnes* catalase antibody (PAC antibody) and localization of the protein in *P. acnes* cells. Western blot analysis with the PAC antibody was performed using sonicated whole-cell lysate of *P. acnes* (lanes **a**1–3 and **b**1–2), other propionibacterial (**b**3–7), other catalase-positive bacteria (**b**8–13) or fungi (**b**15–16), *M. tuberculosis* (**b**14), and mammalian cells (**b**17–20) together with recombinant *P. acnes* catalase protein as a positive control sample (**a**4 and **b**21). Panel **a** is accompanied with Coomassie brilliant blue (CBB) stain of the identical membrane to show that each lane is loaded by nearly the same amount of *P. acnes* lysate protein. FFPE samples of *P. acnes* cell pellets recovered from 48-h cultures of the C1 strain sarcoid isolate were immuno-stained with PAC antibody (**c**) and subjected to immuno-electron microscopy (**d**) together with negative control samples without the primary antibody (**e**). Scale bar: 1 μm (**c**), 100 nm (**d** and **e**). Lane **a**1: *P. acnes* (clinical isolate C1); lanes **a**2 and **b**1: *P. acnes* (ATCC 6919); lanes **a**3 and **b**2: *P. acnes* (ATCC 11828); lane **b**3: *P. acidipropionicum*; lane **b**4: *P. avidum*; lane **b**5: *P. granulosum*; lane **b**6: *P. jensenii*; lane **b**7: *P. thoenii*; lane **b**8: *E. coli*; lane **b**9: *H. pylori*; lane **b**10: *N. asteroids*; lane **b**11: *S. aureus*; lane **b**12: *S. epidermidis*: lane **b**13: *S. warneri*; lane **b**14: *M. tuberculosis*; lane **b**15: *A. fumigatus*; lane **b**16: *C. albicans*; lane **b**17: A549; lane **b**18: HeLa; lane **b**19: Raw264.7; lane **b**20: THP-1

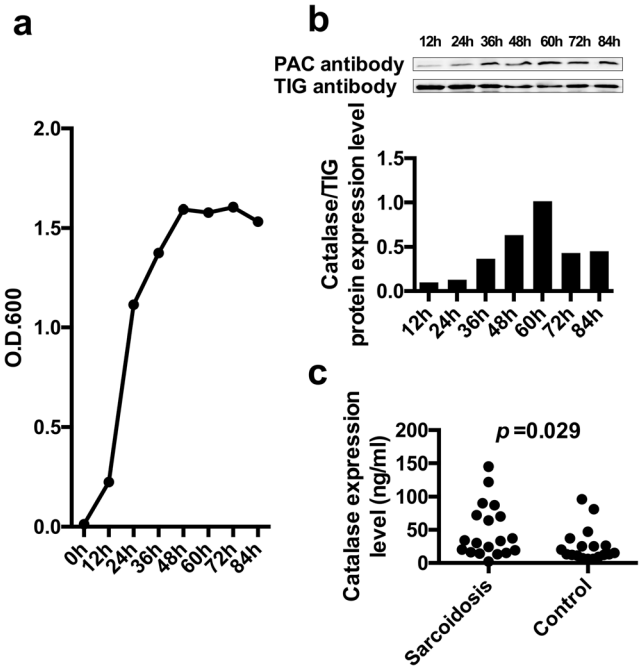

Fig. 2 Catalase expression analysis in cultured *P. acnes* of C1 strain and clinical isolates from sarcoid and non-sarcoid lymph nodes. (**a**) Growth of *P. acnes* C1 strain in culture at 0 to 84 h was measured by OD_{600}, (**b**) Catalase expression of *P. acnes* C1 strain was analyzed at each time-point of culture by Western blotting with PAC antibody and TIG antibody, and the ratio of the catalase expression for the trigger-factor was calculated at each time-point of culture. (**c**) Catalase expression level of *P. acnes* clinical isolates from sarcoid and control lymph nodes was measured at 48 h of culture by sandwich ELISA

Results

Specificity of anti-*P. acnes* catalase antibody

The specificity of the novel PAC antibody examined by Western blotting and IHC is summarized in Table 2. A single clear band with a molecular size of approximately 54 kDa, consistent with a recombinant *P. acnes* catalase protein, was observed in all three *P. acnes* Western blot lanes from a C1 strain sarcoid isolate and ATCC strains of phylotypes I and II (Fig. 1a). We observed no cross-reactivity with other propionibacteria, several catalase-positive bacteria or fungi, *Mycobacterium tuberculosis*, or mammalian cells (Fig. 1b). In *P. acnes* cells immuno-stained with the PAC antibody (Fig. 1c), positive signals were observed on the cell-wall surface of *P. acnes* cells (Fig. 1d), and this reaction was not observed without the primary antibody (Fig. 1e).

P. acnes catalase expression in culture

The proliferative phase of *P. acnes* C1 strain lasts from 12 to 36 h, and the stationary phase occurs after 48 h (Fig. 2a). Catalase expression of the C1 strain was low in the early proliferative phase (< 24 h) and increased after 36 h with a

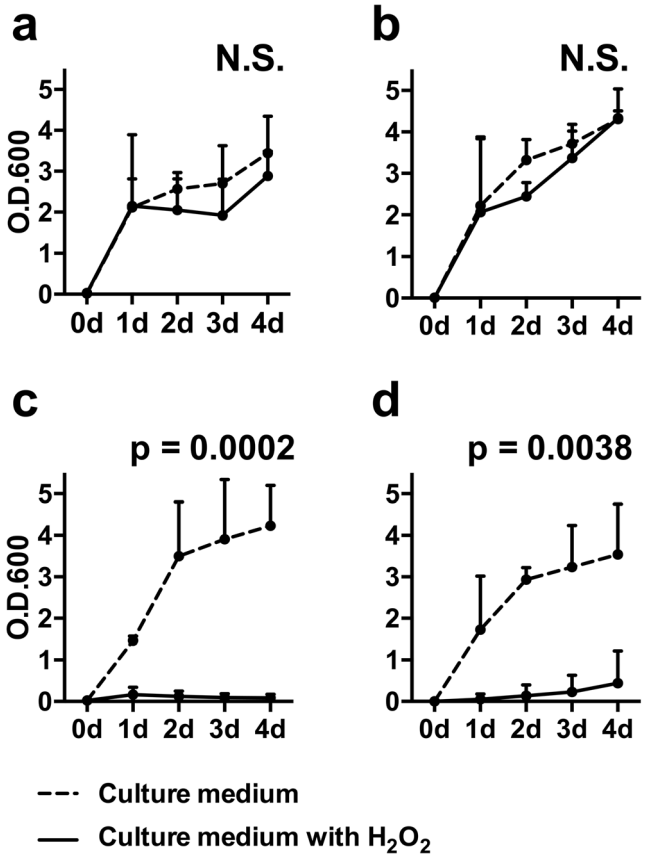

Fig. 3 Growth curves in culture medium with or without H_2O_2 of the representative *P. acnes* isolates with different catalase expression competency. Growth of *P. acnes* in culture medium with or without H_2O_2 was measured by OD_{600} using representative *P. acnes* clinical isolates with high catalase expression from sarcoid lymph nodes (**a**) or control lymph nodes (**b**) and those with low catalase expression from sarcoid lymph nodes (**c**) or control lymph nodes (**d**). Assay results were determined as the mean ± standard deviation of three identical experiments. N.S., not significant

peak at 60 h in the stationary phase, although the expression levels of *P. acnes* trigger-factor protein as a control remained constant (Fig. 2b) during the culture. Catalase expression levels in the stationary phase (48 h) of *P. acnes* clinical isolates were generally higher in isolates from sarcoid than those from control lymph nodes ($P = 0.029$), although the expression level ranged from low to high in each group of isolates (Fig. 2c).

Two representative strains of *P. acnes* with high catalase expression proliferated in culture medium with H_2O_2, and the growth curve did not differ significantly from that in culture without H_2O_2 (Fig. 3a, b). In the other two strains with low catalase expression, bacterial growth was remarkably inhibited in culture with H_2O_2 and significantly different from that in culture without H_2O_2 (Fig. 3c, d).

Detection frequency of *P. acnes* catalase in lymph nodes from each location

P. acnes catalase was detected in many FFPE lymph node samples by IHC with the novel PAC antibody. The detection frequencies of *P. acnes* in each location of the sarcoid and non-sarcoid lymph nodes are summarized in Table 3. In the lymphatic sinus, PAC-reactive large spheroidal bodies were observed in sinus macrophages of many sarcoid and some control lymph nodes (60% vs 4%, $P < 0.0001$). PAC-reactive small-round bodies were infrequently observed in clustered paracortical macrophages of sarcoid and control lymph nodes (9% vs 7%, $P = 0.73$). PAC-reactive small-round bodies or fine-granular signals were also infrequently observed in some granuloma cells of sarcoid lymph nodes (7%).

Table 3 Frequency and localization of *P. acnes* detected by PAC, PAB, or TIG antibody in sarcoid and control lymph nodes

	Number (%) of samples with *P. acnes* detected in each location of lymph nodes from:				
			Control disease		
Localization	Patients with sarcoidosis ($n = 43$)	Patients with control disease ($n = 102$)	Gastric cancer ($n = 35$)	Necrotizing lymphadenitis ($n = 29$)	Reactive lymphadenitis ($n = 38$)
In sinus macrophages					
PAC antibody	26 (60)	4 (4)*	3 (9)*	0*	1 (3)*
PAB antibody	26 (60)	4 (4)*	3 (9)*	0*	1 (3)*
TIG antibody	26 (60)	4 (4)*	3 (9)*	0*	1 (3)*
In paracortical macrophages					
PAC antibody	4 (9)	7 (7)	4 (11)	0	3 (8)
PAB antibody	11 (26)	38 (37)	14 (40)	4 (14)	20 (53)
TIG antibody	3 (7)	2 (2)	0	0	2 (5)
In granuloma cells					
PAC antibody	3 (7)	–	–	–	–
PAB antibody	40 (93)	–	–	–	–
TIG antibody	1 (2)	–	–	–	–

*$P < 0.0001$ compared with sarcoidosis patients (Fisher's exact test)

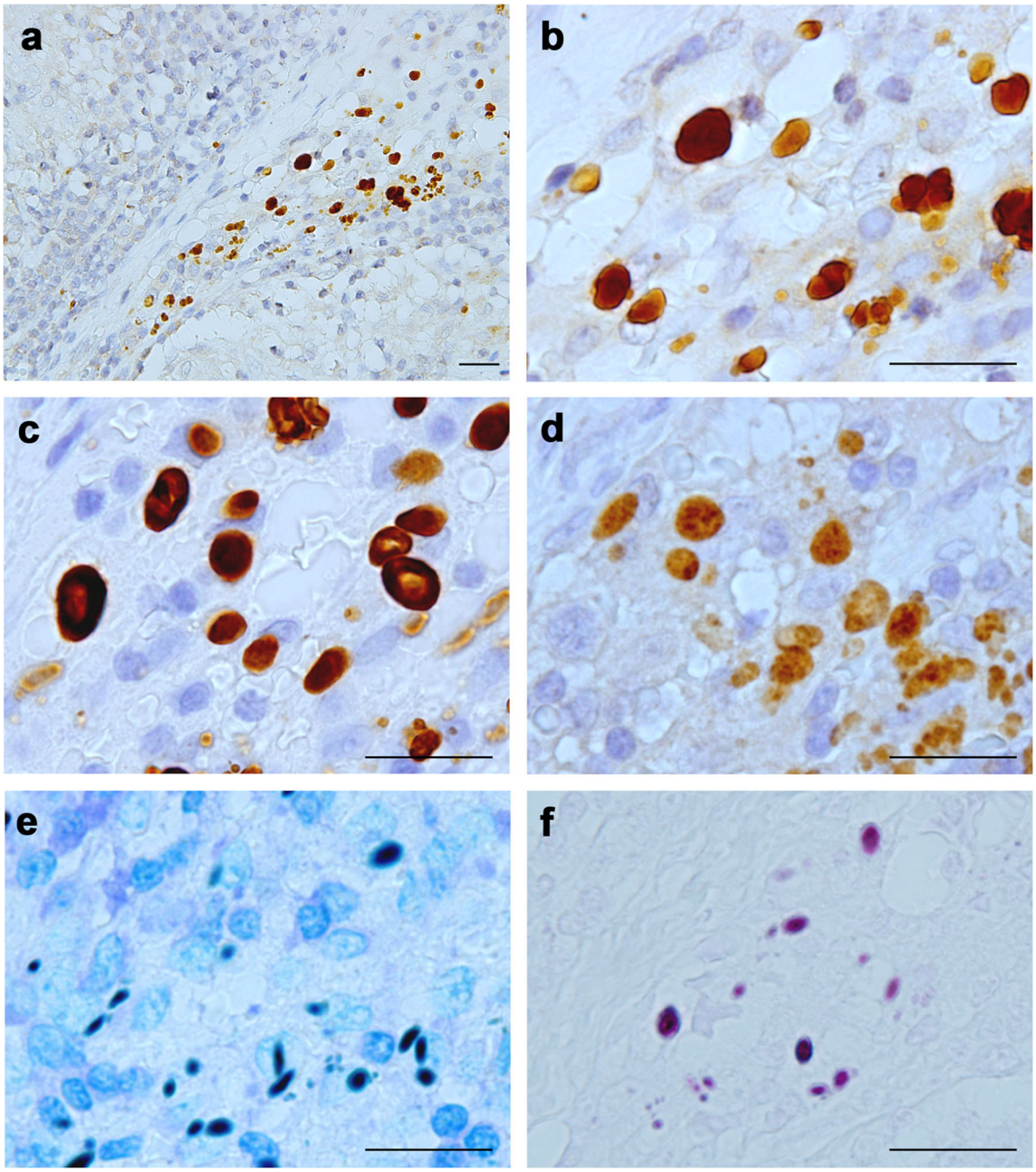

Fig. 4 *P. acnes* catalase expression consistent with the localization of HW bodies in the lymphatic sinus of sarcoid lymph nodes. Identical areas of lymphatic sinus are shown in semi-serial sections of a sarcoid lymph node sample. The size of the HW bodies detected by IHC with the PAC antibody (**a** and **b**), PAB antibody (**c**), or TIG antibody (**d**) was larger than that detected by Giemsa staining (**e**) or Fite staining (**f**). Scale bar: 10 μm

Association of catalase expression with *P. acnes* cell components in lymph nodes

In sinus macrophages, the PAC antibody reacted with large spheroid bodies called Hamazaki–Wesenberg (HW) bodies that are stained by Giemsa and Fite staining (Fig. 4). These HW bodies also reacted with the PAB and TIG antibodies that recognize *P. acnes*-specific lipoteichoic acid and trigger-factor protein, respectively. The detection frequencies by these three antibodies were identical in the lymphatic sinus, regardless of the disease (Table 3).

Double-fluorescence IHC revealed that fluorescein isothiocyanate-positive signals of the PAC or PAB antibody were confined to the outer margins of the HW bodies, and the tetramethylrhodamine-positive signals of the TIG antibody were diffusely distributed throughout the inner areas of the HW bodies (Fig. 5a, b). Immuno-electron microscopy revealed positive PAC or

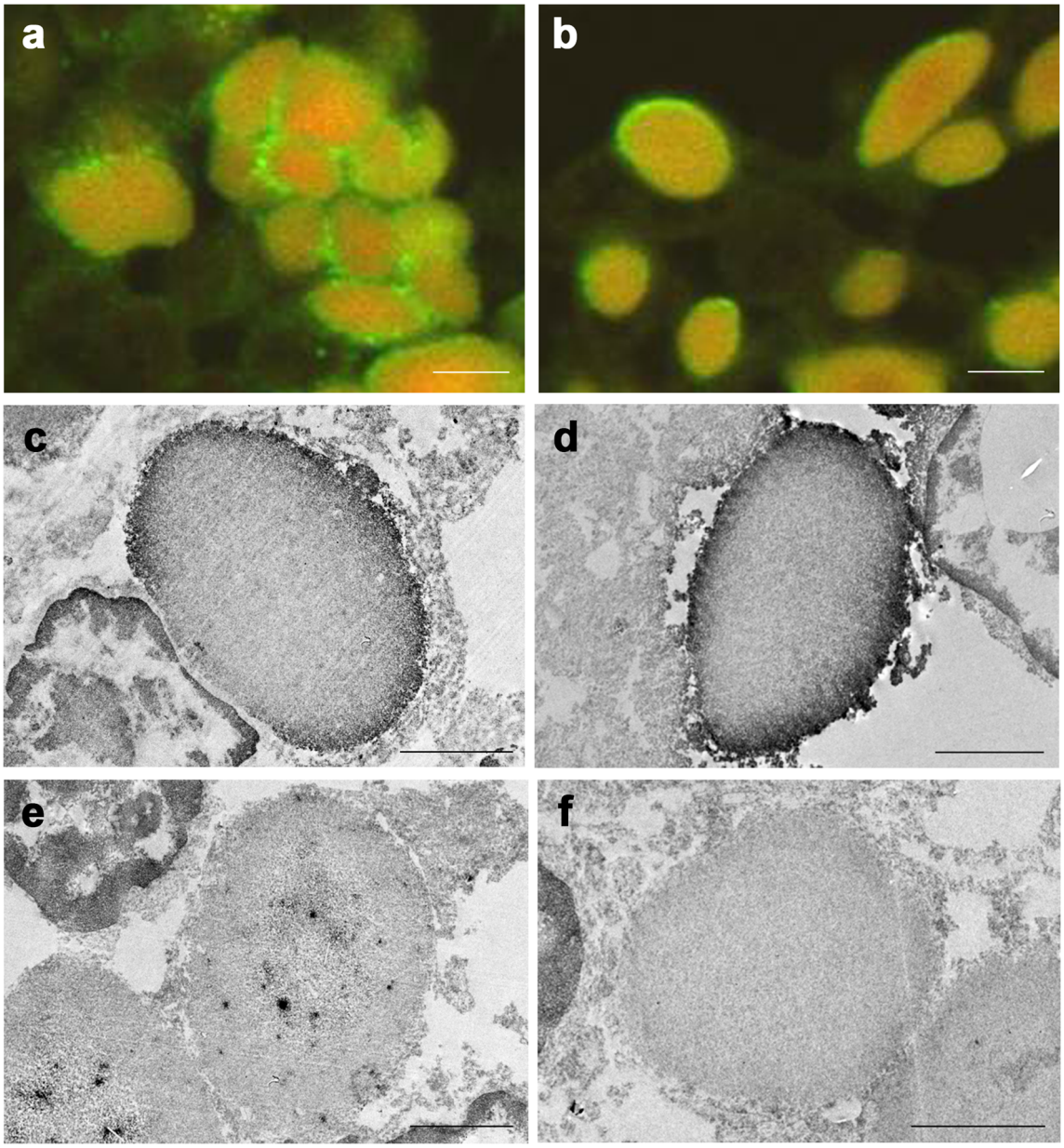

Fig. 5 Localization of *P. acnes* catalase expression in HW bodies is identical with that of membrane-bound lipoteichoic acid and different from ribosome-bound trigger-factor protein. HW bodies in sinus macrophages of sarcoid lymph nodes were examined by double-fluorescence IHC (**a** and **b**) and immuno-electron microscopy (**c**–**f**). Double-fluorescence IHC; (**a**) PAC antibody (green) vs TIG antibody (red) and (**b**) PAB antibody (green) vs TIG antibody (red). Immuno-electron microscopy with PAC antibody (**c**), PAB antibody (**d**), or TIG antibody (**e**) and control without the primary antibody (**f**). Scale bar: 2 μm

PAB antibody signals at the outer margins of HW bodies, and the distribution was almost identical between the two antibodies (Fig. 5c, d). Spotty positive TIG antibody signals were scattered throughout the inner areas of the HW bodies (Fig. 5e). The reaction products of these antibodies were not detected by immuno-electron microscopy in which the primary antibody was omitted (Fig. 5f).

In paracortical areas, the PAB antibody detected clusters of swollen macrophages containing many small-round bodies (Fig. 6a) in 26% of sarcoid and 37% of non-sarcoid lymph nodes. Regardless of the disease, some or none of the PAC or TIG reactivity was observed in these paracortical macrophages (Fig. 6c, e), with detection frequencies of 9 or 7% in sarcoid lymph nodes and 7 or 2% in control lymph nodes, respectively (Table 3). Similarly, PAB-reactive small-round bodies or fine-granular signals in granuloma cells of many (93%) sarcoid lymph nodes were negative for PAC or TIG antibody (Fig. 6d, f), with a few exceptions, as described previously.

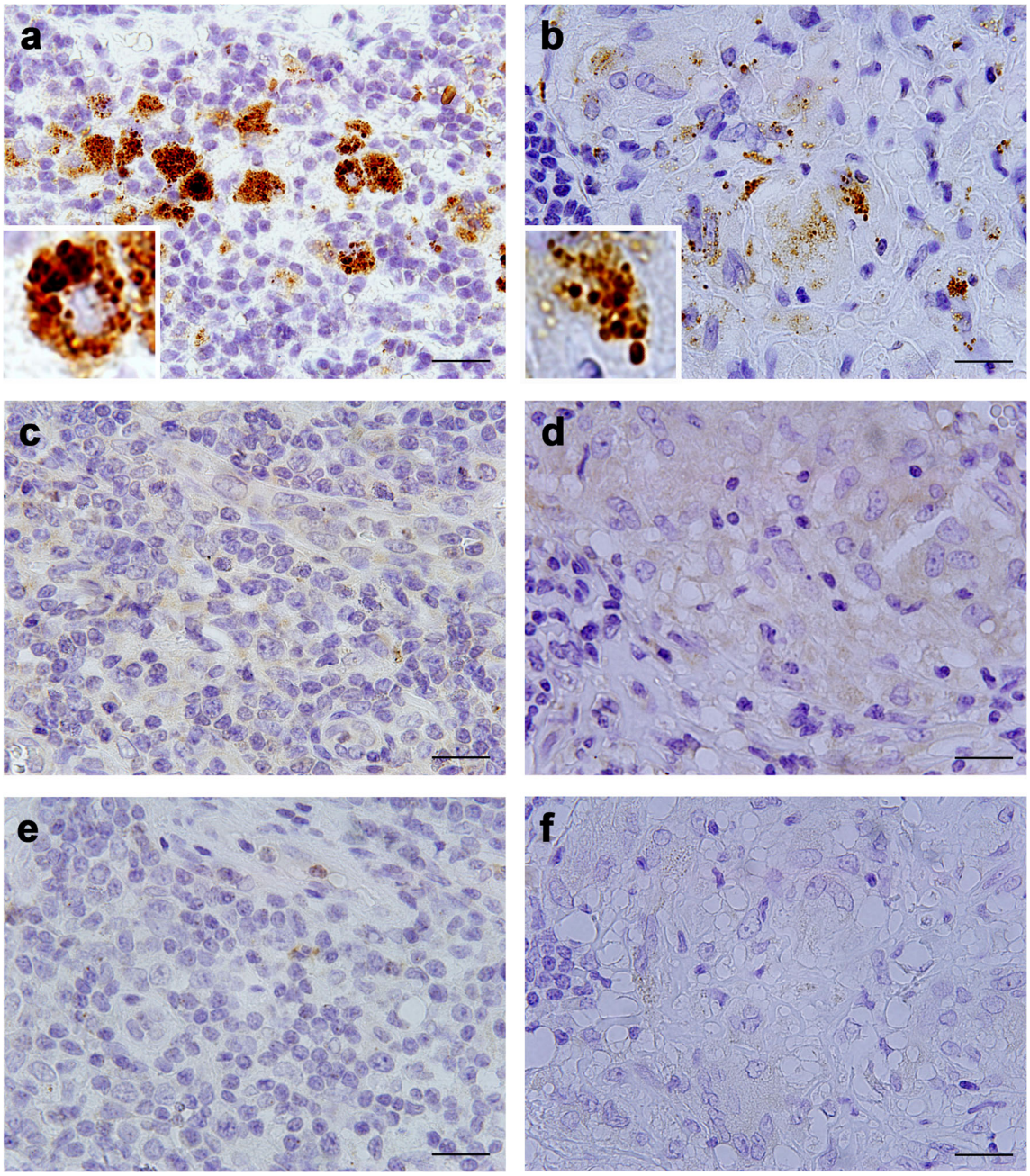

Fig. 6 PAB-reactive small-round bodies in paracortical macrophages or sarcoid granuloma cells are negative for PAC and TIG antibodies. Identical areas of the paracortex (**a**, **c**, and **e**) or the sarcoid granuloma (**b**, **d**, and **f**) are shown in semi-serial sections of a sarcoid lymph node sample; IHC with PAB antibody (**a** and **b**), PAC (**c** and **d**), and TIG antibody (**e** and **f**). Magnification of a paracortical macrophage (**a**, inset) or a granuloma cell (**b**, inset) with PAB-reactive small round bodies. Scale bar: 20 μm

Discussion

P. acnes, a ubiquitous Gram-positive anaerobic bacterium [24], is classified as a catalase-positive bacterium [25]. In the present study, PAC antibody was used to evaluate the catalase expression of *P. acnes* cells isolated from sarcoid and control lymph nodes in culture by Western blotting and sandwich ELISA. Holland et al. detected catalase protein in the culture supernatant of *P. acnes* at the stationary phase by matrix-assisted-laser-desorption/ionization mass spectrometry analysis [26]. We found that catalase expression in the *P. acnes* C1 strain was low during the proliferative phase and increased during the stationary phase. This result is consistent with a previous finding by Prapagdee et al. [27] that the catalase activity of *Agrobacterium tumefaciens* is higher during the stationary phase than the proliferative phase.

Catalase expression levels of *P. acnes* isolates were generally higher in isolates from sarcoid than in isolates from

control lymph nodes. *P. acnes* with high catalase expression was resistant to H_2O_2-induced oxidative stress. Similar observations were made in previous culture experiments using other intracellular pathogens, *Leptospira interrogans* [28] and *Mycobacterium tuberculosis* [29]. Bacterial catalase is important for detoxifying H_2O_2 [30, 31] and facilitating survival in macrophages [32] and confers resistance to phagocyte-mediated killing [16]. Production of intracellular reactive oxygen species was identified by experiments using mouse macrophages infected with *P. acnes* [33]. Thus, *P. acnes* with high catalase expression may be more competent for intracellular survival or persistence, such as in macrophages.

Immuno-electron microscopy demonstrated *P. acnes* catalase protein at the outer margins of the bacterium, and the distribution was almost identical with that observed by immuno-electron analysis of other bacteria with high catalase expression, such as *Vibrio rumoiensis* [34] and *Exiguobacterium oxidotolerans* [35]. The location and high expression of the catalase by this bacterium in the stationary phase allow for the elimination of H_2O_2 activity.

The PAC antibody strongly reacted with HW bodies in sinus macrophages of many sarcoid and some control lymph nodes. Negi et al. [10] suggested that HW bodies are cell wall-deficient *P. acnes* that are persistent in sinus macrophages. In the present study, we used double-fluorescence IHC and immuno-electron microscopy to identify *P. acnes* catalase protein at the outer margin of the HW bodies. The almost identical localization of the catalase with cell membrane-bound lipoteichoic acid indicates that the catalase protein distributes on the cell-membrane surface of cell wall-deficient HW bodies, consistent with the distribution of catalase expression of *P. acnes* cells in the stationary phase. Thus, HW bodies seem to be cell wall-deficient *P. acnes* in the stationary phase with high catalase expression on the cell surface that can establish persistent infection in sinus macrophages.

The PAB-reactive small-round bodies packed in paracortical macrophages were mostly undetectable by IHC with PAC antibody. Negi et al. [10] reported that *P. acnes* can proliferate intracellularly in paracortical macrophages of sarcoid and non-sarcoid lymph nodes. Based on the results of our culture experiments, PAB-reactive but PAC-negative small-round bodies in paracortical macrophages may be activated *P. acnes* in the process of intracellular proliferation. At the same time, PAB-reactive small-round bodies or fine-granular signals in sarcoid granulomas may be a residual form of the proliferated *P. acnes* in the process of degrading in granuloma cells, which have a greater capacity for intracellular digestion compared with macrophages [36]. A few *P. acnes* cells infrequently detected by the PAC antibody in paracortical macrophages or granuloma cells may have reverted to latent *P. acnes* with high catalase expression.

The TIG antibody, which recognizes trigger-factor protein of *P. acnes*, also reacted with HW bodies. The distribution of TIG-reactive signals detected by immuno-electron microscopy seems to be consistent with the ribosome distribution in *P. acnes* cells. The difference in this protein distribution between enzyme or fluorescence IHC and immuno-electron microscopy is likely caused by overlapping positive-signals in the IHC using 4-μm-thick tissue sections. In other bacteria, trigger-factor protein is, like some heat-shock proteins, a ribosome-associated chaperone that prevents protein aggregation [37]. Heat-shock proteins of *Mycobacterium tuberculosis* are associated with its stabilization for survival during the stationary phase [38]. In the present study, HW bodies showed concomitant expression of *P. acnes* catalase and trigger-factor proteins, both of which may be related to the survival of intracellular *P. acnes* and are major target antigens of hypersensitive Th1 immune response in sarcoidosis patients [14, 15].

Three critical conditions essential for *P. acnes*-associated sarcoidosis were proposed by Eishi [11, 12] as follows: latent infection with *P. acnes*; intracellular proliferation of the endogenously-activated latent *P. acnes*; and hypersensitive Th1 immune responses to the bacterial antigens in susceptible subjects. According to the etiology of sarcoidosis as an allergic endogenous infection caused by *P. acnes*, HW bodies expressing catalase seem to be persistent *P. acnes* in sinus macrophages whereas PAB-reactive small-round bodies with undetectable catalase expression may be activated *P. acnes* proliferating in paracortical macrophages. Intracellular proliferation of *P. acnes* in paracortical macrophages may lead to granuloma formation by this commensal bacterium in sarcoidosis patients with Th1 hypersensitivity to certain *P. acnes* antigens, including catalase.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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