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Blockade of Th1 chemokine receptors ameliorates pulmonary granulomatosis in mice

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ABSTRACT: Sarcoidosis is a granulomatous disease of unknown aetiology. We identified immunological targets for the treatment of pulmonary granulomatosis using a murine model generated with *Propionibacterium acnes*.

Sensitisation and challenge using heat-killed *P. acnes* and dendritic cells (DCs) were performed to produce pulmonary granulomatosis in C57BL/6 mice. Immunological analyses using ELISA as well as cDNA microarray analysis were used to search for cytokines or chemokines associated with the formation of granulomas in the lungs.

Co-administration of *P. acnes* and DCs reproducibly induced the formation of pulmonary granulomas, which resembled sarcoid granulomas. The cDNA microarray assay demonstrated that the gene expression of CXCL9 and CXCL10, ligands for CXCR3, and of CCL4, a ligand for CCR5, was strongly upregulated during granulomatosis. ELISA confirmed that levels of CXCL9 and CXCL10 as well as T-helper (Th)1 cytokines and chemokines including tumour necrosis factor-α and interferon-γ were elevated in bronchoalveolar lavage fluid (BALF). The blockade of Th1 chemokine receptors using TAK-779, a dual blocker for CXCR3 and CCR5, led to reduced numbers of CXCR3+CD4+ and CCR5+CD4+ T-cells in BALF. Furthermore, administration of TAK-779 ameliorated the granulomatosis.

The targeted inhibition of Th1 chemokines might be useful for inhibiting Th1-biased granulomatous diseases, including sarcoidosis.

KEYWORDS: CCR5, CXCR3, *Propionibacterium acnes*, pulmonary granulomatosis, Th1 chemokines

Sarcoidosis is a systemic granulomatous disease of unknown aetiology that affects mainly the lungs and lymphatic system [1–4]. Although spontaneous remission occurs in nearly two-thirds of cases, chronic and progressive courses are observed in 10–30% of patients [1–4]. Corticosteroids are used to treat sarcoidosis, but their effects are controversial, particularly in cases complicated with lung diseases [1–4]. In addition, long-term therapy with corticosteroids can result in serious adverse events [4]. Therefore, a new approach to the treatment of sarcoidosis with intensive organ involvement is needed.

The sarcoid granuloma is characterised by non-caseating epitheloid cells with the dominant accumulation of CD4+ T-cells and macrophages [1–4]. CD4+ T-lymphocytes are divided into two subgroups, T helper (Th)1 and Th2 cells, on the basis of cytokine production [5]. Based on the Th1/Th2 paradigm, sarcoidosis is considered a typical Th1-dominant disease, since T-lymphocytes in bronchoalveolar lavage fluid (BALF) and lymph nodes from patients with sarcoidosis predominantly produce interferon (IFN)-γ, interleukin (IL)-2 and tumour necrosis factor (TNF)-α or -β [6–8]. We and others have reported that levels of Th1 chemokines, including CXCL9/IFN-γ (Mig), CXCL10/interferon-γ-inducible protein-10 (IP-10) and CXCL11/interferon-inducible T-cell α-chemoattractant (I-TAC), were elevated in BALF of patients with sarcoidosis [9–13]. However, it is still not clear whether these Th1-related molecules would be useful targets in the treatment of pulmonary granulomatosis. The blockade of these molecules would probably benefit patients: in clinical

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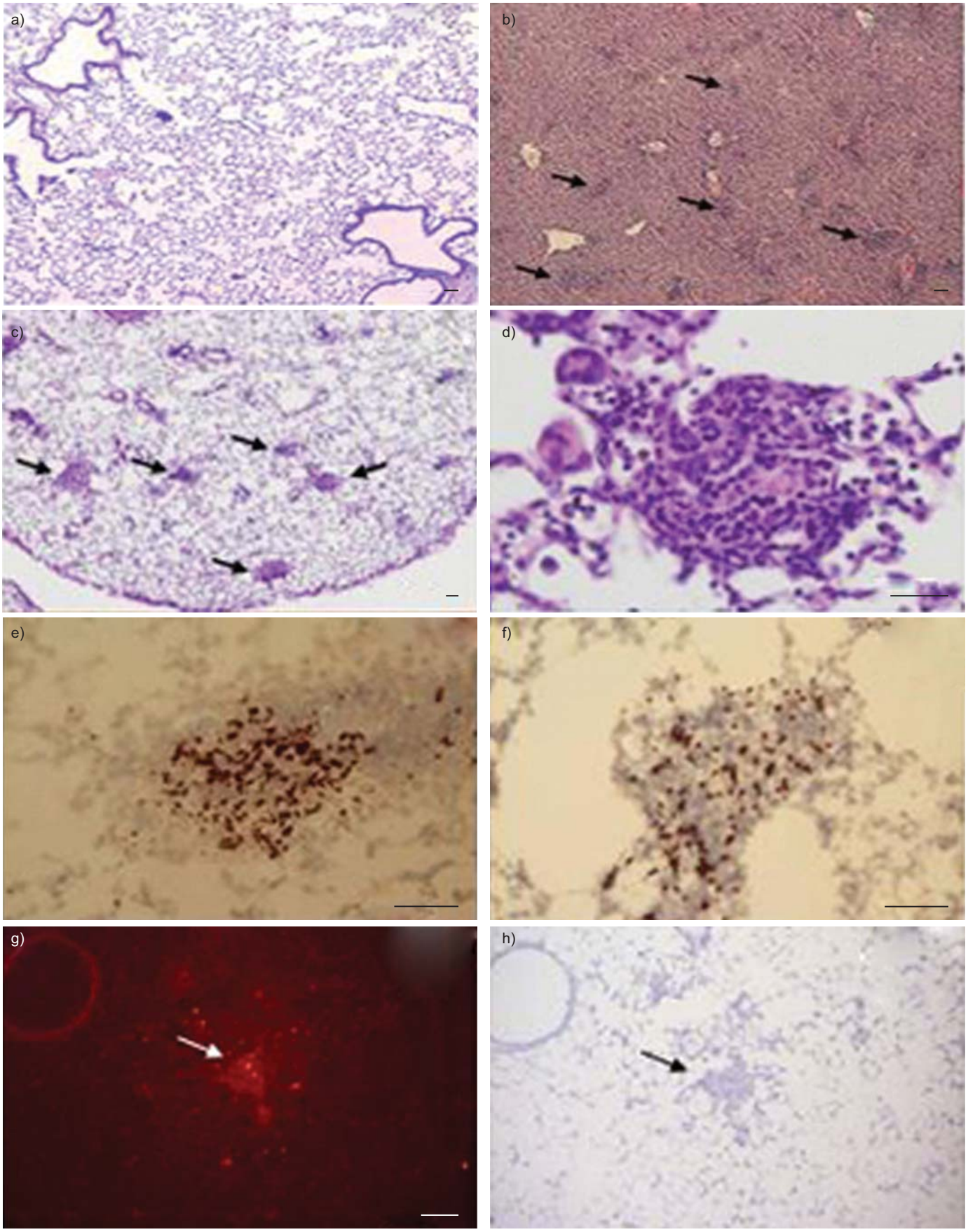

FIGURE 1. Figure legend is presented on the following page.

FIGURE 1. Histological findings in the lungs and livers of mice administered with heat-inactivated *Propionibacterium acnes*. Mice were subcutaneously immunised twice with 1 mg of heat-killed *P. acnes* at a 2-week interval with or without incomplete Freund's adjuvant (IFA), and immature or mature dendritic cells (DCs) (10^6 cells). 2 weeks after the second immunisation, mice were challenged intravenously with heat-killed *P.acnes* (1 mg) and DCs. To obtain the best results, immature DCs were matured by treatment with heat-killed *P. acnes* (100 μg·mL^{-1}) for 24 h before injection into the mice. 7 days after the challenge, mice were sacrificed, and haematoxylin and eosin staining was performed with sections of the lungs and liver. Histological examination of a) the lungs and b) the liver in mice immunised with *P. acnes* and IFA. Arrows indicate granulomas in the liver. c, d) Histological examination of the lungs in mice immunised with *P. acnes* and mature DCs. Arrows indicate granulomas in the lungs. Immunostaining with e) anti-CD3 and f) anti-CD4 antibodies. g, h) Trafficking study of injected DCs. DCs stimulated with heat-killed *P.acnes* were labelled with PKH26, and injected into mice *via* the tail vein. 48 h later, the lung section was analysed with a confocal laser scanning microscope equipped with an Ar–Kr laser. g) Fluorescence image. Arrows represent labelled and injected DCs in granulomas in the lungs. h) Haematoxylin staining. The labelled DCs were detected in granulomatous lesions. Arrows represent granulomas in the lungs. Scale bars=100 μm.

studies with anti-TNF-α antibodies (infliximab and adalimumab), favourable results have been obtained in some cases [14, 15].

To identify novel molecular targets for the treatment of pulmonary granulomatosis, we report here the results of a global analysis of the mRNA expression of cytokines and chemokines using a cDNA microarray in a model of pulmonary granulomatosis generated by the systemic administration of *Propionibacterium acne*s and dendritic cells (DCs) in mice, because *P. acnes* has been reported to be involved in the aetiology of sarcoidosis [16–18].

MATERIALS AND METHODS

Detailed methods are described in the online supplementary material.

Mice and materials

8-week-old female C57BL/6 mice were purchased from Charles River Japan Inc. (Yokohama, Japan), and maintained in the animal facility of the University of Tokushima (Tokushima, Japan) [19]. The small nonpeptide compound TAK-779 (*N*,*N*-dimethyl-*N*-[4-[[[2-(4-methylphenyl)-6,7-dihydro-5H-benzocyclohepten-8-yl]carbonyl]amino]benzyl]tetrahydro-2H–pyran-4-aminium chloride) was provided by Takeda Pharmaceutical Company (Osaka, Japan) [20]. TAK-779 is a selective antagonist of CXCR3 and CCR5 [21].

P. acnes

P. acnes was obtained from the American Type Culture Collection (Manassas, VA, USA) (ATCC #6919) and grown in GAM broth (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan). The suspension was heated by autoclaving, and was kept at -70°C prior to use.

Mouse bone marrow-derived DCs

The bone marrow (BM)-derived DCs were generated using recombinant mouse granulocyte–macrophage-colony stimulating factor (Kirin Brewer Inc., Tokyo, Japan) and recombinant mouse IL-4 (Pepro Tech, London, UK) [22].

Sensitisation and challenge of mice with heat-killed P. acnes

Mice were subcutaneously immunised twice with heat-killed *P. acnes* with or without incomplete Freund's adjuvant (IFA) or DCs. Mice were then challenged intravenously with heat-killed *P. acnes* with or without DCs.

Bronchoalveolar lavage

Bronchoalveolar lavage was performed as described previously [23].

Measurements of CXCR3 ligands and Th1 and Th2 cytokines

Levels of CXCL9 and CXCL10 were examined by ELISA (R&D System, Minneapolis, MN, USA) [23]. Th1 and Th2 cytokines were measured using the BD Cytometric Beads Array System mouse Th1/Th2 cytokine kit (Becton Dickinson Company, Franklin Lakes, NJ, USA).

Trafficking study of injected DCs

DCs (10^6 cells) were stained with PKH26 (Sigma, St. Louis, MO, USA), and injected into the tail vein. Fluorescence images of sections excited at 568 nm were captured with a confocal laser scanning microscope (Leica TCS NT; Leica, Heidelberg,

TABLE 1 Pulmonary granulomatosis in mice immunised with heat-killed *Propionibacterium acnes* and dendritic cells (DCs)

Method of immunisation	Experiment	Granulomas	Size μm^2
***P. acnes* + IFA**			
	1	5	2880±1731
	2	4	2137±1132
	3	2	1800±1272
	Total	3.6±1.5	2413±1426
***P. acnes* + immature DCs**			
	1	22	5195±4172
	2	9	2025±1153
	3	8	3318±2054
	Total	13±7.8	4132±3933
Mature DCs pulsed with *P. acnes*			
	1	32	7980±8941
	2	21	7682±6999
	3	21	8207±4693
	Total	24.7±6.3*	7960±7311*

Data are presented as n or mean±SD. Mice were subcutaneously immunized twice with 1 mg of heat-killed *Propionibacterium acnes* at a 2-week interval. 2 weeks after the second immunisation, mice were challenged intravenously with heat-killed *P. acnes* (1 mg). The heat-killed *P. acnes* was administered with or without incomplete Freund's adjuvant (IFA), immature or mature DCs (10^6 cells). Mature DCs were generated by pulsing with heat-killed *P. acnes* (100 μg·mL^{-1}) for 24 h before their injection into mice. 7 days after the challenge, mice were sacrificed, and haematoxylin and eosin staining was performed on sections of the lungs. The granulomatosis was evaluated by measuring the number and size of granulomas in the lungs. Similar results were obtained in three separate experiments. *: $p<0.05$ *versus P. acnes* plus IFA group.

Germany) equipped with an Ar-Kr laser and a ×10 dry objective (Leica Plan Apochromat).

Isolation of total RNA

Total RNA was isolated from the lungs of mice using Isogen (Wako K.K., Kyoto, Japan) [24].

DNA microarrays

A description of mouse cytokine and chemokine microarrays that contained probes for the genes of 29 cytokines, 34 cytokine receptors, 33 chemokines and 21 chemokine receptors (Kakengeneqs Co. Ltd, Chiba, Japan) is given in online supplementary table E1 [25].

cDNA preparation and array hybridisation

Total RNA samples (100 μg) were converted to double-stranded cDNA using a custom kit (LavelStar Array kit; Qiagen, Valencia, CA, USA) and labelled with cyanine 3-conjugated deoxyuridine triphosphate (dUTP) [25]. Reference total RNA was labelled with cyanine 5-conjugated dUTP (PerkinElmer, Boston, MA, USA). Array hybridisation was performed according to the manufacturer's instructions [25].

Microarray quantification

Fluorescence images of hybridised microarrays were obtained using an array scanner (model 428; Affymetrix, Santa Clara, CA, USA) [25]. Raw fluorescence intensity data were used to calculate signal intensities of the spots (DNASIS Array; Hitachi Software Engineering Co., Ltd, Tokyo, Japan). A two-fold change in gene expression was used as the cut-off.

Histopathology

Left lungs were fixed in buffered 10% formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin [23].

Immunohistochemistry

6-μm sections of the left lungs were stained using the R.T.U. VECTASTAIN Universal Quick Kit (Vector Laboratories, Burlingame, CA, USA) [25].

Administration of TAK-779

TAK-779 was dissolved in 5% mannitol solution, and was administered subcutaneously into the mice.

Flow cytometry and immunofluorescence staining

Flow cytometric analysis was performed using FACSCalibur (BD Biosciences, Franklin Lakes, NJ, USA) [19, 22]. Immunofluorescence staining was visualised using a fluorescence microscope (Olympus BX61; Olympus Optical Co. Ltd, Tokyo, Japan).

Statistical analysis

Comparisons among multiple groups were performed using one-way ANOVA with Newman–Keuls *post hoc* correction (GraphPad Prism, version 3.0; GraphPad Software, La Jolla, CA, USA). Values of $p<0.05$ were considered statistically significant. Data are presented as mean±SD.

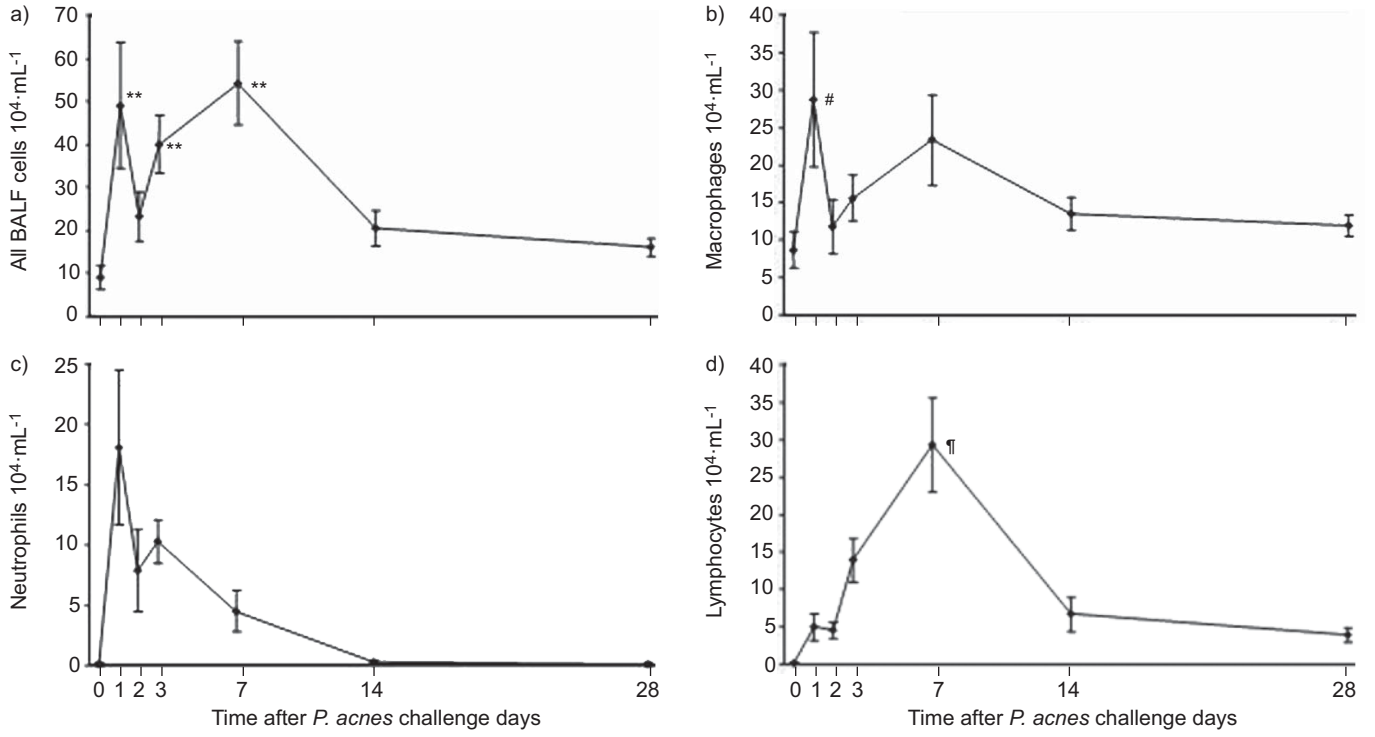

FIGURE 2. Analysis of bronchoalveolar lavage fluid (BALF) of mice with pulmonary granulomatosis induced by *Propionibacterium acnes* and dendritic cells (DCs). Mice were subcutaneously immunised twice with 1 mg of heat-killed *P. acnes* and mature DCs at a 2-week interval. 2 weeks after the second immunisation, mice were challenged intravenously with heat-killed *P. acnes* (1 mg) and mature DCs (10^6 cells). On days 0, 1, 2, 3, 7, 14 and 28 after the challenge, bronchoalveolar lavage was performed. Data are presented as mean±SD for the group of four mice. Similar results were obtained in three separate experiments. **: $p<0.001$ *versus* day 0; #: $p=0.02$; ¶: $p=0.01$.

RESULTS

Administration of heat-inactivated P. acnes *with DCs enhanced the formation of pulmonary granulomas in mice*

We first examined whether immunisation with heat-inactivated *P. acnes* could induce granulomas to form in mouse lungs. C57BL/6 mice were subcutaneously sensitised twice with *P. acnes* and IFA at a 2-week interval. 2 weeks after the second sensitisation, mice were challenged with an *i.v.* injection of heat-killed *P. acnes*. As reported previously [26], many granulomatous lesions were developed in the liver, but not in the lungs, after immunisation with heat-inactivated *P. acnes* and IFA (fig. 1a and b). When BM-DCs were co-administered with *P. acnes* in the immunisation as well as the challenge process, the number and size of pulmonary granulomas were significantly increased (table 1; fig. 1c and d). These granulomas consisted of lymphocytes, epithelioid cells, and multinucleated giant cells (fig. 1d; online supplementary figs E1 and E2), quite similar to those observed in the lungs of patients with sarcoidosis. Granuloma size was largest on day 7, and gradually reduced until day 14 (online supplementary fig. E2). Immunohistochemical analysis showed CD3+ and CD4+ lymphocytes in the pulmonary granulomas (fig. 1e and f). The trafficking study of labelled DCs showed that the injected DCs were distributed into the lungs 6 h after injection, and the granuloma appeared 24 h later. In the granulomatous lesion, some labelled DCs were detected until 72 h after injection. These data demonstrated that the injected DCs migrated into the lungs *via* blood circulation and induced an immune reaction to generate the granulomatous lesions *in situ* (online supplementary fig. E5).

Analysis of BALF of mice with pulmonary granulomatosis induced by P. acnes

To clarify which type of cell is involved in the granulomatosis caused by *P. acnes*, we analysed cells in the BALF of mice. As shown in figure 2, neutrophils in BALF increased in number rapidly 1 day after the challenge with *P. acnes*, but had

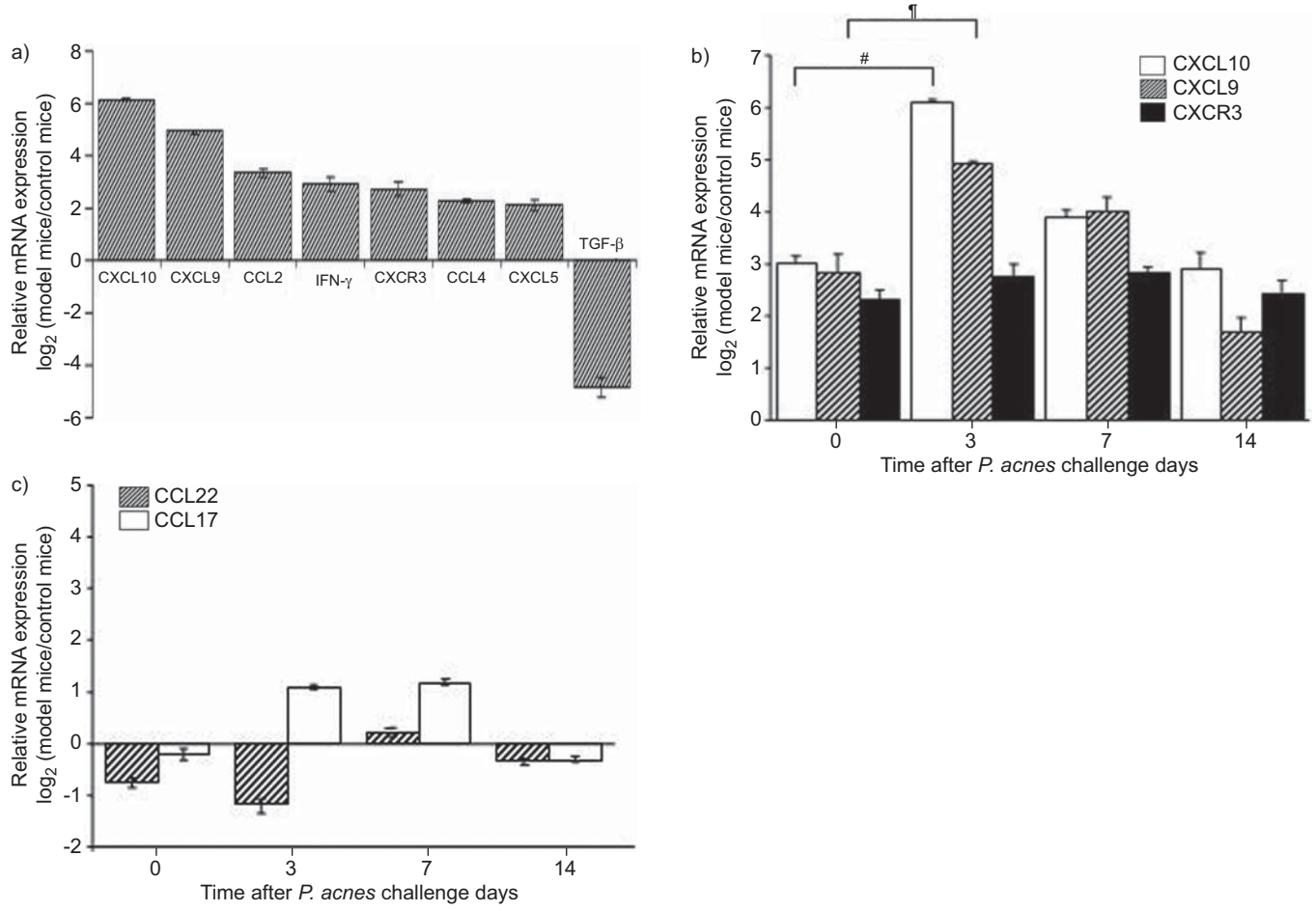

FIGURE 3. Change in gene expression in mice with pulmonary granulomatosis induced by *Propionibacterium acnes* and dendritic cells (DCs). Total RNA was isolated from the lungs of untreated mice as well as *P. acnes*-immunised mice before or 3, 7 or 14 days after *i.v.* administration of *P. acnes* with DCs. Briefly, the lungs were quickly perfused with 5 mL of cold PBS through the right ventriculum. The lungs were then homogenised in 1 mL of Isogen (Wako K.K., Kyoto, Japan), total RNA was extracted and a cDNA microarray analysis was performed as described in the online supplement. Data are presented as mean±SD. a) Major upregulated genes in the lungs of mice 3 days after *P. acnes* challenge. b) Time course of the gene expression of CXCL9, CXCL10 and CXCR3 in the lungs of mice after *P. acnes* challenge. c) Time course of the gene expression of CCL17 and CCL22 in the lungs of mice after the *P. acnes* challenge. IFN: interferon; TGF: transforming growth factor. #: p=0.007; ¶: p=0.008.

disappeared by day 14. However, the number of lymphocytes rose gradually and reached a peak 7 days after the challenge, then decreased until day 28. Two peaks in the number of macrophages were found, 1 and 7 days after the *P. acnes* challenge, with a gradual decline from day 7 to day 28. These changes were correlated with the histological findings (data not shown).

Levels of Th1 chemokines were elevated during pulmonary granulomatosis induced by P. acnes

Next, we analysed which cytokines and chemokines are involved in the pulmonary granulomatosis induced by *P. acnes*, using a cDNA microarray focusing on cytokines and chemokines (online supplementary table E1). As shown in figure 3a, mRNA expression of the Th1 chemokines CXCL9 and CXCL10 was strongly stimulated. mRNAs of other Th1-related molecules including CCL4, CXCL5, CXCR3 and IFN-γ were also expressed strongly after *P. acnes* challenge. The mRNA of CCL2 also increased in the granulomatous lungs, consistent with reports describing pulmonary granulomas induced by *P. acnes* in rabbits [27]. The increase in CXCL9 and CXCL10 occurred 3 to 7 days after the challenge with *P. acnes* (fig. 3b). However, levels of the Th2 chemokines CCL17 and CCL22 did not increase during the course of the pulmonary granulomatosis (fig. 3c).

To confirm the stimulation of Th1 chemokines and cytokines, we performed an ELISA with BALF. As shown in figure 4, CXCL9, CXCL10 and IFN-γ levels were markedly elevated after challenge with *P. acnes*. TNF-α also increased in BALF, but the difference did not reach statistical significance. However, Th2 cytokines, including IL-4 and IL-5, were not detected in the course of pulmonary granulomatosis (data not shown).

Therapy targeting Th1 chemokines ameliorates the pulmonary granulomatosis caused by P. acnes

As we confirmed the elevation in levels of the Th1 chemokines CXCL9, CXCL10 and CCL4 in this model, we hypothesised that the blockade of Th1 chemokines might be effective in inhibiting pulmonary granulomatosis. To test this, we used TAK-779, a selective blocker of the Th1 chemokine receptors CXCR3 and CCR5.

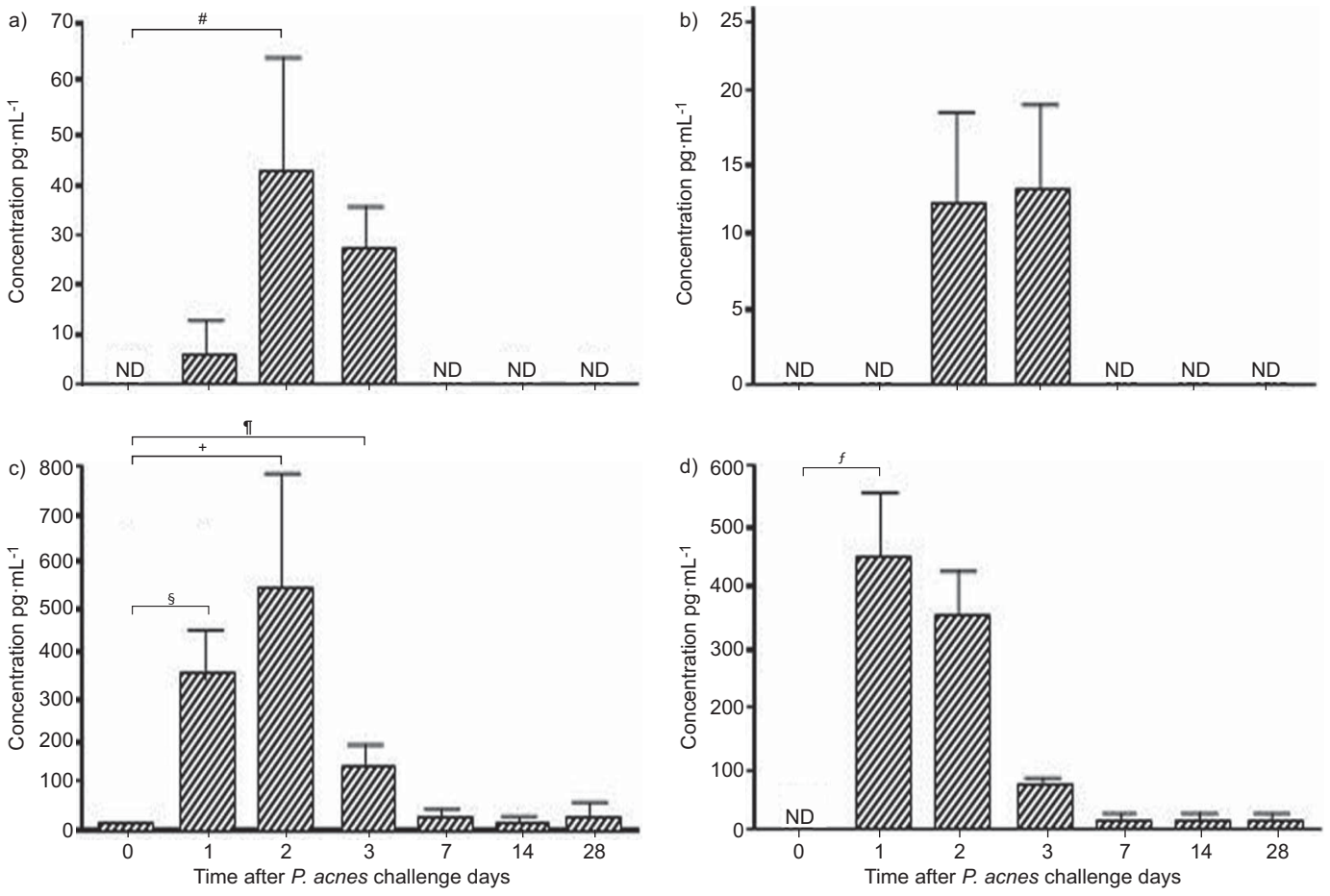

FIGURE 4. Elevation in levels of T helper (Th)1 cytokines and chemokines in bronchoalveloar lavage fluid (BALF) of mice with pulmonary granulomatosis generated by *Propionibacterium acnes*. Mice were anaesthetised and a soft cannula (23G) was inserted into the trachea. Bronchoalveolar lavage was performed five times with the instillation and withdrawal of 1 mL of saline at various time points. BALF was stored at -80°C until used. The frozen BALF was quickly thawed and used to examine concentrations of cytokines and chemokines. a) Th1 (interleukin (IL)-2, IL-4, IL-5 and interferon-γ) and b) Th2 (tumour necrosis factor-α) cytokines were measured using the BD Cytometric Beads Array System. Mouse c) CXCL9 and d) CXCL10 levels were measured by ELISA. Data are presented as mean±SD for the group of four mice. Similar results were obtained in three separate experiments. #: p=0.034; ¶: p=0.025; +: p=0.009; §: p=0.018; ƒ: p=0.05.

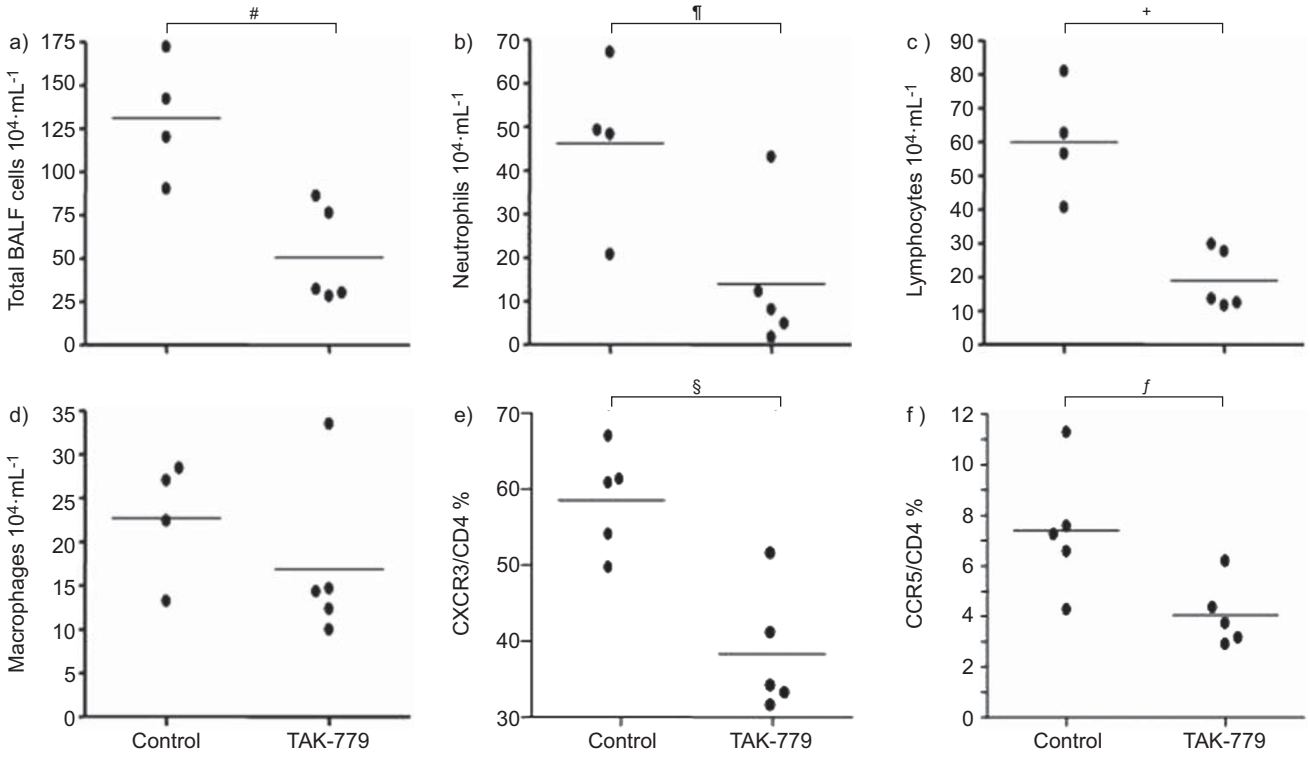

FIGURE 5. Administration of TAK-779 blocks the trafficking of T helper 1 lymphocytes into the lungs. TAK-779 (150 μg·day^{-1}) was administered subcutaneously from 4 days before to 6 days after challenge with heat-inactivated *Propionibacterium acnes* and mature dendritic cells every other day. Bronchoalveolar lavage (BAL) analysis was performed 7 days after the challenge. Mice were anaesthetised and a soft cannula (23G) was inserted into the trachea. BAL was performed five times with the instillation and withdrawal of 1 mL of saline at various time points. The total cell count of the BAL fluid (BALF) was determined using Turk staining solution. BALF was centrifuged, and the cell pellets were resuspended into saline and cytospun onto glass slides. These cells were stained with Diff-Quick staining solution (Baxter, Miami, FL, USA), and 200 cells were counted for cell classification. The numbers of a) all cells, b) neutrophils, c) lymphocytes and d) macrophages in BALF of mice treated with control (5% mannitol solution) or TAK-779. Data are presented as mean±SD for each group of four or five mice. The percentages of e) CXCR3+CD4+ and f) CCR5+CD4+ T-cells in BALF of mice treated with control (5% mannitol solution) or TAK-779. BAL cells were analysed by flow cytometry. CXCR3+CD4+ and CCR5+CD4+ T-cells were determined with two-colour fluorescence staining. Data are presented as the mean±SD for the group of five mice. Data are representative of three separate experiments. #: p=0.011; ¶: p=0.015; +: p=0.005; §: p=0.016; ƒ: p=0.02.

Mice were treated every other day with TAK-779 from 4 days before to 6 days after the challenge with heat-inactivated *P. acnes* and DCs. TAK-779 significantly reduced the total number of cells as well as numbers of both neutrophils and lymphocytes (fig. 5a–d). The proportions of CXCR3+CD4+ T-cells and CCR5+CD4+ T-cells in BALF determined using flow cytometry were 50–68% and 4–12%, respectively, indicating Th1-skewed immune responses in this model (fig. 5e and f). Treatment with TAK-779 clearly reduced the numbers of both CXCR3+CD4+ and CCR5+CD4+ T-cells (control *versus* TAK-779: CXCR3+CD4+; 58.54±6.75% *versus* 38.29±8.24%, p=0.016; CCR5+CD4+; 7.37±2.52% *versus* 4.05±1.31%, p=0.020). Furthermore, histological examination confirmed major reductions in both the number and size of pulmonary granulomas generated by *P. acnes* following the administration of TAK-779 (control *versus* TAK-779: size; 7,033.14±1,889.6 *versus* 4,233.27±576.57 μm^2, p=0.008; number; 25.4±5.89 *versus* 15.2±4.20, p=0.032) (fig. 6).

DISCUSSION

In the present study, we generated a murine model of pulmonary granulomatosis by co-administering *P. acnes* and DCs. Examination of the expressions of cytokines and chemokines in the lungs demonstrated that levels of Th1-type cytokines and chemokines were strongly elevated in this model. In addition, the Th1 chemokine receptor blocker TAK-779 inhibited the formation of granulomas in the lungs by reducing the numbers of CXCR3+CD4+ and CCR5+CD4+ T-cells accumulated in the lungs.

In this study, mice were sensitised twice with systemic administrations of heat-inactivated *P. acnes* and BM-derived DCs to generate pulmonary granulomas. A series of microbiological and molecular investigations have suggested that *Propionibacteria* spp. are aetiologically linked to sarcoidosis [16–18]. Using heat-inactivated *P. acnes*, several models of pulmonary granulomatosis have been established [26, 28]. McCaskill *et al.* [28] showed that the intratracheal administration of heat-killed *P. acnes* induced granulomatosis in the lungs of mice. Since it is likely that the intratracheal administration of heat-killed *P. acnes* induces nonspecific inflammation in the lungs, we selected systemic administration as it is more relevant to the condition in humans. We found that the challenge with *P. acnes*-pulsed DCs, which have the phenotype of mature DCs (online supplementary fig. E3), without heat-inactivated *P. acnes* also could induce many large

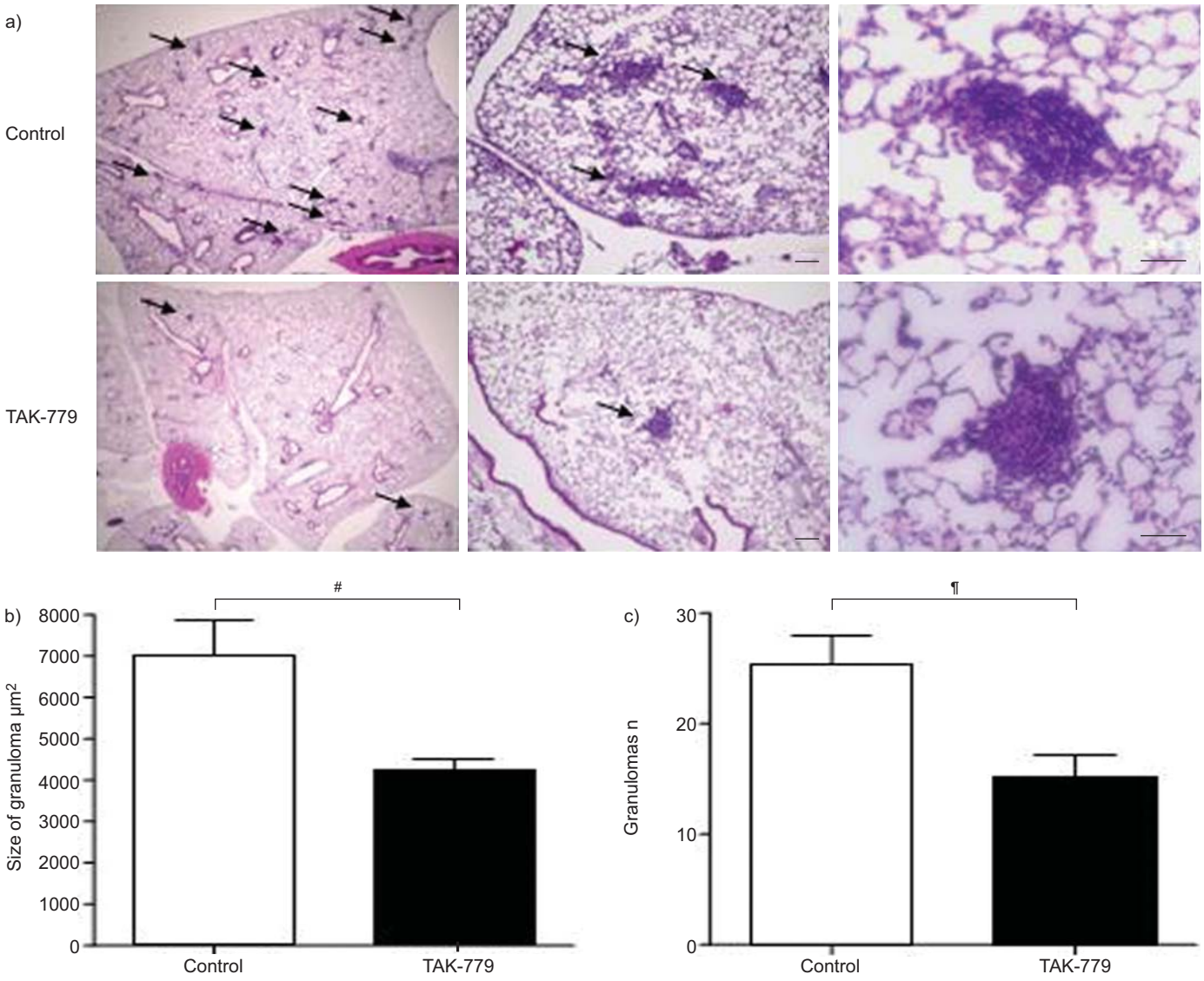

FIGURE 6. Administration of TAK-779 ameliorates the formation of granulomas in the lungs. TAK-779 (150 μg·day^{-1}) was administered subcutaneously from 4 days before to 6 days after challenge with heat-inactivated *Propionibacterium acnes* and mature dendritic cells every other day. Histological analysis was performed 7 days after the challenge. a) The histology of the lungs treated with control (5% mannitol solution) or TAK-779. Arrows indicate granulomas in the lungs. Scale bar=100 μm. Quantitative analysis of pulmonary granulomatosis. Granulomatosis was evaluated by measuring the b) size and c) number of granulomas in the lungs. Data are presented as mean±SD of five mice in the group. Similar results were obtained in three separate experiments. #: p=0.008; ¶: p=0.032.

granulomas in the lungs, indicating that the pulmonary granulomatosis seen in this model was mediated by immune reactions against *P. acnes*. NISHIWAKI *et al.* [26] also reported that repeated immunisation with heat-killed *P. acnes* using complete Freund's adjuvant (CFA) induced pulmonary granulomatosis [26], although we could not reproducibly generate granulomas using similar methods with IFA. The reason for this discrepancy is not clear, but it is likely to be due to the difference in the route and dose of immunisation with heat-killed *P. acnes* or adjuvant. In particular, CFA may not be suitable for inducing specific immune responses to *P. acnes*, since it contains derivatives of *Mycobacterium tuberculosis*. The granulomas found in previous reports consisted of lymphocytes, macrophages and epithelioid cells without multinucleated cells. However, the granulomas observed in our study contained multinucleated giant cells, being most similar to those in cases of human sarcoidosis. The DCs used in the immunisation might play a critical role because DCs were reported to be key immune cells in initiating granulomatous cell-mediated immunity [29].

In previous reports, levels of Th1 cytokines including IFN-γ, TNF-α and IL-12 were elevated in models of pulmonary granulomatosis induced by *P. acnes* [26, 29], whereas there is no published report analysing chemokines. Here, we showed significant upregulation of CXCL9 and CXCL10 expression during pulmonary granulomatosis induced by heat-killed *P. acnes*. Furthermore, these changes were strongest among cytokines and chemokines tested in the cDNA microarray system. This suggests these chemokines are ideal molecular

targets for regulating Th1-type granulomas in the lungs, because they play critical roles in the trafficking of immune cells, particularly Th1 cells, into the lungs. In our model, CXCR3+CD4+ T-cells probably play more of a role than CCR5+CD4+ T-cells since the proportions of these cells were 50–68% and 4–12%, respectively, which is consistent with an analysis in patients with sarcoidosis [30].

TAK-779 has a unique profile, selectively blocking both CXCR3 and CCR5 [19, 20]. To test whether the blockade of Th1 chemokines attenuates pulmonary granulomatosis caused by *P. acnes*, we treated the sensitised mice with TAK-779 during the challenge with *P. acnes*. Administration of TAK-779 strongly inhibited pulmonary granulomatosis by preventing the accumulation of CXCR3+CD4+ and CCR5+CD4+ T-cells into the lungs. There are two ways to inhibit the interaction between a ligand and a receptor: neutralise the ligand or block the receptor. With chemokines, unlike cytokines, many ligands can bind a single receptor. Consequently, blocking the receptor would be more effective than blocking the ligands. Furthermore, TAK-779 has the advantage of blocking both Th1 chemokine receptors. This characteristic of TAK-779 might lead to the significant reduction in the accumulation of Th1 cells, and inhibit granulomatosis in the lungs. In turn, these observations also indicate that Th1 chemokines are crucial regulators in the formation of pulmonary granulomas by *P. acnes*. Furthermore, we found a reduced number of neutrophils in the BALF of TAK-779-treated mice. This is not surprising: it has been reported that the neutrophils can also express the Th1 chemokine receptors CXCR3 and CCR5 [31, 32]. In addition, we could not rule out the possibility that the inhibitory effects of TAK-779 were partly mediated by blocking the influx of injected DCs into the lungs, since *P. acnes*-stimulated DCs also express CCR5 and CXCR3 (online supplementary fig. E4). Unlike human sarcoidosis, the condition in our model is not chronic and improved within 2 weeks, which is consistent with the previous reports, but is a limitation of our study [26, 28]. Recently, SAMOKHIN *et al.* [33] reported a model of prolonged pulmonary granulomatosis like human sarcoidosis in ApoE -/- mice. Further study is required to confirm the efficacy of TAK-779 in chronic models with therapeutic protocols.

In summary, we have demonstrated that production of Th1 chemokines is strongly stimulated during pulmonary granulomatosis caused by *P. acnes* with DCs, and targeted inhibition of Th1 chemokines with receptor blockers might be useful for inhibiting Th1-biased granulomatous diseases including sarcoidosis.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

None declared.

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