ORIGINAL RESEARCH

Myocardial Immunocompetent Cells and Macrophage Phenotypes as Histopathological Surrogates for Diagnosis of Cardiac Sarcoidosis in Japanese

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Background—The histological diagnosis of cardiac sarcoidosis (CS) is based on the presence of myocardial granulomas; however, the sensitivity of endomyocardial biopsy is relatively low. We investigated whether immunocompetent cells including dendritic cells (DC) and macrophages in nongranuloma sections of endomyocardial biopsy samples could be histopathological surrogates for CS diagnosis.

Methods and Results—The numbers of DC and macrophages were investigated in 95 consecutive CS patients and 50 patients with nonischemic cardiomyopathy as controls. All patients underwent endomyocardial biopsy, and immunohistochemical staining was performed on all samples. We examined these immunocompetent cells in nongranuloma sections in CS patients diagnosed by the presence of myocardial granulomas (n=26) and in CS patients without myocardial granulomas diagnosed by the Japanese Ministry of Health Welfare 2007 criteria (n=65) or the Heart Rhythm Society 2014 criteria (n=26). In CS patients with and without myocardial granulomas, $CD209^+$ DC and $CD68^+$ macrophages were more frequently observed ($P<0.01$) and $CD163^+$M2 macrophages were less frequently observed ($P<0.01$) in nongranuloma sections compared to controls. Furthermore, the combination of decreased $CD163^+$M2/$CD68^+$ macrophage ratio and increased number of $CD209^+$ DC in nongranuloma sections of CS patients demonstrated high specificity (100%, 95% CI 92.7−100) for CS diagnosis with each diagnostic criteria and the presence of myocardial granulomas.

Conclusions—Increased number of DC and decreased M2 among all macrophages in nongranuloma sections of myocardium showed high specificity for CS diagnosis, suggesting DC and macrophage phenotypes as histopathological surrogates for the diagnosis of CS. (*J Am Heart Assoc*. 2016;5:e004019 doi: 10.1161/JAHA.116.004019)

Key Words: cardiac sarcoidosis • dendritic cell • diagnosis • diagnostic method • histopathology • inflammation • macrophage

Cardiac involvement has been recognized as a determinant of worse clinical outcomes in sarcoidosis patients;[1,2] therefore it should be detected earlier and treated

with steroids for a long-term period.[3,4] However, clinicians often have difficulty in diagnosing cardiac sarcoidosis (CS) accurately because of few currently accepted international guidelines, especially in extracardiac sarcoidosis patients with latent myocardial damage.[5] To date, there are 3 proposed major diagnostic guidelines: the Japanese Ministry of Health and Welfare's criteria modified in 2007 (JMHW 2007),[6] the World Association for Sarcoidosis and Other Granulomatous Disorders' criteria modified in 2014 (WASOG 2014),[7] and the Heart Rhythm Society expert consensus in 2014 (HRS 2014).[8] Of them, the WASOG 2014 and HRS 2014 criteria mandate the presence of granulomas in either myocardium or other extracardiac tissue for the diagnosis of CS. Nevertheless, endomyocardial biopsy (EMB) and extracardiac biopsy have low sensitivity because of the focal nature of the disease, and indeed granulomas in myocardium can be identified in less than 30% of CS patients.[9] On the other hand, the JMHW 2007 criteria do not mandate positive biopsy for CS diagnosis, but

they may in time afford overdiagnosis. To overcome these limitations, novel histopathological surrogates for the diagnosis of CS are strongly warranted.

Dendritic cells (DC) are professional antigen-presenting cells, which are found in all organ systems, including the myocardium, and play crucial roles not only in the postinfarction healing process after myocardial infarction,[10,11] but also in the pathogenesis of sarcoidosis, based on immunohistochemical analyses of lung and muscle.[12,13] Macrophages are another key player in the granuloma formation following the stimuli of DC and T-cells. Recently, macrophages have been able to be divided into 2 phenotypes, M1 (pro-inflammatory) and M2 (anti-inflammatory), which differ phenotypically and, almost certainly, functionally.[14]

Therefore, we hypothesized that DC and macrophages might also be associated with the development of granulomas in the myocardium and provide a histopathological clue for the diagnosis of CS. The purpose of this study was first to investigate whether DC and macrophages are observed in sarcoid granulomas and their surrounding areas in myocardium, and second to examine the clinical application of DC and macrophage phenotypes in relation to CS diagnosis by evaluating these immunocompetent cells in nongranuloma sections of EMB samples from CS patients with and without myocardial granulomas.

Figure 1. Study population. CS indicates cardiac sarcoidosis; DCM, dilated cardiomyopathy; EMB, endomyocardial biopsy; HCM, hypertrophic cardiomyopathy; HHD, hypertensive heart disease; HRS, Heart Rhythm Society; JMHW, Japanese Ministry of Health and Welfare.

Methods

Study Population and Clinical Data Collection

We examined 186 consecutive patients with newly diagnosed CS based on the clinical manifestations and/or histological findings, without coronary artery disease, who were admitted to the National Cerebral and Cardiovascular Center between September 1979 and December 2015, and the Hokkaido University Hospital between April 2001 and June 2015. Patients who did not undergo EMB at the time of CS diagnosis and before immunosuppressive treatment (n=72), did not have appropriate EMB samples for immunohistochemical analyses (n=11), or did not meet the diagnostic criteria defined in the JMHW 2007 (n=8) were excluded. Briefly, according to the JMHW 2007 criteria, a definite diagnosis of CS was made on the basis of the presence of granulomas in the myocardium, or clinical and/or histological findings meeting clinical criteria for CS with extracardiac involvement of at least 1 organ. Finally, 95 CS patients were included in this study (Figure 1). We also examined 50 consecutive patients with nonischemic and noninflammatory cardiomyopathy who underwent EMB at Hokkaido University Hospital between October 2012 and January 2015, and at National Cerebral and Cardiovascular Center Hospital between February 2015 and April 2015, based on a widely accepted
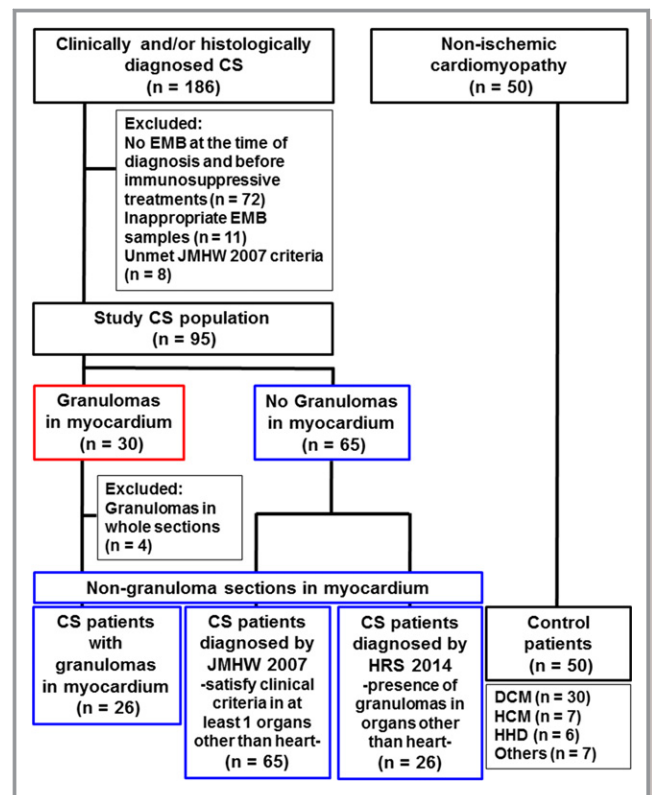
scientific statement (Figure 1).[15] The study protocol was approved by our institutional review committee (M27-021) and conformed to the principles of the Declaration of Helsinki, and the subjects gave informed consent.

We collected the following data: age, sex, echocardiographic findings, baseline fatal ventricular tachycardia, advanced atrioventricular block, extracardiac organ involvement, laboratory findings, cardiovascular medication, and findings of imaging modalities including gallium scintigraphy and ^{18}F-fluorodeoxyglucose–positron emission tomography (FDG-PET) at the time of diagnosis. Regarding FDG-PET findings, specific focal uptake of FDG was defined as positive based on previous reports.[16,17] The findings of gallium scintigraphy and FDG-PET were determined by the consensus of 2 experienced radiologists. Venous blood samples were serially obtained to measure plasma angiotensin-converting enzyme activity, lysozyme, and brain natriuretic peptide levels.

Histological and Immunohistochemical Staining

We examined immunocompetent cells in myocardial granulomas (n=30), and then examined those in nongranuloma

sections of myocardium in CS patients with myocardial granulomas (n=26) and CS patients without myocardial granulomas diagnosed by the JMHW 2007 criteria (n=65) or the HRS 2014 criteria (n=26) (Figure 1).

We used a transcatheter method to collect EMB samples from 3 to 5 different sites in the right ventricular septum. For histological analyses, the EMB samples were fixed in formalin and embedded in paraffin using standard histological procedures. The tissue was cut to yield 5-µm-thick cross sections. The sections were subsequently stained with hematoxylin and eosin and Masson's trichrome stain to determine the extent of fibrosis.

Immunohistochemical examinations were performed on 5-µm-thick formalin-fixed and paraffin-embedded tissue sections. All steps were performed on a Leica Bond III automated system (Leica Microsystems, Wetzlar, Germany) according to the manufacturer's instructions as previously reported.[11] Briefly, specimens were deparaffinized and antigen was retrieved on the instrument. All slides were incubated with primary antibodies against CD3 (diluted 1:10; Dako, Glostrup, Denmark), CD68 (diluted 1:1000, Dako), CD163 (diluted 1:10; Abcam, Cambridge, UK), and CD209 (1:1000, BD Pharmingen, Franklin Lakes, NJ) for 16 minutes, followed by incubation with a mouse–rabbit–horseradish peroxidase polymer and 3,3′-diaminobenzidine substrate. The sections were then incubated in primer (anti-rabbit and anti-mouse) for 8 minutes. The primary antibody was omitted from these protocols as a negative control. The sections were subsequently counterstained with hematoxylin.

Quantitative Analyses of Myocardial Immunocompetent Cells

Quantitative analyses were performed by 2 trained technicians without knowledge of the patients' backgrounds. In nongranuloma sections in the 3 CS and control groups, a representative section for analysis was randomly chosen by 1 technician in a low-magnification whole view of 3 to 5 sections obtained by EMB. Stained immunocompetent cells were counted at a magnification of ×100 in 10 representative parts (0.01 mm^2/part) that were randomly chosen by 2 technicians in the representative section, using ImageJ software (version 1.50b; National Institutes of Health, Bethesda, MD). For each part, total cell numbers/10 representative parts (0.01 mm^2/part) in high-power fields were counted and demonstrated the cell numbers per 0.1 mm^2.

Statistical Analyses

Continuous data were expressed as median (interquartile range). The 2 groups were compared using the Wilcoxon rank sum test for continuous variables. Categorical variables were reported as frequencies with percentages and compared between the 2 groups using the Fisher's exact test. Receiver-operating characteristic (ROC) curve analysis was used to determine optimal cut-off values for selected variables regarding immunocompetent cell counts in granuloma-negative sections in each group, to obtain a definite CS diagnosis with JMHW 2007 and HRS 2014 criteria and the presence of myocardial granulomas. The best cut-off point was calculated by obtaining the maximum value of Youden index (sensitivity+specificity−1). The area under the ROC curve was determined as a summary measure for diagnostic accuracy of the variables. Sensitivity and specificity were calculated for each cut-off value, using standard techniques. A logistic regression analysis was also performed to check the relationship between diagnostic accuracy of myocardial DC and macrophage phenotypes, and sex. All statistical analyses were performed with SPSS® for Windows version 23.0 (IBM Corp, Armonk, NY) and STATA® 14 (Stata Corp, College Station, TX). Statistical significance was defined as a *P* value of <0.05.

Results

Baseline Characteristics

Patients with CS had significantly higher prevalence of female sex, basal interventricular thinning, positive findings of gallium scintigraphy and FDG-PET, and higher serum angiotensin-converting enzyme level compared with the control group, regardless of the presence of granulomas in the myocardium and diagnostic criteria (Table 1). Other characteristics with regard to age and left ventricular ejection fraction did not differ statistically in the CS and control groups (Table 1).

Immunocompetent Cells in Myocardium With Sarcoid Granulomas

Figure 2 shows a representative section showing immunocompetent cells in myocardium with sarcoid granuloma. A large number of CD68-positive macrophages were found in the central core of granulomas, and CD3-positive T-cells were present in the central core and its surrounding area. CD209-positive DC were found mainly in the lymphocyte layer of granulomas (median number=19.5 [interquartile range: 14.3–22.0]/0.1 mm^2), and were rarely found in the central core. On the other hand, CD163-positive M2 macrophages were less frequently observed in the center and the surrounding area of granulomas (median number=7.0 [interquartile range: 2.8–8.3]/0.1 mm^2).

Table 1. Baseline Characteristics of Study Population

Variable	Cardiac Sarcoidosis			Control (n=50)
	Myocardial Granulomas (n=26)	No Myocardial Granulomas		
		Diagnosed by JMHW 2007 (n=65)	Diagnosed by HRS 2014 (n=26)	
Age, y	56 (48–66)	62 (55–68)	59 (54–68)	59 (44–70)
Female sex, n (%)	20 (77)*	46 (71)*	18 (69)*	17 (34)
LVEF, %	34 (27–42)	38 (31–49)	40 (33–53)	33 (22–55)
Basal IVS thinning, n (%)	15 (58)*	31 (48)*	14 (54)*	0 (0)
AVB, n (%)	9 (35)*	13 (20)	8 (31)	5 (10)
Sustained VT/VF, n (%)	4 (15)	15 (23)	9 (35)*	5 (10)
Organ involvements, n (%)				
Lung	15 (58)	31 (48)	13 (50)	—
Skin	1 (4)	11 (17)†	11 (42)†	—
Eye	4 (15)	22 (34)†	11 (42)†	—
Laboratory data				
BNP, pg/mL	253 (118–561)	128 (62–285)	99 (72–212)	131 (43–296)
ACE, IU/L	19.2 (14.2–25.5)*	13.0 (8.3–17.5)*	13.1 (10.3–17.6)*	9.1 (6.0–13.6)
Medications, n (%)				
ACE-Is or ARBs	12 (46)*	38 (58)	17 (65)	38 (76)
β-Blockers	17 (65)	36 (55)*	15 (58)*	41 (82)
Diuretics	14 (54)	23 (35)	7 (27)	24 (48)
Statins	6 (27)	19 (29)	7 (27)	10 (20)
Imaging modalities				
Ga scintigraphy positive, positive/n (%)	11/21 (52)*	25/45 (56)*	6/13 (46)*	0/6 (0)
FDG-PET positive, positive/n (%)	17/22 (77)*	40/50 (80)*	13/19 (68)*	0/6 (0)

Continuous variables are presented as median (interquartile range). ACE indicates angiotensin-converting enzyme; ACE-Is, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; AVB, atrioventricular block; BNP, brain natriuretic peptide; FDG-PET, ^{18}F-fluorodeoxyglucose–positron emission tomography; Ga, gallium; HRS, Heart Rhythm Society; IVS, interventricular septum; JMHW, Japanese Ministry of Health and Welfare; LVEF, left ventricular ejection fraction; VF, ventricular fibrillation; VT, ventricular tachycardia.
*$P<0.05$ vs control.
†$P<0.05$ vs granulomas in myocardium.

Immunocompetent Cells in Nongranuloma Sections of CS Patients With and Without Myocardial Granulomas

From immunohistochemical examination of nongranuloma sections of CS patients with and without myocardial granulomas, the numbers of CD209-positive DC and CD68-positive macrophages were significantly higher, and that of CD163-positive M2 macrophages was lower in the CS groups compared to the control group. Moreover, the CD163/CD68 macrophage ratio was also significantly lower in the CS groups compared to the control group. However, the number of CD3-positive T-cells was not statistically different among the CS and control groups, except for nongranuloma sections of CS patients with myocardial granulomas (Figure 3A and 3B).

Diagnostic Accuracy of Myocardial DC and Macrophage Phenotypes in Nongranuloma Sections for CS Diagnosis

ROC curves for the CD163/CD68 macrophage ratio, number of CD68-positive macrophages, and CD209-positive DC for predicting CS with each of the JMHW 2007 and HRS 2014 criteria and the presence of myocardial granulomas are shown in Figure 4A through 4C. The area under the ROC curve of the ROC curve for CD163/CD68 macrophage ratio showed the best area under the ROC curve with each criterion and the presence of myocardial granulomas. At the cut-off value, the combination of decreased CD163/CD68 macrophage ratio and increased number of CD209-positive DC in nongranuloma sections of CS patients with and without myocardial granulomas also demonstrated the highest
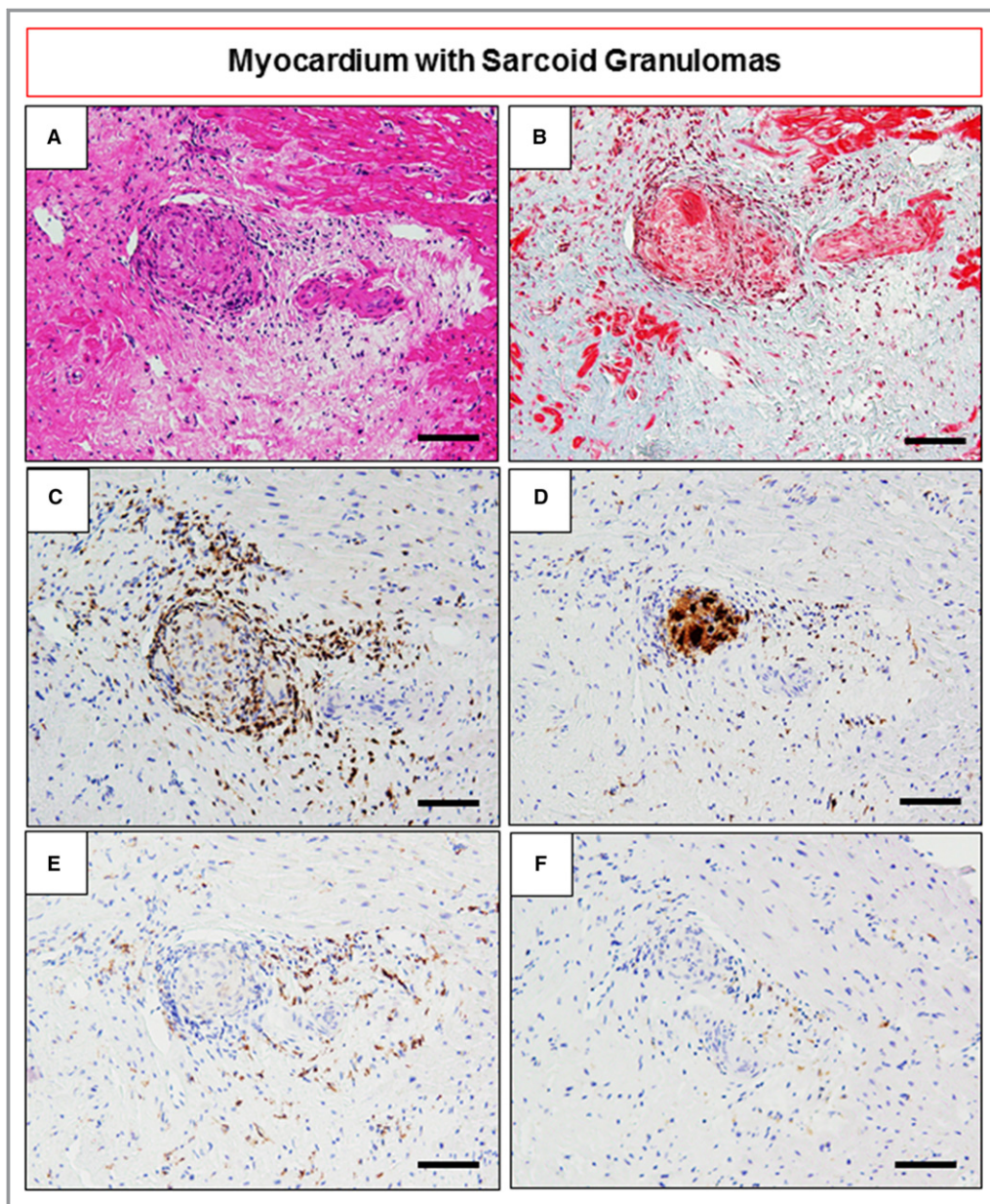
Figure 2. Immunocompetent cells in myocardium with sarcoid granulomas. A, Hematoxylin and eosin staining. B, Masson's-trichrome staining. C, CD3-positive T-cells. D, CD68-positive macrophages. E, CD209-positive dendritic cells. F, CD163-positive M2 macrophages. Scale bar indicates 100 μm (A through F).

specificity with each criterion and the presence of myocardial granulomas (Table 2). However, these variables had limited sensitivity for CS diagnosis (Table 2). There were no statistically significant interactions between diagnostic accuracy of myocardial DC and macrophage phenotypes, and sex (for the JMHW 2007 criteria; CD163/68 macrophage ratio [odds ratio: OR 0.07, 95% CI 0.005–1.01, P=0.051], CD68-positive macrophages [OR 1.02, 95% CI 0.83–1.27, P=0.84], CD209-positive DC [OR 0.94, 95% CI 0.81–1.10, P=0.48], for the HRS 2014 criteria; CD163/68 macrophage ratio [OR 0.02, 95% CI 0.0003–1.19, P=0.061], CD68-positive macrophages [OR 1.07, 95% CI 0.83–1.38, P=0.60], CD209-positive DC [OR

1.00, 95% CI 0.83–1.21, P=1.00], for the presence of myocardial granulomas; CD163/68 macrophage ratio [OR 0.06, 95% CI 0.00008–42.63, P=0.40], CD68-positive macrophages [OR 0.99, 95% CI 0.65–1.51, P=0.97], CD209-positive DC [OR 0.96, 95% CI 0.74–1.27, P=0.80]).

Discussion

The major findings of the present study were as follows: (1) CD209-positive DC were found in the myocardium with sarcoid granulomas, mainly in the lymphocyte layer of granulomas, and were rarely found in the central core. On the other hand, the
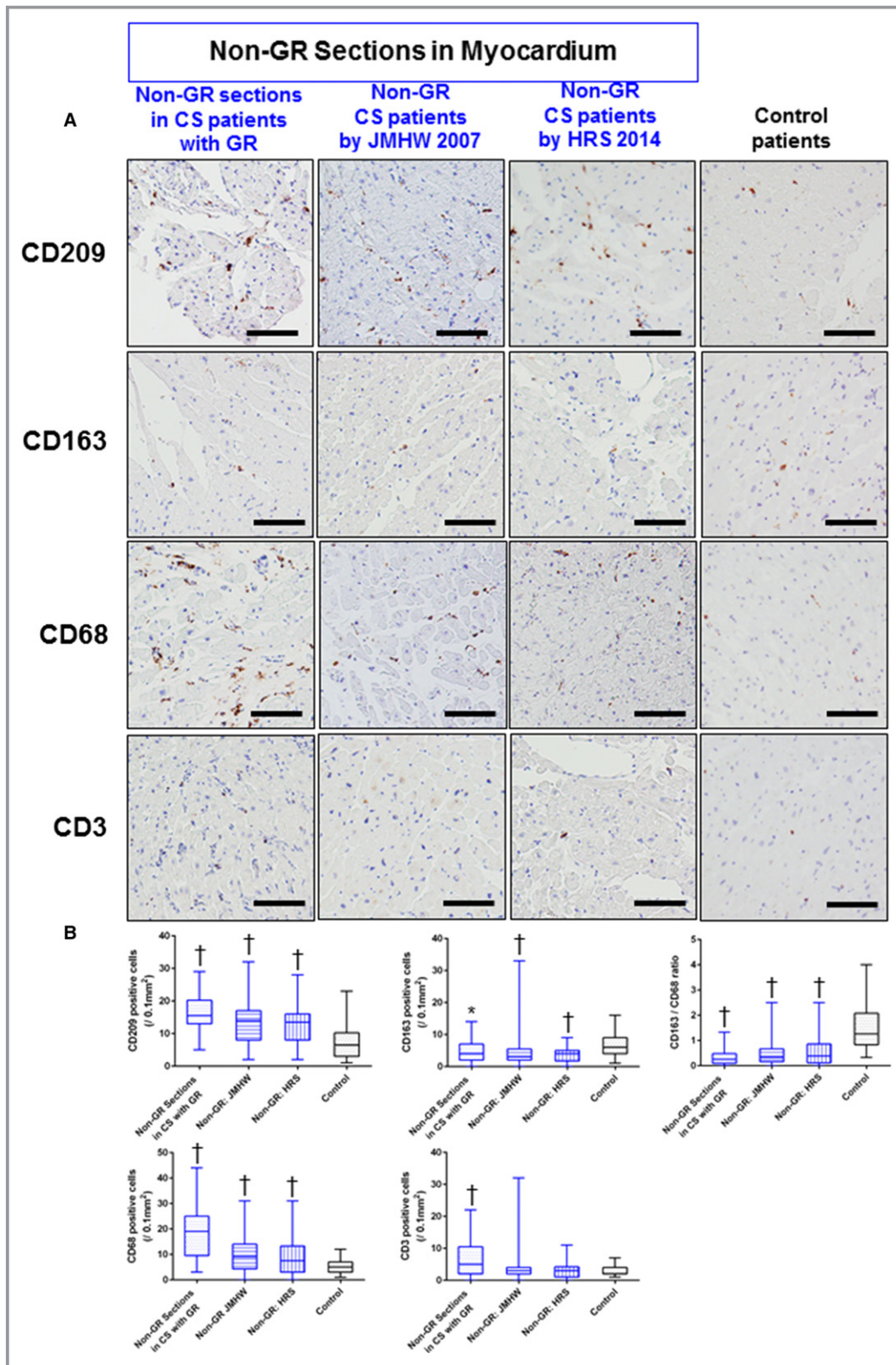
Figure 3. Immunocompetent cells in nongranuloma sections of myocardium. A, Immunohistochemical staining for CD209-positive dendritic cells, CD163-positive M2 macrophages, CD68-positive macrophages, and CD3-positive T-cells. B, Quantitative analyses for CD 209, CD163, CD68, and CD3-positive cells, and CD163/CD68 ratio. CS, cardiac sarcoidosis; GR, granuloma; JMHW, Japanese Ministry of Health and Welfare; HRS, Heart Rhythm Society. Scale bar indicates 100 μm (A). *$P<0.05$, †$P<0.01$ vs control.
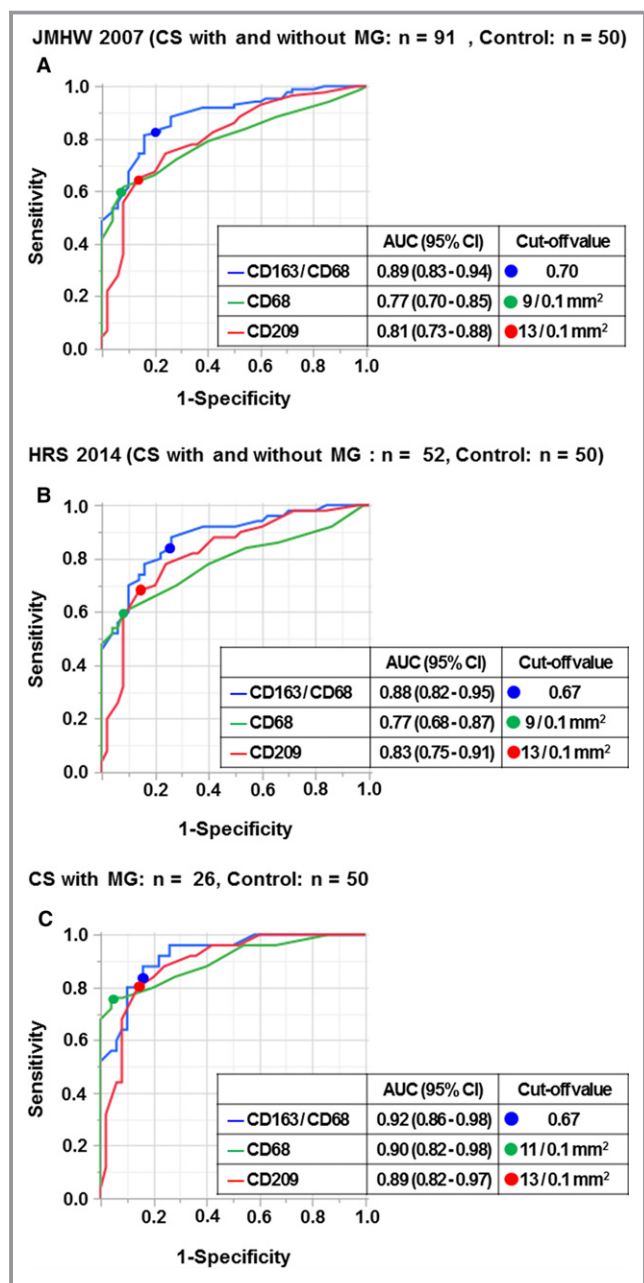
Figure 4. Diagnostic accuracy of myocardial DC and macrophage phenotypes in nongranuloma sections for CS diagnosis. A, JMHW criteria. B, HRS 2014 criteria. C, CS with MG. AUC indicates area under the curve; CS, cardiac sarcoidosis; DC, dendritic cells; HRS, Heart Rhythm Society; JMHW, Japanese Ministry of Health and Welfare; MG, myocardial granulomas.

Table 2. Diagnostic Accuracy of Immunocompetent Cells in Nongranuloma Sections of Myocardium

Variables	Sensitivity, % (95% CI)	Specificity, % (95% CI)
For JMHW 2007 criteria CS with and without MG: n=91 Control: n=50		
CD163/CD68 ratio $\leq$0.70	81.4 (72.6–89.0)	84.0 (70.9–92.8)
CD68 $\geq$9/0.1 mm^2	59.6 (48.6–69.8)	92.0 (80.8–97.8)
CD209 $\geq$13/0.1 mm^2	64.8 (54.1–74.6)	86.0 (73.3–94.2)
CD163/CD68 ratio $\leq$0.70, and CD209 $\geq$13/0.1 mm^2	46.2 (35.6–56.9)	100 (92.9–100)
For HRS 2014 criteria CS with and without MG: n=52 Control: n=50		
CD163/CD68 ratio $\leq$0.67	78.0 (64.0–88.5)	84.0 (70.9–92.8)
CD68 $\geq$9/0.1 mm^2	58.8 (44.2–72.4)	92.0 (80.8–97.8)
CD209 $\geq$13/0.1 mm^2	69.2 (54.9–81.3)	86.0 (73.3–94.2)
CD163/CD68 ratio $\leq$0.67, and CD209 $\geq$13/0.1 mm^2	46.2 (32.2–60.5)	100 (92.9–100)
For CS with MG: n=26 Control: n=50		
CD163/CD68 ratio $\leq$0.67	88.0 (68.8–97.5)	84.0 (70.9–92.8)
CD68 $\geq$11/0.1 mm^2	76.0 (54.9–90.6)	96.0 (86.3–99.5)
CD209 $\geq$13/0.1 mm^2	80.8 (60.6–93.4)	86.0 (73.3–94.2)
CD163/CD68 ratio $\leq$0.67, and CD209 $\geq$13/0.1 mm^2	65.4 (44.3–82.8)	100 (92.9–100)

CS indicates cardiac sarcoidosis; HRS, Heart Rhythm Society; JMHW, Japanese Ministry of Health and Welfare; MG, myocardial granulomas.

number of CD163-positive M2 macrophages was quite low in myocardium with sarcoid granulomas. (2) CD209-positive DC and CD68-positive macrophages were more frequently observed, and CD163-positive M2 macrophages were less frequently observed in nongranuloma sections of CS patients regardless of the presence or absence of myocardial granulomas compared to those in the control group. (3) The

combination of decreased CD163/CD68 macrophage ratio and increased number of CD209-positive DC in nongranuloma sections using those cut-off values by ROC analyses demonstrated the highest specificity (100%, 95% CI 92.9–100) for CS diagnosis with the JMHW 2007 and the HRS 2014 criteria, and the presence of myocardial granulomas. These findings indicated that CD209-positive DC and CD68-positive macrophages might be associated with the development of granulomas in sarcoid myocardial lesions. Furthermore, increased number of DC and decreased M2 macrophages in nongranuloma sections of EMB samples also might be histopathological surrogates for the diagnosis of CS even in patients without myocardial granulomas detected by EMB.

Although the pathogenesis of sarcoidosis remains unclear, several findings from previous studies in sarcoidosis patients, including an increased number of oligoclonal T-cells in bronchoalveolar lavage fluid and skin granulomas, are suggestive of an antigen-driven autoimmune disease.[18,19] DC may play cardinal roles in both innate and adaptive immunity, acting as representative antigen-presenting cells in sarcoidosis. Indeed, DC contribute to granuloma formation in experimental models of granulomatous disease in response to

mycobacterium antigens, and recent clinical studies have suggested that DC are the key antigen-presenting cells in pulmonary and muscular sarcoidosis.[12,13,20] Willart et al have demonstrated that pulmonary granuloma formation is dependent on the presence of DC and DC-induced T-cell proliferation, and DC are also observed in not only the lungs, but also in skin and lymph nodes.[21] In addition, DC recruitment and CD40/CD40L system upregulation in muscular sarcoidosis indicate that DC are involved in granulomatous inflammation through antigen presentation in a Th1 immune system.[13]

Notably, several subtypes of DC have been described thus far, with so-called myeloid DC (mDC) and plasmacytoid DC (pDC) being predominant.[22] Myeloid DC have been reported to capture antigens in peripheral tissues, carry them to draining lymph nodes, and stimulate naive T cells and induce either Th1 or Th2 differentiation, depending on their maturation stage and the type and duration of activation.[23] Plasmacytoid DC are also important in the initiation and regulation of immune responses, and enter lymph nodes during inflammation, where they are able to secrete large amounts of interferon-γ in response to viral stimulation.[24] It is noteworthy that there are a larger number of mDC in sarcoidosis bronchoalveolar lavage fluid compared to normal, and this finding is specific to sarcoidosis and not to other inflammatory lung diseases.[25] Furthermore, pDC were found less frequently than mDC in the lymphocyte layers of granulomas in muscle lesions of sarcoidosis.[13] In sarcoidosis bronchoalveolar lavage fluid and blood,[26,27] pDC also appear with similar frequencies and absolute numbers to normal. These results indicate that mDC rather than pDC play a crucial role in the pathogenesis of sarcoidosis. Consistent with previous reports, our present findings suggested that CD209-positive mDC were frequently observed in the lymphocyte layer of myocardial granulomas.

Interestingly, CD163-positive M2 macrophages were not often found in both the central core and lymphocyte layer of granulomas in the present study. Moreover, the CD163/CD68 macrophage ratio in nongranuloma sections in CS patients was also significantly decreased compared to that in controls. Whereas M1 macrophages have a pro-inflammatory function, M2 macrophages have a suppressive and immunoregulatory function, namely, acting as anti-inflammatory macrophages accompanied by enhanced interleukin-10 and interleukin-1 receptor antagonist production.[28] Thus, the increased CD209-positive DC and decreased CD163/CD68 macrophage ratio in nongranuloma sections in CS patients indicate that the M1 macrophage-dominant pro-inflammatory process might precede the formation of myocardial granulomas in CS patients.

Ideally, CS should be diagnosed based on definite histopathological evidence, representative of the presence of noncaseating granulomas in the heart or other organs.[7,8] However, quite a few cases fail to meet the HRS 2014 criteria

because of negative biopsy findings, albeit with many specific clinical findings of CS in at least 2 involved organs and meeting the JMHW 2007 criteria. Our present findings demonstrated that increased CD209-positive DC and CD68-positive macrophages were more frequently observed and CD163-positive M2 macrophages were less frequently observed in nongranuloma sections in CS patients with and without myocardial granulomas compared to the control group. Moreover, the combination of decreased CD163/CD68 macrophage ratio, which may represent a pro-inflammatory status, and increased number of CD209 above the cut-off value in nongranuloma sections showed high diagnostic accuracy. Therefore, these histopathological findings could be useful surrogates for the diagnosis of CS.

Several limitations of this study warrant mention. First, the number of study patients was relatively small. The statistical power thus might not be adequate for any negative results. Second, we could not find significant proliferation of T-cells in nongranuloma sections in CS patients without myocardial granulomas in parallel with increased DC. However, a previous study has shown that DC isolated from bronchoalveolar lavage fluid in sarcoidosis patients increase pro-inflammatory tumor necrosis factor-α secretion without enhanced T-cell proliferation.[12] Therefore, interactions between DC and T-cell proliferation are still under debate. Third, our study focused on the histopathological findings derived from invasive and expensive procedures. Noninvasive approaches with lower cost (eg, biomarkers and diagnostic imaging) would be more useful practically. Nevertheless, we believe that our current findings may be useful for the diagnosis of CS in patients without myocardial granulomas in EMB samples and could contribute to the development of new diagnostic tools (eg, peripheral immunocompetent cells or molecular imaging). Fourth, both the JMHW and HRS criteria may be nonspecific reference standards because of the insufficient diagnostic accuracy of these criteria for cardiac involvement.[8,29] Although we obtained similar results in CS patients with the presence of myocardial granulomas as a specific reference standard (Figure 4C), the sample size was quite small. Finally, because all study patients were Japanese, our findings may not be generalizable or applicable to non-Japanese patients. Thus, validation studies in another cohort outside Asians are warranted.

In conclusion, we identified that DC and macrophages were more frequently observed, and M2 macrophages were less frequently observed in both myocardial granulomas and nongranuloma sections in CS patients compared to controls. Furthermore, the combination of decreased M2 macrophage ratio among all macrophages and increased DC number in nongranuloma sections of the myocardium demonstrated high specificity for the diagnosis of CS, suggesting the significance of DC and macrophage phenotypes as novel histopathological surrogates for the diagnosis of CS.

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Disclosures

None.

References

1. Silverman KJ, Hutchins GM, Bulkley BH. Cardiac sarcoid: a clinicopathologic study of 84 unselected patients with systemic sarcoidosis. *Circulation*. 1978;58:1204–1211.

2. Yazaki Y, Isobe M, Hiroe M, Morimoto S, Hiramitsu S, Nakano T, Izumi T, Sekiguchi M. Prognostic determinants of long-term survival in Japanese patients with cardiac sarcoidosis treated with prednisone. *Am J Cardiol*. 2001;88:1006–1010.

3. Nagai T, Nagano N, Sugano Y, Asaumi Y, Aiba T, Kanzaki H, Kusano K, Noguchi T, Yasuda S, Ogawa H, Anzai T. Effect of discontinuation of prednisolone therapy on risk of cardiac mortality associated with worsening left ventricular dysfunction in cardiac sarcoidosis. *Am J Cardiol*. 2016;117:966–971.

4. Nagai T, Nagano N, Sugano Y, Asaumi Y, Aiba T, Kanzaki H, Kusano K, Noguchi T, Yasuda S, Ogawa H, Anzai T. Effect of corticosteroid therapy on long-term clinical outcome and left ventricular function in patients with cardiac sarcoidosis. *Circ J*. 2015;79:1593–1600.

5. Nagai T, Kohsaka S, Okuda S, Anzai T, Asano K, Fukuda K. Incidence and prognostic significance of myocardial late gadolinium enhancement in patients with sarcoidosis without cardiac manifestation. *Chest*. 2014;146:1064–1072.

6. Diagnostic standard and guidelines for sarcoidosis. *Jpn J Sarcoidosis Granulomatous Disord*. 2007;27:89–102.

7. Judson MA, Costabel U, Drent M, Wells A, Maier L, Koth L, Shigemitsu H, Culver DA, Gelfand J, Valeyre D, Sweiss N, Crouser E, Morgenthau AS, Lower EE, Azuma A, Ishihara M, Morimoto S, Tetsuo Yamaguchi T, Shijubo N, Grutters JC, Rosenbach M, Li HP, Rottoli P, Inoue Y, Prasse A, Baughman RP; Organ Assessment Instrument Investigators TW. The WASOG Sarcoidosis Organ Assessment Instrument: an update of a previous clinical tool. *Sarcoidosis Vasc Diffuse Lung Dis*. 2014;31:19–27.

8. Birnie DH, Sauer WH, Bogun F, Cooper JM, Culver DA, Duvernoy CS, Judson MA, Kron J, Mehta D, Cosedis Nielsen J, Patel AR, Ohe T, Raatikainen P, Soejima K. HRS expert consensus statement on the diagnosis and management of arrhythmias associated with cardiac sarcoidosis. *Heart Rhythm*. 2014;11:1305–1323.

9. Ardehali H, Howard DL, Hariri A, Qasim A, Hare JM, Baughman KL, Kasper EK. A positive endomyocardial biopsy result for sarcoid is associated with poor prognosis in patients with initially unexplained cardiomyopathy. *Am Heart J*. 2005;150:459–463.

10. Anzai A, Anzai T, Nagai S, Maekawa Y, Naito K, Kaneko H, Sugano Y, Takahashi T, Abe H, Mochizuki S, Sano M, Yoshikawa T, Okada Y, Koyasu S, Ogawa S, Fukuda K. Regulatory role of dendritic cells in postinfarction healing and left ventricular remodeling. *Circulation*. 2012;125:1234–1245.

11. Nagai T, Honda S, Sugano Y, Matsuyama TA, Ohta-Ogo K, Asaumi Y, Ikeda Y, Kusano K, Ishihara M, Yasuda S, Ogawa H, Ishibashi-Ueda H, Anzai T. Decreased myocardial dendritic cells is associated with impaired reparative fibrosis and development of cardiac rupture after myocardial infarction in humans. *J Am Heart Assoc*. 2014;3:e000839 doi: 10.1161/JAHA.114.000839.

12. Ten Berge B, Kleinjan A, Muskens F, Hammad H, Hoogsteden HC, Hendriks RW, Lambrecht BN, Van den Blink B. Evidence for local dendritic cell activation in pulmonary sarcoidosis. *Respir Res*. 2012;13:33.

13. Tateyama M, Fujihara K, Itoyama Y. Dendritic cells in muscle lesions of sarcoidosis. *Hum Pathol*. 2011;42:340–346.

14. Cassetta L, Cassol E, Poli G. Macrophage polarization in health and disease. *Scientific World Journal*. 2011;11:2391–2402.

15. Cooper LT, Baughman KL, Feldman AM, Frustaci A, Jessup M, Kuhl U, Levine GN, Narula J, Starling RC, Towbin J, Virmani R. The role of endomyocardial biopsy in the management of cardiovascular disease: a scientific statement from the American Heart Association, the American College of Cardiology, and the European Society of Cardiology. Endorsed by the Heart Failure Society of America and the Heart Failure Association of the European Society of Cardiology. *J Am Coll Cardiol*. 2007;50:1914–1931.

16. Ishimaru S, Tsujino I, Takei T, Tsukamoto E, Sakaue S, Kamigaki M, Ito N, Ohira H, Ikeda D, Tamaki N, Nishimura M. Focal uptake on ^{18}F-fluoro-2-deoxyglucose positron emission tomography images indicates cardiac involvement of sarcoidosis. *Eur Heart J*. 2005;26:1538–1543.

17. Manabe O, Ohira H, Yoshinaga K, Sato T, Klaipetch A, Oyama-Manabe N, Ito YM, Tsujino I, Nishimura M, Tamaki N. Elevated ^{18}F-fluorodeoxyglucose uptake in the interventricular septum is associated with atrioventricular block in patients with suspected cardiac involvement sarcoidosis. *Eur J Nucl Med Mol Imaging*. 2013;40:1558–1566.

18. Forman JD, Klein JT, Silver RF, Liu MC, Greenlee BM, Moller DR. Selective activation and accumulation of oligoclonal V beta-specific T cells in active pulmonary sarcoidosis. *J Clin Invest*. 1994;94:1533–1542.

19. Mempel M, Flageul B, Suarez F, Ronet C, Dubertret L, Kourilsky P, Gachelin G, Musette P. Comparison of the T cell patterns in leprous and cutaneous sarcoid granulomas. Presence of Valpha24-invariant natural killer T cells in T-cell-reactive leprosy together with a highly biased T cell receptor Valpha repertoire. *Am J Pathol*. 2000;157:509–523.

20. Tsuchiya T, Chida K, Suda T, Schneeberger EE, Nakamura H. Dendritic cell involvement in pulmonary granuloma formation elicited by bacillus Calmette-Guerin in rats. *Am J Respir Crit Care Med*. 2002;165:1640–1646.

21. Willart MA, Jan de Heer H, Hammad H, Soullie T, Deswarte K, Clausen BE, Boon L, Hoogsteden HC, Lambrecht BN. The lung vascular filter as a site of immune induction for T cell responses to large embolic antigen. *J Exp Med*. 2009;206:2823–2835.

22. Steinman RM. Lasker Basic Medical Research Award. Dendritic cells: versatile controllers of the immune system. *Nat Med*. 2007;13:1155–1159.

23. Schuurhuis DH, Fu N, Ossendorp F, Melief CJ. Ins and outs of dendritic cells. *Int Arch Allergy Immunol*. 2006;140:53–72.

24. Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A, Colonna M. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med*. 1999;5:919–923.

25. Zaba LC, Smith GP, Sanchez M, Prystowsky SD. Dendritic cells in the pathogenesis of sarcoidosis. *Am J Respir Cell Mol Biol*. 2010;42:32–39.

26. Lommatzsch M, Bratke K, Bier A, Julius P, Kuepper M, Luttmann W, Virchow JC. Airway dendritic cell phenotypes in inflammatory diseases of the human lung. *Eur Respir J*. 2007;30:878–886.

27. Mathew S, Bauer KL, Fischoeder A, Bhardwaj N, Oliver SJ. The anergic state in sarcoidosis is associated with diminished dendritic cell function. *J Immunol*. 2008;181:746–755.

28. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol*. 2002;23:549–555.

29. Blankstein R, Osborne M, Naya M, Waller A, Kim CK, Murthy VL, Kazemian P, Kwong RY, Tokuda M, Skali H, Padera R, Hainer J, Stevenson WG, Dorbala S, Di Carli MF. Cardiac positron emission tomography enhances prognostic assessments of patients with suspected cardiac sarcoidosis. *J Am Coll Cardiol*. 2014;63:329–336.