

## ORIGINAL ARTICLE

# Adaptive immune system in pulmonary sarcoidosis—Comparison of peripheral and alveolar biomarkers

Miriana d'Alessandro <sup>1</sup> | Laura Bergantini<sup>1</sup> | Paolo Cameli<sup>1</sup> | Fabrizio Mezzasalma<sup>2</sup> | Rosa Metella Refini<sup>1</sup> | Maria Pieroni<sup>1</sup> | Piersante Sestini<sup>1</sup> | Elena Bargagli<sup>1</sup>

<sup>1</sup>Respiratory Diseases and Lung Transplantation, Department of Medical and Surgical Sciences & Neurosciences, Siena University Hospital, Siena, Italy

<sup>2</sup>Diagnostic and Interventional Bronchoscopy Unit, Cardio-Thoracic and Vascular Department, University Hospital of Siena (Azienda Ospedaliera Universitaria Senese, AOUS), Siena, Italy

## Correspondence

Miriana d'Alessandro, Respiratory Diseases Unit, Department of Medical and Surgical Sciences and Neurosciences, University of Siena, Viale Bracci 1, 53100 Siena, Italy.  
 Email: dalessandro.miriana@gmail.com

## Abstract

Sarcoidosis is a multi-systemic granulomatous disease of unknown origin. Recent research has focused upon the role of autoimmunity in its development and progression. This study aimed to determine and define the disturbance and distribution of T and B cell subsets in the alveolar and peripheral compartments. Thirteen patients were selected for the study [median age, interquartile range (IQR) = 57 years (48–59); 23% were male]. Twelve healthy controls [median age, IQR = 53 years (52–65); 16% male] were also enrolled into the study. Cellular and cytokine patterns were measured using the cytofluorimetric approach. Peripheral CD8 percentages were higher in sarcoidosis patients (SP) than healthy controls (HC) ( $p = 0.0293$ ), while CD4 percentages were lower ( $p = 0.0305$ ). SP showed low bronchoalveolar lavage (BAL) percentages of CD19 ( $p = 0.0004$ ) and CD8 ( $p = 0.0035$ ), while CD19<sup>+</sup>CD5<sup>+</sup>CD27<sup>-</sup> percentages were higher ( $p = 0.0213$ ); the same was found for CD4 ( $p = 0.0396$ ), follicular regulatory T cells (T<sub>reg</sub>) ( $p = 0.0078$ ) and T<sub>reg</sub> ( $p < 0.0001$ ) cells. Low T helper type 17 (Th17) percentages were observed in BAL ( $p = 0.0063$ ) of SP. Peripheral CD4<sup>+</sup> C-X-C chemokine receptor (CXCR)5<sup>+</sup>CD45RA<sup>-</sup> percentages and follicular T helper cells (Tfh)-like Th1 (Tfh1) percentages ( $p = 0.0493$  and  $p = 0.0305$ , respectively) were higher in the SP than HC. Tfh1 percentages and Tfh-like Th2 percentages were lower in BAL than in peripheral blood ( $p = 0.0370$  and  $p = 0.0078$ , respectively), while CD4<sup>+</sup> C-X-C motif CXCR5<sup>+</sup>CD45RA<sup>-</sup> percentages were higher ( $p = 0.0011$ ). This is the first study, to our knowledge, to demonstrate a link between an imbalance in circulating and alveolar Tfh cells, especially CCR4-, CXCR3- and CXCR5-expressing Tfh subsets in the development of sarcoidosis. These findings raise questions about the pathogenesis of sarcoidosis and may provide new directions for future clinical studies and treatment strategies.

## KEYWORDS

biomarkers, bronchoalveolar lavage, follicular cells, regulatory cells, sarcoidosis

## INTRODUCTION

Sarcoidosis is a multi-systemic granulomatous disease of unknown origin with common lung involvement, variable clinical course and no universally accepted treatment algorithm [1,2].

The incidence of sarcoidosis is higher in women, peaking between 30 and 60 years [2]. Recent research has focused upon the etiology and pathogenesis of sarcoidosis and the role of autoimmunity in its development and progression. Genetic predisposition, infections, environmental factors and exposure to inorganic

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors. *Clinical & Experimental Immunology* published by John Wiley & Sons Ltd on behalf of British Society for Immunology

substances may trigger an autoimmune response in the disease [2]. The pathogenesis of autoimmune diseases may be orchestrated by T cell immunity and the corresponding lymphocyte subsets. CD4<sup>+</sup> T cells from the bronchoalveolar lavage (BAL) of sarcoidosis patients are also highly T helper type 1 (Th1) polarized. CD4<sup>+</sup> T cells from the BAL of sarcoidosis patients are also highly Th1 polarized. Considering the infiltration of Th1-polarized CD4<sup>+</sup> T cells in the lung, it is widely accepted that these T cells are trafficking to and proliferating in the lung in response to an unknown antigen. In contrast to CD4<sup>+</sup> T cell alveolitis in BAL of sarcoidosis patients [3], T cell lymphopenia in the peripheral blood is characteristic of the disease [4]. CD4<sup>+</sup> T cells with a Th17 phenotype and T cells expressing both interleukin (IL)-17 and interferon (IFN)- $\gamma$  (i.e. Th17.1 cells) have been found in lung tissue and BAL from sarcoidosis [5,6]. Recently, a significant increase was demonstrated in CCR6<sup>+</sup> Th17.1 cells in both BAL fluid and mediastinal lymph nodes of sarcoidosis patients compared to controls. Interestingly, these cells contribute to IFN- $\gamma$  production [7]. It is believed that disturbance of Th17-stimulated regulatory T cells (T<sub>regs</sub>) can lead to autoimmune inflammation, characterized by the presence of autoantigen-specific T and B lymphocytes producing autoantibodies [8]. Disturbances in T and B cell immune responses have been reported in several studies of sarcoidosis, suggesting that the triggering factor for inflammation is the contact of a foreign antigen with antigen-presenting cells, leading to activation of T and B lymphocytes which migrate to the inflammatory foci [9–16]. If the antigen persists, macrophages undergo epithelioid differentiation and lymphocytes continue to migrate to the foci, forming granulomas. Peripheral blood B cell subsets of patients with sarcoidosis are often altered with respect to healthy controls [17]. Although there is no evidence that B cells can play a direct role in disease pathogenesis in sarcoidosis, several studies have reported altered antibody responses in these patients [18]. Increased numbers of memory B cells producing immunoglobulin (Ig)A are found in patients with sarcoidosis, raising the possibility that IgA could be involved in granuloma formation [19]. A new regulatory B cell (B<sub>reg</sub>) (CD19<sup>+</sup>CD1d<sup>+</sup>CD5<sup>+</sup>) subset was discovered in the last decade, suggesting a potential function in the development of T cell immune responses, including inhibition of naive T and Th17 cells [20]. B cell involvement in sarcoidosis is therefore well documented [21,22], but nothing is known about T follicular helper (Tfh) cells in this disease. Tfh is the major cell subset considered to be involved in humoral adaptive immune response, due to its essential role in generating plasma and memory B cells during the germinal center reaction. Tfh cells express high levels of the C-X-C chemokine receptor (CXCR)5 necessary for Tfh migration towards B cell follicles rich in CXCL13 (B cell chemoattractant). Additionally, specific stimulation of Tfh cells induces secretion of IL-21, thereby promoting B cell growth, differentiation and class-switching. Recent studies have demonstrated that alterations in circulating Tfh cell subsets have significant effects on the progression of various autoimmune diseases [23].

The aim of this study was to determine and define disturbance and distribution of T and B cell subsets in the alveolar and peripheral compartments, focusing upon the proportions of Tfh, including T follicular regulatory cells, in patients with sarcoidosis.

## MATERIALS AND METHODS

### Study population

Forty patients, monitored at the Siena Regional Referral Centre for Sarcoidosis and other Interstitial Lung Diseases, were enrolled retrospectively. We excluded patients with active respiratory infections, malignant diseases, Lofgren syndrome, acute disease onset or spontaneous remission of sarcoidosis, as well as those patients for whom simultaneous BAL and peripheral blood samples were not available. Thirteen were selected for the study. The median age [interquartile range (IQR)] was 57 years (range = 48–59); 23% were male. All had undergone bronchoscopy for interstitial lung disease (ILD) diagnostic work-up. Diagnosis was based on international criteria [24,25]. In all cases, medical history, lung function parameters and serum angiotensin-converting enzyme (ACE) concentrations were recorded in a specific database. Bronchoscopy with BAL and chest X-ray with radiological staging, performed in the framework of the sarcoidosis diagnostic work-up, were also available. The radiological classification was according to Scadding [26]: stage 0, normal; stage 1, bilateral hilar adenopathy without parenchymal involvement; stage 2, bilateral adenopathy and parenchymal infiltration; stage 3, parenchymal infiltration; and stage 4, pulmonary fibrosis associated with sarcoidosis. All patients were negative for tuberculosis (TB), bacterial infections, mycological infections and malignant diseases. Twelve healthy controls [median age (IQR) = 53 years (52–65); 16% male] were also enrolled into the study. They had no history of concomitant pathologies and were not on any medication, and had normal lung function test parameters.

Peripheral blood was sampled from both patients and controls while BAL samples were collected only from sarcoidosis patients, because BAL cannot be performed on healthy subjects for scientific purposes in Italy.

All patients and healthy individuals gave their written informed consent to the study, which was approved by the local ethics committee (CEAVSE, Markerlung 17431, Tuscany, Italy).

### Peripheral blood mononuclear cell (PBMC) preparation

Preparation and storage of PBMCs was performed at the Respiratory Diseases Laboratory, University Hospital of Siena. Peripheral blood samples were collected after 8-h fasting in a tube containing ethylenediamine tetraacetic acid

(EDTA) anti-coagulant (BD Vacutainer® EDTA tubes; BD Biosciences, San Jose, California, USA) and processed within 8 h, as previously reported [27].

## BAL processing

Bronchoscopy with BAL was performed for diagnostic purposes according to the European Respiratory Society BAL Task Force Group guidelines [28]. Briefly, BAL was filtered through sterile gauze and cell count was determined by cytocentrifuge smear (600 g for 5 min) with a Thermo Shandon Cytospin 3 (Marshall Scientific, Hampton, New Hampshire, USA) and stained with Diff Quik Stain Kit (modified Giemsa) (Diapath, Bergamo, Italy); a total of 500 cells were counted. Cell viability was determined by trypan blue exclusion in a Burkner chamber.

## Regulatory cell detection

The phenotypes of T<sub>reg</sub> and B<sub>reg</sub> cells through flow cytometric analysis were performed as previously reported [29–31]. The panel of monoclonal antibodies, including T<sub>regs</sub> (human regulatory T cell cocktail; BD Biosciences), fluorescein isothiocyanate anti-human CD4 (clone SK3), phycoerythrin-cyanin 7 (PE-Cy7) anti-human CD25 (clone 2A3) and Alexa Fluor® 647 anti-human CD127 (clone HIL-7R-M21), were used. V450 anti-CD8, allophycocyanin (APC)-Cy7 anti-CD3 and peridinin chlorophyll (PerCP)-Cy5.5 anti-CXCR5 were also added. Cells were stained for 30 min at 4°C, measured with a fluorescence activated cell sorter (FACS)Canto II flow cytometer and analyzed using Kaluza Analysis version 2.1 (Beckman Coulter Life Sciences, Indianapolis, Indiana, USA). Supporting information, Figure S1 reports the gating strategy for T<sub>reg</sub> panel, while Supporting information, Table S1 summarizes the main cell subsets identified from the analysis.

To detect B<sub>regs</sub>, anti-human monoclonal antibodies (mAbs) were used as follows: APC anti-CD45, fluorescein isothiocyanate (FITC)-conjugated anti-CD38, PE anti-CD1d, PE-Cy7-conjugated anti-CD19, PerCP-CY5.5-conjugated CD5, APC-H7-conjugated anti-CD24 and BV510-conjugated anti-CD27. Cells were stained for 30 min at 4°C, measured with a FACSCanto II flow cytometer and analyzed using Kaluza Analysis version 2.1 (Beckman Coulter Life Sciences). Supporting information, Figure S2 reports the gating strategy for the B<sub>reg</sub> panel, while Supporting information, Table S1 summarizes the main cell subsets identified from the analysis.

## Central and effector cell phenotyping (Th17, Th17.1, Th1, Th2)

The following fluorochrome-labeled monoclonal anti-human antibodies were used to identify central and effector

cells: CD3-APC-Cy7, CD4-V450 (REA623), CD45RA-PE-Vio770 (REA562), CD196 (CCR6)-APC (REA190), CD183 (CXCR3)-VioBright FITC (REA232), CD194 (CCR4)-PE (REA279) and PerCP-Cy5.5 anti-CXCR5 (all from Miltenyi Biotec, Bergisch Gladbach, Germany). Cells were stained for 30 min at 4°C, measured with a FACSCanto II flow cytometer and analyzed using Kaluza Analysis version 2.1 (Beckman Coulter Life Sciences). Supporting information, Figure S3 reports the gating strategy for central and effector cell phenotyping panel, while Supporting information, Table S1 summarizes the main cell subsets identified from the analysis.

## Follicular T cell phenotyping

The following fluorochrome-labeled monoclonal anti-human antibodies were used to identify follicular T cells: CD3-APC-Cy7, CD4-V450 (REA623), CD45RA-PE-Cy7, CD196 (CCR6)-APC (REA190), CD183 (CXCR3)-VioBright FITC (REA232) and CD185 (CXCR5) (all from Miltenyi Biotec). Cells were stained for 30 min at 4°C, measured with a FACSCanto II flow cytometer and analyzed using Kaluza Analysis version 2.1 (Beckman Coulter Life Sciences). Supporting information, Figure S3 reports the gating strategy for follicular T cell phenotyping, while Supporting information, Table S1 summarizes the main cell subsets identified from the analysis.

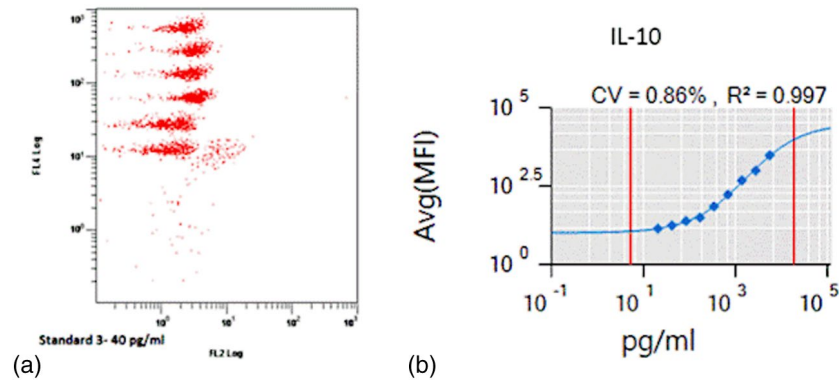
## Cytometric bead array analysis

A human Th1/Th2/Th17 cytokine kit was used to measure serum concentrations of IL-2, -4, -6, -10, tumor necrosis factor (TNF), IFN- $\gamma$  and IL-17A in blood drawn from controls and sarcoidosis patients at the same time as that drawn for cell analysis, before and after anti-fibrotic therapy, as previously reported [31]. The tests were performed according to the manufacturer's protocols [32].

FACSCanto II flow cytometer and LEGENDplex™ version 8.0 software (BioLegend, San Diego, California, USA) were used to analyze all samples. Concentrations were expressed in pg/ml and entered into the database. Before analysis, the cytometer was calibrated using set-up beads according to the manufacturer's protocol [33].

Cytometric analysis of standards generated 10 cytograms fluorescence (FL)4 *versus* FL2) for each kit. A dot-plot (cytogram) example is shown in Figure 1a. LEGENDplex™ version 8.0 software was used to calculate mean fluorescence intensity (MFI) for each population of molecules that bound a given cytokine. As a result, six standard curves (four parameter logistic) were obtained and an example of a standard curve for IL-10 is shown in Figure 1b.

The linear part of the curves allowing repetitive interpolation of fluorescence intensity began above 20 pg/ml.



**FIGURE 1** (a) Fluorescence (FL)4 fluorescence distinguished cell populations and FL2 (*x*-axis) for the studied cytokines. (b) An example of a standard curve for interleukin (IL)-10

## Statistical analysis

All the results were expressed as medians and quartiles (25th and 75th percentiles) for continuous variables, as appropriate. For multiple comparisons, Kruskal–Wallis test [one-way analysis of variance (ANOVA) non-parametric test with Bonferroni correction to avoid a type I error] and Dunn's comparison test were performed. The Mann–Whitney *U*-test was used for comparison between the two groups. The  $\chi^2$  test was used for categorical variables. Spearman's test was used to look for correlations. A *p* value less than 0.05 was considered statistically significant. Statistical analysis was performed by GraphPad Prism version 9.0 software.

## RESULTS

### Study population

Demographic features, lung function parameters and Scadding radiological stages of our sarcoidosis patients are reported in Table 1. Tables 2 and 3 report peripheral and alveolar cell and cytokine patterns of sarcoidosis patients and HC, respectively. As expected [34–37], disease onset mainly occurred in the 5th decade ( $44.6 \pm 7.7$  years), while most patients were female (77%) and never-smokers (54%). In our population, lung parameters were in the normal range with no significant alterations with respect to reference values.

### Regulatory cell detection

Peripheral percentages of CD1d<sup>+</sup>CD5<sup>+</sup> cells were higher in sarcoidosis patients than healthy controls (*p* = 0.0085), as were those of CD8 cells (*p* = 0.0293) (Figure 2). Lower peripheral percentages of CD3 and CD4 cells were observed

**TABLE 1** Demographic and clinical data of sarcoidosis patients and healthy controls (HC) group

Parameters	Sarcoidosis ( <i>n</i> = 13)	Healthy controls ( <i>n</i> = 12)	<i>p</i> value
Age	57 (48–59)	53 (52–65)	0.129
Gender (M/F)	3/10	4/8	0.099
Smoking status (years/ <i>n</i> )	6/7	5/7	0.089
Scadding stage			
0	4/13		
1	0/13		
2	6/13		
3	2/13		
4	1/13		
ACE (U/l)	58 (43–75)		
Pulmonary function test			
FEV1 %	93 (81–106)		
FEV1 ml	2384 (1902–2715)		
FVC %	94 (82–106)		
FVC ml	2999 (2425–3392)		
DLCO %	71 (65–81)		
Extrapulmonary localization			
Skin	3		
Lymphonodes	6		
Hearth	2		
Brain	1		
Liver	1		

M/F = male/female; ACE = angiotensin-converting enzyme; FEV1 = forced expiratory volume in 1 second; DLCO = fusing capacity of the lungs for carbon monoxide.

**TABLE 2** Peripheral and alveolar distribution of cellular patterns in sarcoidosis patients and peripheral cell populations in healthy controls

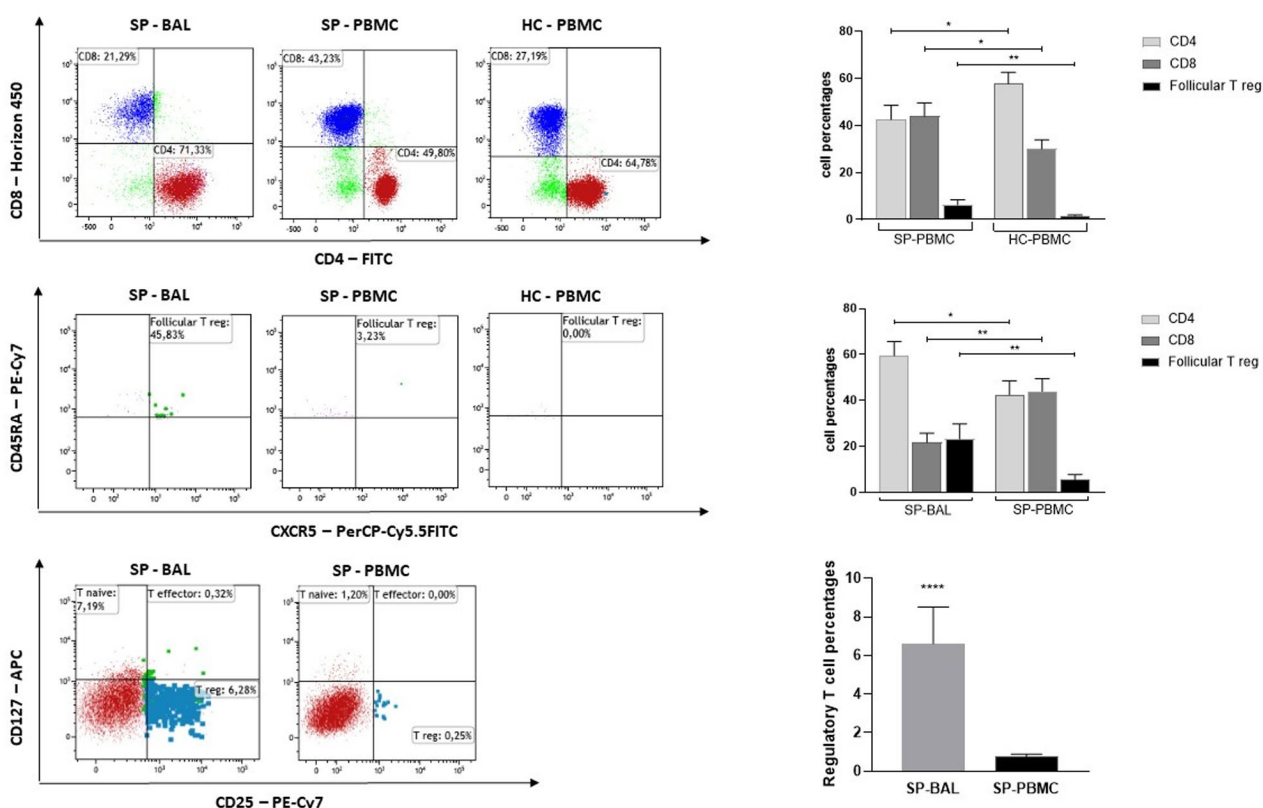
	BAL cellular pattern in sarcoidosis patients ( <i>n</i> = 13)	Peripheral cellular pattern in sarcoidosis patients ( <i>n</i> = 13)	<i>p</i> value: sarcoidosis group, BAL versus peripheral	Healthy controls ( <i>n</i> = 12)	<i>p</i> value: peripheral cellular pattern, sarcoidosis versus healthy controls
B cell	0.93 (0.57–2.52)	21.16 (14.33–27.41)	0.0004	16.75 (12.17–20.88)	0.0167
B <sub>reg</sub>	0 (0–0.90)	0.36 (0.14–1.99)		0.26 (0.1–0.36)	
CD1d <sup>+</sup> CD5 <sup>+</sup>	1.93 (0–3.25)	0.91 (0.41–1.95)		0.09 (0.03–0.42)	0.0085
CD5 <sup>+</sup> CD27 <sup>-</sup>	28.05 (11.14–77.47)	12.62 (2.88–19.95)	0.0213	1.09 (0.66–2.35)	0.0051
Transitional B cell	3.18 (0.24–5.41)	3.2 (1.45–3.57)		1.46 (1.15–2.53)	0.0051
T cell	24.19 (18.56–60.52)	41.02 (33.93–47.88)		66.61 (62.09–67.89)	0.0215
T helper cell	70.36 (38.45–75.16)	49.22 (20.62–49.8)	0.0396	61.15 (47.89–65.71)	0.0305
CD4 <sup>+</sup> CD25 <sup>+</sup> CXCR5 <sup>-</sup>	9.39 (3.40–13.35)	1.12 (0.3–2.51)	0.0002	0.84 (0.4–2.11)	
T cytotoxic cell	19.59 (10.48–33.75)	40.7 (33.76–59.04)	0.0035	26.31 (23.94–36.83)	0.0293
CD8 <sup>+</sup> CD25 <sup>+</sup> CD127 <sup>-</sup>	0 (0–0.10)	0 (0–0.02)		0 (0–0.01)	
CD8 <sup>+</sup> CD25 <sup>-</sup> CD127 <sup>+</sup>	2.11 (0.18–9.68)	9.88 (1.62–19.35)		1.85 (0.45–14.19)	
Follicular T <sup>+</sup>	16.31 (3.65–44.06)	3.23 (2–7.55)	0.0078	0.87 (0.36–2.93)	0.0039
T effector	0.27 (0.01–1.14)	0.11 (0–0.36)		0.05 (0–0.38)	
T naive	4.31 (1.70–10.86)	12.95 (6.65–25.1)		5.12 (1.64–12.68)	
T <sub>reg</sub>	4.98 (2.34–8.49)	0.63 (0.39–1.08)	<0.0001	0.7 (0.32–1.08)	
CCR6 <sup>-</sup>	5.24 (2.24–18.96)	64.85 (36.79–89.81)	0.0006	95.31 (76.06–98.51)	0.0121
CCR6 <sup>+</sup>	89.15 (49.85–97.81)	33.88 (9.84–68.17)	0.0043	5.1 (1.62–31.73)	0.0121
CD4 <sup>+</sup> CD45RA <sup>-</sup>	99.14 (97.36–99.43)	90.5 (74.12–97.96)	0.0159	40.75 (31.35–57.61)	0.0079
CD4 <sup>+</sup> CD45RA <sup>+</sup>	0.86 (0.57–2.64)	9.5 (2.04–25.88)	0.0219	57.09 (17.18–65.14)	0.0305
CD4 <sup>+</sup> CXCR5 <sup>+</sup> CD45RA <sup>-</sup>	58.80 (28.23–84.11)	15.33 (12.43–19.73)	0.0011	10.46 (4.35–16.57)	0.0493
Tfh1	8.09 (2.42–20.29)	20.99 (7.89–33.27)	0.0370	6.73 (2.58–23.39)	0.0305
Tfh17	30.55 (8.83–68.44)	28.59 (22.36–52.14)		22.24 (14.02–32.14)	0.0360
Tfh17.1	19.19 (6.96–31.54)	12.35 (6.19–15.66)		14.12 (8.59–17)	
Tfh2	12.73 (0.85–39.87)	40.57 (27.43–55.7)	<0.0001	59.01 (24.26–68.72)	0.0076
Th1	32.74 (1.91–57.45)	32.11 (21.8–41.77)		21.36 (17.58–27.82)	0.0305
Th17	8.42 (0.02–11.51)	17.33 (10.76–30.89)	0.0063	16.90 (4.88–24.31)	
Th17-1	17.98 (5.93–32.86)	16.09 (11.46–27.7)		18.28 (1.12–21.51)	
Th2	0.47 (0–4.87)	7.99 (3.63–12.88)	0.0016	15.15 (9.37–21.26)	0.0360

B<sub>reg</sub> = regulatory B cells; T<sub>reg</sub> = regulatory T cells; Th = T helper; Tfh = follicular T helper cells; CXCR = C-X-C chemokine receptor.

**TABLE 3** Cytokine concentrations including interleukin (IL)-2, -4, -6, -10, tumor necrosis factor (TNF), interferon (IFN)-gamma and IL-17A in serum and bronchoalveolar lavage (BAL) from sarcoidosis patients and serum samples from healthy controls

Parameters (pg/ml)	Alveolar concentrations in sarcoidosis patients (n = 13)	Serum concentrations in sarcoidosis patients (n = 13)	Serum concentrations in healthy controls (n = 12)
IL-2	91.09 (75.37–156.9)	121.3 (106.4–218.7)	30.38 (26.44–54.76) <sup>a</sup>
IL-4	16.06 (16.06–17.6)	20.75 (13.08–24)	10.4 (9.45–12.38) <sup>b</sup>
IL-6	12.2 (11.23–14.19)	13.19 (12.2–16.74)	8.37 (7.73–9.06) <sup>c</sup>
IL-10	6.74 (6.17–7.9)	7.9 (6.74–8.79)	4.8 (4.15–5.43) <sup>d</sup>
TNF	8.2 (6.66–10.64)	8.2 (7.04–11.48)	3.81 (2.55–4.9) <sup>e</sup>
IFN-γ	10.15 (9.27–12.88) <sup>f</sup>	11.96 (11.51–14.76)	8.4 (7.04–9.65) <sup>g</sup>
IL-17A	7.7 (6.41–9.47)	8.29 (6–10.66)	5.42 (4.9–5.42) <sup>h</sup>

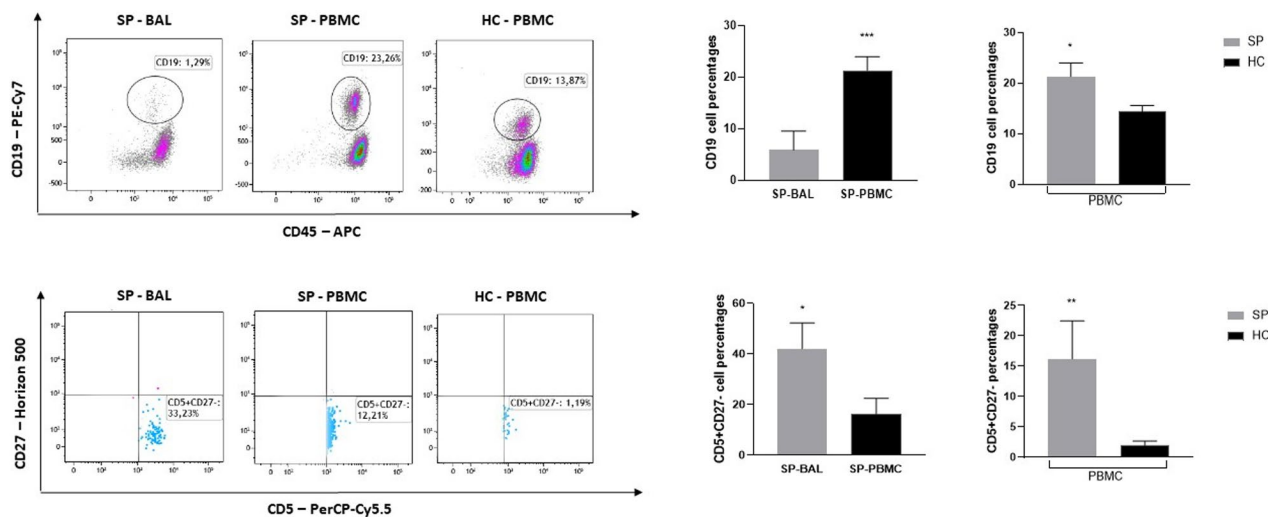
<sup>a</sup>*p* < 0.0001; <sup>b</sup>*p* = 0.0003; <sup>c</sup>*p* = 0.0006; <sup>d</sup>*p* = 0.0003; <sup>e</sup>*p* = 0.0002; <sup>f</sup>*p* = 0.0244; <sup>g</sup>*p* < 0.0001; <sup>h</sup>*p* = 0.0252.



**FIGURE 2** Significant results of CD4, CD8, follicular regulatory T cells (T<sub>reg</sub>) and regulatory T cell percentages from comparison analysis between peripheral and alveolar compartments of sarcoidosis patients. The significant comparisons between peripheral cell subsets in healthy controls and sarcoidosis patients. Lymphocytes were distinguished on the basis of forward (FSC)- versus side-(SSC) scatters and CD3 was identified. A secondary dot-plot was subsequently assessed to distinguish CD4<sup>+</sup> and CD8<sup>+</sup> cells. Accordingly, on CD4<sup>+</sup> cells, CD25<sup>bright</sup>CD127<sup>low</sup> (T<sub>regs</sub>), CD25<sup>high</sup>CD127<sup>high</sup> (T effector) and CD4<sup>high</sup>CD25<sup>high</sup>C-X-C chemokine receptor CXCR5<sup>high</sup> (follicular T<sub>regs</sub>) were identified. SP = sarcoidosis patients; PBMC = peripheral blood mononuclear cells; BAL = bronchoalveolar lavage; HC = healthy controls. \**p* = 0.0332; \*\**p* = 0.0021; \*\*\**p* = 0.0002; \*\*\*\**p* < 0.0001

in the sarcoidosis group than in healthy controls (*p* = 0.0215 and *p* = 0.0305, respectively) (Figure 2). Comparative analysis of peripheral and alveolar cell patterns in sarcoidosis patients showed low BAL percentages of CD19 (*p* = 0.0004) (Figure 3) and CD8 cells (*p* = 0.0035) (Figure

2). The sarcoidosis group showed higher BAL than peripheral percentages of CD5<sup>+</sup>CD27<sup>-</sup> cells (*p* = 0.0213) (Figure 3); the same was found for CD4 (*p* = 0.0396) (Figure 2), CD4<sup>+</sup>CD25<sup>+</sup>CXCR5<sup>-</sup> (*p* = 0.0002), follicular T<sub>reg</sub> (*p* = 0.0078) (Figure 2) and T<sub>reg</sub> (*p* < 0.0001) cells (Figure



**FIGURE 3** Peripheral B cell and CD5<sup>+</sup>CD27<sup>-</sup> cell distribution in sarcoidosis patients and healthy controls. The significant comparison results between alveolar and peripheral cell subsets in sarcoidosis patients. Lymphocytes were distinguished on the basis forward (FSC)- versus side (SSC)-scatters and CD19 were identified. On CD19<sup>+</sup> cells, regulatory B cells (B<sub>regs</sub>) were identified as CD5<sup>+</sup>CD27<sup>-</sup>. SP = sarcoidosis patients; PBMC = peripheral blood mononuclear cells; BAL = bronchoalveolar lavage; HC = healthy controls. \* $p = 0.0332$ ; \*\* $p = 0.0021$ ; \*\*\* $p = 0.0002$ ; \*\*\*\* $p = < 0.0001$

2). Significant correlations between immunological and clinical parameters are reported in Supporting information, Table S2.

### Central and effector cell phenotyping (Th17, Th17.1, Th1, Th2)

Peripheral percentages of CD4<sup>+</sup>CD45RA<sup>-</sup> and Th1 cells were higher in sarcoidosis patients than healthy controls ( $p = 0.0079$  and  $p = 0.0305$ , respectively). Peripheral percentages of Th2 cells were lower in sarcoidosis patients than controls ( $p = 0.0360$ ). Comparative analysis of alveolar and peripheral cell patterns in sarcoidosis patients revealed high percentages of CD4<sup>+</sup>CD45RA<sup>+</sup> ( $p = 0.0219$ ) and low percentages of Th17 (Figure 4) and Th2 cells in BAL ( $p = 0.0063$  and  $p = 0.0016$ , respectively). Significant correlations between immunological and clinical parameters are reported in Supporting information, Table S2.

### Follicular T cell subsets

Peripheral percentages of CCR6<sup>-</sup> cells were lower ( $p = 0.0121$ ) and those of CCR6<sup>+</sup> cells were higher ( $p = 0.0121$ ) in the sarcoidosis group than in healthy controls. The same was found for percentages of CXCR5<sup>+</sup>CD45RA<sup>-</sup> and Tfh1 cells ( $p = 0.0493$  and  $p = 0.0305$ , respectively) (Figure 4). Percentages of CCR6<sup>-</sup>, Tfh1 and Tfh2 (Figure 4) cells were lower in BAL than in peripheral blood ( $p = 0.0006$ ,

$p = 0.0370$  and  $p = 0.0078$ , respectively). Conversely, percentages of CCR6<sup>+</sup> and CXCR5<sup>+</sup>CD45RA<sup>-</sup> cells (Figure 4) were higher in BAL than in peripheral blood ( $p = 0.0043$  and  $p = 0.0011$ , respectively). Significant correlations between immunological and clinical parameters are reported in Supporting information, Table S2.

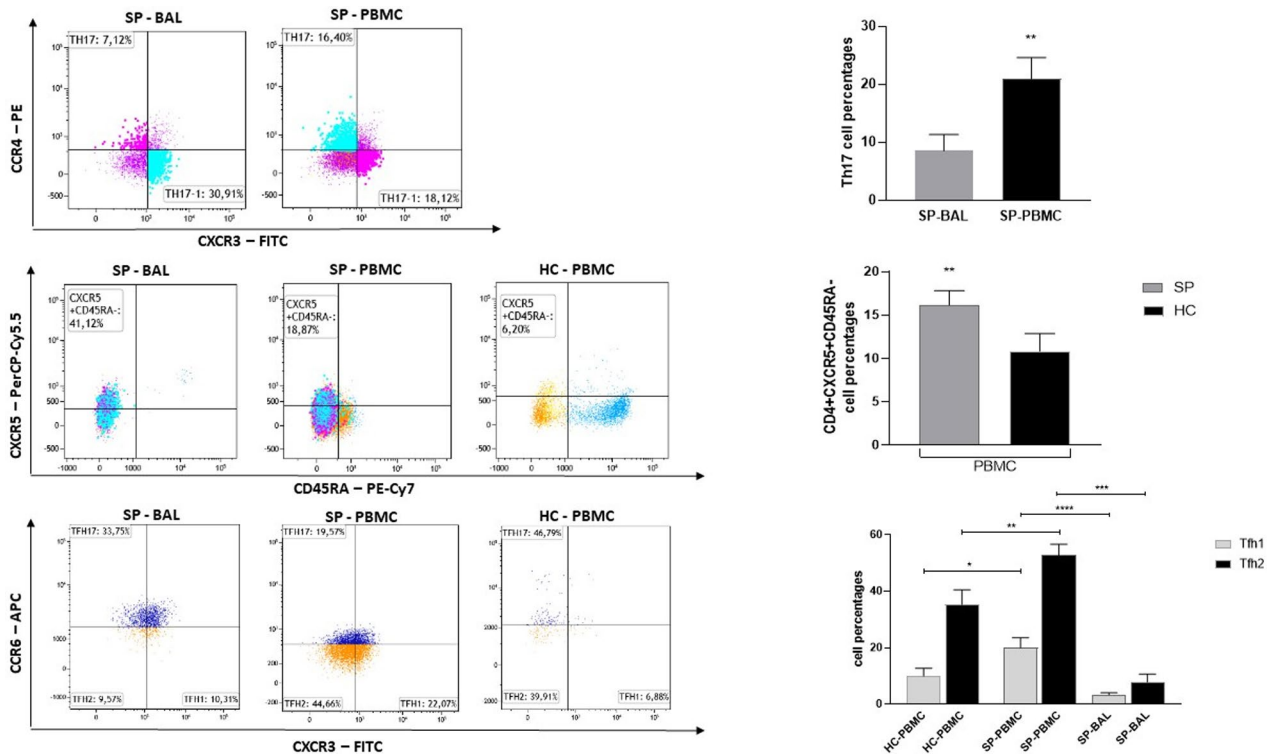
### Cytometric bead array analysis

Peripheral blood concentrations of IL-2, -4, -6, -10, TNF, IFN- $\gamma$  and IL-17A were higher in sarcoidosis patients than in healthy controls ( $p < 0.0001$ , Table 3). Significant correlations between cell subsets and cytokine concentrations in HC and sarcoidosis patients are reported in Supporting information, Table S3.

Comparative analysis of BAL and serum concentrations of cytokines in sarcoidosis patients showed lower concentrations of IFN- $\gamma$  in BAL than in serum ( $p = 0.0244$ ). Supporting information, Table S3 reports the significant correlations between cytokines and cell subsets in peripheral and alveolar compartments of sarcoidosis patients.

## DISCUSSION

This is the first study, to our knowledge, to analyze the frequency and distribution of peripheral and alveolar B and T lymphocyte subsets with a special focus on Tfh cells in pulmonary sarcoidosis patients. According to the currently



**FIGURE 4** Significant comparison results of T helper type 17 (Th17), CD4<sup>+</sup> C-X-C chemokine receptor CXCR5<sup>+</sup>CD45RA<sup>-</sup>, T follicular helper (Tfh)1 and Tfh2 cell percentages in sarcoidosis patients and healthy controls. Additionally, significant differences in peripheral and alveolar cell distribution from sarcoidosis patients were analysed. Lymphocytes were distinguished on the basis of forward (FSC)- versus side (SSC) scatters and CD4 cells were identified. Accordingly, CD4<sup>+</sup>CD45RA<sup>+</sup> and CD4<sup>+</sup>CXCR5<sup>+</sup>CD45RA<sup>-</sup> were distinguished on secondary dot-plots. Th17 cells were defined on the basis of markers CD45 RA and CCR6, with and without expression of CXCR3 and CCR4. Central cells (CD4<sup>+</sup>CD45RA<sup>-</sup>) were divided according to the expression of CCR6: Th17 (CXCR3<sup>+</sup>CCR4<sup>+</sup>). A dot-plot was also assessed to identify CXCR5<sup>+</sup>CD45RA<sup>-</sup>, CD4<sup>high</sup>CXCR5<sup>high</sup> (Tfh cells) and a secondary do-plot according to CXCR3 and CCR6 distinguished Tfh1 (CXCR3<sup>+</sup>CCR6<sup>-</sup>) and Tfh2 (CXCR3<sup>-</sup>CCR6<sup>-</sup>). SP = sarcoidosis patients; PBMC = peripheral blood mononuclear cells; BAL = bronchoalveolar lavage; HC = healthy controls. \**p* = 0.0332; \*\**p* = 0.0021; \*\*\**p* = 0.0002; \*\*\*\**p* < 0.0001

proposed model of sarcoidosis, antigen-presenting cells are the first in the cascade of inflammatory reactions, and B and T lymphocytes actively migrate to the focus of inflammation [2,13,14,35–38]. T cells undergo differentiation into T helper and T<sub>reg</sub> cells, depending on secreted cytokines [14]. Our sarcoidosis patients showed higher serum concentrations of cytokines (including IL- 2, -4, -6, -10, TNF and IFN-γ) than did controls.

As expected, higher percentages of CD4 and lower percentages of CD8 cells were observed in BAL than in peripheral blood from sarcoidosis patients [15,16]. Moreover, altered Th subsets were identified in sarcoidosis patients: percentages of Th2 and Th17 cells were higher in serum than in BAL of our patients, while percentages of T<sub>regs</sub> were lower. As Th17 cells contribute to fibrosis [31] and T<sub>regs</sub> reveal anti-proliferative activity [31,39], variations in their ratios may explain the proliferation of connective tissue in affected organs and be a confirming factor of the autoimmune nature of sarcoidosis [40–42]. Percentages of T<sub>regs</sub> in BAL were positively correlated with alveolar concentrations of IL-10,

demonstrating their regulatory effects. However, percentages of Th2 cells were lower and those of Th1 cells higher in serum of sarcoidosis patients than controls.

Concerning B cells, our study demonstrated alterations in the alveolar and peripheral blood compartments in sarcoidosis patients, in line with previous observations [17]. CD5-expressing B cells were fewer in peripheral blood than in BAL. This trend was also demonstrated in peripheral blood of controls and sarcoidosis patients. The role of CD5<sup>+</sup> B cells in sarcoidosis is unclear. The literature suggests that CD5<sup>+</sup> B cells can be found in various human tissues, produce autoantibodies (including rheumatoid factors and anti-ssDNA antibodies) and proliferate in autoimmune diseases such as rheumatoid arthritis and systemic sclerosis [43]. However, little is known about their functional capacities and their precise role in the mechanisms of human autoimmune diseases. In humans, CD5 expression can be found on the plasma membrane of CD24<sup>high</sup>CD38<sup>high</sup> cells which, however, produce lower levels of IL-10 than do other transitional B cell subsets [44].



Our findings suggest that several  $B_{reg}$  subsets (including  $CD19^+CD24^+CD27^+$ ,  $CD19^+CD5^+CD27^-$  and  $CD19^+CD1d^+CD5^+$ ) could be involved in the pathogenesis of sarcoidosis. Note that sarcoidosis is a granulomatous disease with focused, targeted migration of the cells to form lymphoid and other granulomata. Accordingly, any changes in their relative content in peripheral blood may not only be due to altered production but may also be associated with their selective redistribution into granulomatous foci. Thus, any changes in the spectrum of peripheral blood cells may reflect pathogenic as well as compensatory body processes. The increase in peripheral percentages of  $B_{reg}$  cells ( $CD19^+CD5^+CD27^-$  and  $CD19^+CD1d^+CD5^+$ ) in sarcoidosis patients, with respect to controls, may reflect an increase in their compensatory production or a pathological decrease in their migration to foci of inflammation. Imbalance in B cell subsets could be closely linked to the abnormal distribution of Tfh cells, known to have distinct capacities to help B cells, besides generating plasma and memory B cells through the germinal center reaction [45]. Relative increases in the number of  $CD4^+CXCR5^+CD45RA^-$  Th cells were observed in the peripheral and alveolar compartments of sarcoidosis patients with respect to peripheral cell distribution in controls. Furthermore, sarcoidosis patients showed an altered balance of Tfh subsets. An abnormal distribution or imbalance between 'regulatory' Tfh1-like cells and 'proinflammatory' Tfh2 and Tfh17 cells is reported to be closely linked to the pathogenesis of a number of autoimmune diseases [46]. In line with this, our patients showed higher percentages of Tfh1, Tfh2 and Tfh17 cells in peripheral blood than did controls. These findings may be consistent with an abnormal humoral immune response, further demonstrated by low alveolar concentrations of IFN- $\gamma$ .

Recent studies have identified a subpopulation of regulatory T cells, known as follicular regulatory T (Tfr) cells. These cells can inhibit Tfh and/or  $B_{reg}$  cells in a variety of ways, specifically regulating germinal center reactions. Dysfunction of Tfr cells may lead to immune disorders and a variety of autoimmune diseases [47,48]. Indeed, our sarcoidosis patients showed higher percentages of Tfr cells in peripheral blood than did controls. In addition, percentages of Tfr and Tfh17 cells were higher and Tfh1 and Tfh2 lower in BAL of our patients than in peripheral blood. Alveolar percentages of Tfr cells were also positively correlated with Scadding stages, suggesting that these cells may have a role in disease progression.

A predominance of the Tfh17 subset over that of Tfh1 cells is typical of different systemic autoimmune diseases and of sarcoidosis [49]. These observations support an association between the altered balance of Tfh subsets and pathogenesis of sarcoidosis. Targeting Tfh cells could be a strategy for treating sarcoidosis and other autoimmune diseases, as recently demonstrated by two experimental models of rheumatoid arthritis and multiple sclerosis, in which an

anti-CXCL13 antibody blocking the main ligand for CXCR5 receptors showed good efficacy [50].

Similarities with autoimmune diseases suggest that autoimmune components play an important part in the pathogenesis of sarcoidosis. This is the first study, to our knowledge, to demonstrate a link between the imbalance in circulating and alveolar Tfh cells, especially CCR4-, CXCR3- and CXCR5-expressing Tfh subsets, in the development of sarcoidosis. These findings raise questions about the pathogenesis of sarcoidosis and may provide new directions for future clinical studies and treatment strategies.

## ACKNOWLEDGEMENTS

This study received no funding.

## CONFLICTS OF INTEREST

No conflicts of interest to declare.

## AUTHOR CONTRIBUTIONS

Miriana d'Alessandro: conceptualization, sample and statistical analysis; Laura Bergantini: sample analysis; Paolo Cameli, Rosa Metella Refini, Maria Pieroni, Piersante Sestini, Elena Bargagli and Fabrizio Mezzasalma clinically followed patients and revised the manuscript; Miriana d'Alessandro, Laura Bergantini, Paolo Cameli and Elena Bargagli wrote the manuscript. All authors read and approved the final version of the manuscript.

## ETHICAL APPROVAL

Ethical approval was waived by the local Ethics Committee (CEAVSE) of the University of Siena in view of the retrospective nature of the study. Informed consent was approved by our local ethics committee (CEAVSE; Tuscany, Italy, Markerlung number 17431.

## DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

## ORCID

Miriana d'Alessandro  <https://orcid.org/0000-0002-2368-5722>

## REFERENCES

1. Culver DA, Judson MA. New advances in the management of pulmonary sarcoidosis. *BMJ* 2019;22:i5553.
2. Grunewald J, Grutters JC, Arkema EV, Saketkoo LA, Moller DR, Müller-Quernheim J. Sarcoidosis. *Nat Rev Dis Primers*. 2019;5:45.
3. Hunninghake GW, Crystal RG. Pulmonary sarcoidosis: a disorder mediated by excess helper T-lymphocyte activity at sites of disease activity. *N Engl J Med*. 1981;305:429–34.
4. Sweiss NJ, Salloum R, Ghandi S, Alegre M-L, Sawaged R, Badaracco M, et al. Significant CD4, CD8, and CD19 lymphopenia

- in peripheral blood of sarcoidosis patients correlates with severe disease manifestations. *PLOS ONE*. 2010;5:e9088.
5. Facco M, Cabrelle A, Teramo A, Olivieri V, Gnoato M, Teolato S, et al. Sarcoidosis is a Th1/Th17 multisystem disorder. *Thorax* 2011;66:144–50.
  6. Ramstein J, Broos CE, Simpson LJ, Ansel KM, Sun SA, Ho ME, et al. IFN- $\gamma$ -producing T-helper 17.1 cells are increased in sarcoidosis and are more prevalent than T-helper type 1 cells. *Am J Respir Crit Care Med*. 2016;193:1281–91.
  7. Broos CE, Koth LL, van Nimwegen M, in 't Veen JCCM, Paulissen SMJ, van Hamburg JP, et al. Increased T-helper 17.1 cells in sarcoidosis mediastinal lymph nodes. *Eur Respir J*. 2018;51:1701124.
  8. Rossman MD, Thompson B, Frederick M, Iannuzzi MC, Rybicki BA, Pander JP, et al. HLA and environmental interactions in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis*. 2008;25:125–32.
  9. Patterson KC, Chen ES. The pathogenesis of pulmonary sarcoidosis and implications for treatment. *Chest* 2018;153:1432–42.
  10. Celada LJ, Rotsinger JE, Young A, Shaginurova G, Shelton D, Hawkins C, et al. Programmed death-1 inhibition of phosphatidylinositol 3-kinase/AKT/mechanistic target of rapamycin signaling impairs sarcoidosis CD4<sup>+</sup> T cell proliferation. *Am J Respir Cell Mol Biol*. 2017;56:74–82.
  11. Parasa VR, Forsslund H, Enger T, Lorenz D, Kullberg S, Eklund A, et al. Enhanced CD8<sup>+</sup> cytolytic T cell responses in the peripheral circulation of patients with sarcoidosis and non-Löfgren's disease. *Respir Med*. 2018;138S:S38–44.
  12. Kaiser Y, Lepzien R, Kullberg S, Eklund A, Smed-Sörensen A, Grunewald J. Expanded lung T-bet<sup>+</sup>ROR $\gamma$ T<sup>+</sup> CD4<sup>+</sup> T-cells in sarcoidosis patients with a favourable disease phenotype. *Eur Respir J*. 2016;48:484–94.
  13. Crouser ED. Role of imbalance between Th17 and regulatory T-cells in sarcoidosis. *Curr Opin Pulm Med*. 2018;24:521–6.
  14. Agostini C, Meneghin A, Semenzato G. T-lymphocytes and cytokines in sarcoidosis. *Curr Opin Pulm Med*. 2002;8:435–40.
  15. d'Alessandro M, Carleo A, Cameli P, Bergantini L, Perrone A, Vietri L, et al. BAL biomarkers' panel for differential diagnosis of interstitial lung diseases. *Clin Exp Med*. 2020;20:207–16.
  16. Bergantini L, Cameli P, d'Alessandro M, Vagaggini C, Refini RM, Landi C, et al. NK and NKT-like cells in granulomatous and fibrotic lung diseases. *Clin Exp Med*. 2019;19:487–94.
  17. Lee N-S, Barber L, Akula SM, Sigounas G, Kataria YP, Arce S. Disturbed homeostasis and multiple signaling defects in the peripheral blood B-cell compartment of patients with severe chronic sarcoidosis. *Clin Vaccine Immunol*. 2011;18:1306–16.
  18. Hunninghake GW, Crystal RG. Mechanisms of hypergammaglobulinemia in pulmonary sarcoidosis. Site of increased antibody production and role of T lymphocytes. *J Clin Invest*. 1981;67:86–92.
  19. Kamphuis LS, van Zelm MC, Lam KH, Rimmelzwaan GF, Baarsma GS, Dik WA, et al. Perigranuloma localization and abnormal maturation of B cells: emerging key players in sarcoidosis? *Am J Respir Crit Care Med*. 2013;187:406–16.
  20. Qin J, Zhou J, Fan C, Zhao N, Liu Y, Wang S, et al. Increased circulating Th17 but decreased CD4<sup>+</sup>Foxp3<sup>+</sup> Treg and CD19<sup>+</sup>CD1dhiCD5<sup>+</sup> Breg subsets in new-onset Graves' disease. *Biomed Res Int*. 2017;2017:8431838.
  21. Kudryavtsev I, Serebriakova M, Starshinova A, Zinchenko Y, Basantsova N, Malkova A, et al. Imbalance in B cell and T follicular helper cell subsets in pulmonary sarcoidosis. *Sci Rep*. 2020;10:1059.
  22. Ando M, Goto A, Takeno Y, Yamasue M, Komiya K, Umeki K, et al. Significant elevation of the levels of B-cell activating factor (BAFF) in patients with sarcoidosis. *Clin Rheumatol*. 2018;37:2833–8.
  23. Gensous N, Charrier M, Duluc D, Contin-Bordes C, Truchetet M-E, Lazaro E, et al. T follicular helper cells in autoimmune disorders. *Front Immunol*. 2018;9:1637.
  24. Costabel U, Hunninghake GW, on behalf of the Sarcoidosis Statement Committee. ATS/ERS/WASOG statement on sarcoidosis. Sarcoidosis Statement Committee. American Thoracic Society. European Respiratory Society. World Association for Sarcoidosis and Other Granulomatous Disorders. *Eur Respir J*. 1999;14:735–7.
  25. Statement on Sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med*. 1999;160:736–55.
  26. Scadding JG. Prognosis of intrathoracic sarcoidosis in England. A review of 136 cases after five years' observation. *BMJ* 1961;2:1165–72.
  27. Riedhammer C, Halbritter D, Weissert R. Peripheral blood mononuclear cells: isolation, freezing, thawing, and culture. *Methods Mol Biol*. 2016;1304:53–61.
  28. European Society of Pneumology. Clinical guidelines and indications for bronchoalveolar lavage (BAL): report of the European Society of Pneumology Task Group on BAL. *Eur Respir J*. 1990;3:937–76.
  29. Bergantini L, d'Alessandro M, Cameli P, Bono C, Perruzza M, Biagini M, et al. Regulatory T cell monitoring in severe eosinophilic asthma patients treated with mepolizumab. *Scand J Immunol*. 2021;19:e13031.
  30. Bergantini L, Cameli P, d'Alessandro M, Vietri L, Perruzza M, Pieroni M, et al. Regulatory T cells in severe persistent asthma in the era of monoclonal antibodies target therapies. *Inflammation* 2020;43:393–400.
  31. d'Alessandro M, Bergantini L, Cameli P, Fanetti M, Alderighi L, Armati M, et al. Immunologic responses to antifibrotic treatment in IPF patients. *Int Immunopharmacol*. 2021;11:107525.
  32. Human,Th1/Th2/Th17,Kit,RUO - 560484 | BD Biosciences – US [internet] [cited 2021 Mar 31]. Available at: <https://www.bdbiosciences.com/us/applications/research/bead-based-immunoassays/cba-kits/human-th1th2th17-kit/p/560484> (accessed 12 April 2021).
  33. LEGENDplex™ Data Analysis [internet] [cited 2021 Mar 31]. Available at: <http://www.vigenetech.com/LEGENDplex7.htm> (accessed 12 April 2021).
  34. Reich JM. Epidemiology of sarcoidosis. *Mayo Clin Proc*. 2016;91:822–3.
  35. O'Regan A, Berman JS. Sarcoidosis. *Ann Intern Med*. 2012;156:ITC5-1, ITC5-2, ITC5-3, ITC5-4, ITC5-5, ITC5-6, ITC5-7, ITC5-8, ITC5-9, ITC5-10, ITC5-11, ITC5-12, ITC5-13, ITC5-14, ITC5-15; quiz ITC5-16.
  36. Salah S, Abad S, Monnet D, Brézin AP. Sarcoidosis. *J Fr Ophtalmol*. 2018;41:e451–e467.
  37. Llanos O, Hamzeh N. Sarcoidosis. *Med Clin North Am*. 2019;103:527–34.
  38. Bennett D, Bargagli E, Refini RM, Rottoli P. New concepts in the pathogenesis of sarcoidosis. *Expert Rev Respir Med*. 2019;13:981–91.

39. Dowling MR, Kan A, Heinzel S, Marchingo JM, Hodgkin PD, Hawkins ED. Regulatory T Cells suppress effector t cell proliferation by limiting division destiny. *Front Immunol.* 2018;9:2461.
40. Weng Y, Wang H, Lv D, Fu Z, Yu W. Th17 and Treg cell levels in patients with sarcoidosis and their relation to disease activation. *Zhejiang Da Xue Xue Bao Yi Xue Ban.* 2015;44:154–61.
41. Liu Y, Qiu L, Wang Y, Aimurolo H, Zhao Y, Li S, et al. The circulating Treg/Th17 cell ratio is correlated with relapse and treatment response in pulmonary sarcoidosis patients after corticosteroid withdrawal. *PLOS ONE.* 2016;11:e0148207.
42. Miyara M, Amoura Z, Parizot C, Badoual C, Dorgham K, Trad S, et al. The immune paradox of sarcoidosis and regulatory T cells. *J Exp Med.* 2006;203:359–70.
43. Dauphinée M, Tovar Z, Talal N. B cells expressing CD5 are increased in Sjögren's syndrome. *Arthritis Rheum.* 1988;31:642–7.
44. Simon Q, Pers J-O, Cornec D, Le Pottier L, Mageed RA, Hillion S. In-depth characterization of CD24(high)CD38(high) transitional human B cells reveals different regulatory profiles. *J Allergy Clin Immunol.* 2016;137:1577–1584.e10.
45. Vinuesa CG, Linterman MA, Yu D, MacLennan ICM. Follicular helper T cells. *Annu Rev Immunol.* 2016;20:335–68.
46. Morita R, Schmitt N, Bentebibel S-E, Ranganathan R, Bourdery L, Zurawski G, et al. Human blood CXCR5(+)CD4(+) T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. *Immunity* 2011;34:108–21.
47. Gong Y, Tong J, Wang S. Are follicular regulatory T cells involved in autoimmune diseases? *Front Immunol.* 2017;8:1790.
48. Linterman MA, Pierson W, Lee SK, Kallies A, Kawamoto S, Rayner TF, et al. Foxp3<sup>+</sup> follicular regulatory T cells control the germinal center response. *Nat Med.* 2011;17:975–82.
49. Ly NTM, Ueda-Hayakawa I, Nguyen CTH, Okamoto H. Exploring the imbalance of circulating follicular helper CD4<sup>+</sup> T cells in sarcoidosis patients. *J Dermatol Sci.* 2020;97:216–24.
50. Klimatcheva E, Pandina T, Reilly C, Torno S, Bussler H, Scrivens M, et al. CXCL13 antibody for the treatment of autoimmune disorders. *BMC Immunol.* 2015;12:6.

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

**How to cite this article:** d'Alessandro M, Bergantini L, Cameli P, Mezzasalma F, Refini RM, Pieroni M, et al. Adaptive immune system in pulmonary sarcoidosis—Comparison of peripheral and alveolar biomarkers. *Clin Exp Immunol.* 2021;205:406–416. <https://doi.org/10.1111/cei.13635>