Increased T helper 17.1 cells in sarcoidosis mediastinal lymph nodes

Caroline E. Broos¹, Laura L. Koth², Menno van Nimwegen¹, Johannes C.C.M. in ’t Veen³, Sandra M.J. Paulissen⁴, Jan Piet van Hamburg⁵, Jouke T. Annema⁶, Roxane Heller-Baan⁷, Alex Kleinjan¹, Henk C. Hoogsteden¹, Marlies S. Wijsenbeek¹, Rudi W. Hendriks¹, Bernt van den Blink¹⁸, Mirjam Kool¹²

¹Department of Pulmonary Medicine, Erasmus MC, Rotterdam, The Netherlands; ²Department of Medicine, Division of Pulmonary and Critical Care, University of California, San Francisco, California 94143; ³Department of Pulmonology, Franciscus Gasthuis & Vlietland, Rotterdam, The Netherlands; ⁴Department of Rheumatology, Erasmus MC, Rotterdam, The Netherlands; ⁵Department of Experimental Immunology, Amsterdam Rheumatology & Immunology Center, Academic Medical Center/University of Amsterdam, Amsterdam, The Netherlands; ⁶Department of Pulmonology, Academic Medical Center/University of Amsterdam, Amsterdam, The Netherlands; ⁷Department of Pulmonology, Ikazia Hospital, Rotterdam, The Netherlands.

⁸ Shared senior authors

* Reprint requests and address correspondence to: Mirjam Kool, Department of Pulmonary Medicine, Erasmus MC, ‘s-Gravendijkwal 230, 3015 CE, Rotterdam, Netherlands, Phone: +3110-7038526, Email: m.kool@erasmusmc.nl

Online Data Supplement
Supplementary Figure E1.

Flowchart describing the donated material (BALF, MLN, and/or PB) by the 55 sarcoidosis patients in this study.
Supplementary Figure E1

Total patients that donated material for this study
n=55

Patients that donated material of multiple organs
n=30

MLN and PB
n=15

BALF and PB
n=13

Patients that donated material of a single organ
n=25

PB
n=4

BALF
n=21

MLN, BAL and PB
n=2
**Supplementary Table E1. Overview antibodies**

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<th>Company</th>
<th>Antigen</th>
<th>Fluor chrome</th>
<th>Clone</th>
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<td>CD4</td>
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<td></td>
<td>FoxP3</td>
<td>PE</td>
<td>236A/E7</td>
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<td>CTLA4</td>
<td>PerCp-eFluor710</td>
<td>14D3</td>
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<td>CCR6</td>
<td>APC</td>
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<td>Pe-Cy7</td>
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<td>PE-Texas Red</td>
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Supplementary Figure E2

A. Five CD3\(^+\)CD4\(^+\) T cell populations were characterized according to CD45RA and intracellular FoxP3 expression\(^1\), i.e. CD45RA\(^+\)FoxP3\(^-\) naïve T cells, CD45RA\(^+\)FoxP3\(^-\) memory T cells, CD45RA\(^+\)FoxP3\(^{int}\) activated (non-suppressive/-regulatory) T cells, CD45RA\(^+\)FoxP3\(^{int}\) naïve regulatory T cells and CD45RA\(^-\)FoxP3\(^{high}\) activated Tregs. The total memory T cell pool consists of memory T cells plus activated T cells. The total (non-suppressive/-regulatory) T cell pool consists of naïve T cells plus total memory T cells. B. Th cell subsets can be classified according to chemokine-receptor expression\(^2\)\(-6\). CCR6 memory T cells subsets, including CCR4\(^+\)CXCR3\(^+\) Th1 cells and CCR4\(^+\)CXCR3\(^-\) Th2 cells; and CCR6\(^+\) T cell subsets, which include CCR4\(^+\)CXCR3\(^-\) Th17 cells, CCR4\(^+\)CXCR3\(^+\) DP Th cells and CCR4\(^+\)CXCR3\(^+\) Th17.1 cells. Th17.1 cells (i.e. IFN-\(\gamma\)-producing Th17 cells) contain both IL-17A\(^+/\)IFN-\(\gamma\)\(^+\) (Th17/Th1) cells and IFN-\(\gamma\)-single-positive cells. CCR6\(^+\)CCR4\(^+\)CXCR3\(^+\) DP cells are thought to reflect an intermediate Th17 and Th17.1 cell population\(^7\)\(^,\)\(^4\)\(^5\), expressing both ROR\(\gamma\)t/IL-17A and T-bet/IFN-\(\gamma\) in patients with rheumatoid arthritis (RA)\(^7\). Abbreviations: CCR: C-C chemokine receptor, CXCR: CXC chemokine receptor, DP: double-positive, Th: T helper, Treg: regulatory T cells.
A.

Supplementary Figure E2.

CD3^+CD4^+ T cells

Naive T cells

CD45RA

FoxP3

Memory T cells + Activated T cells

Total memory T cells

Total T cells

B.

Contains:
- IL17A^+ classic Th17 cells

Transcription factors:
- RORC

CCR6^- Th cells

Th2

Th1

CCR6^+ Th cells

Th17

DP

Th17.1

i.e. 'IFN-γ-producing Th17 cells'

Contains:
- IL17A^+ classic Th17 cells

Transcription factors:
- RORC

Contains:
- IL17A^+/IFN-γ^+ (Th17/Th1) cells
- IL17A^-/IFN-γ^- cells

Transcription factors:
- RORC and TBX21

Contains:
- IFN-γ^+ classic Th1 cells

Transcription factors:
- TBX21

Contains:
- IL17A^+/IFN-γ^- cells

Transcription factors:
- RORC and TBX21
**Supplementary Figure E3**

Representative flow cytometry analysis of BALF (A) and MLN (B) from one sarcoidosis patient and one control. *Abbreviations: CCR: C-C chemokine receptor, BALF: broncho-alveolar lavage fluid, MLN: mediastinal lymph nodes, SRC: sarcoidosis, Ctrl: control.*
Supplementary Figure E3.

**A. BALF**

**Total memory CD4+ T cells**

- **SRC**
  - CD45RA vs. CCR4
  - CCR6 vs. CXCR3

**CCR6-**

- Th2
- Th1

**CCR6+**

- Th17
- DP

**B. MLN**

**Total memory CD4+ T cells**

- **Ctrl**
  - CD45RA vs. CCR4
  - CCR6 vs. CXCR3

**SRC**

- CD45RA vs. CCR4
- CCR6 vs. CXCR3

**CCR6-**

- Th2
- Th1

**CCR6+**

- Th17
- DP
Supplementary Figure E4

Proportions of total CCR6⁺ and Th1 cells were determined in total memory CD4⁺ T cells from sarcoidosis PB, MLN and BALF. A-B. Proportions of total CCR6⁺ and Th1 cells of total memory CD4⁺ T cells. Statistics: Horizontal lines indicate median values and significance was determined using a Mann-Whitney U test, * p < 0.05 ** p < 0.01 *** p < 0.001. Data are from 34 (PB), 17 (MLN) and 36 (BALF) patients. Abbreviations: CCR: C-C chemokine receptor, PB: peripheral blood, MLN: mediastinal lymph nodes, BALF: broncho-alveolar lavage fluid, DP: double-positive, Th: T helper.
Supplementary Figure E4.

A. BALFPB MLN

B. BALFPB MLN

**CCR6**

(% of total memory T cells)

0 20 40 60 80 100

PB MLN BALF

***

Th1

(% of total memory T cells)

0 20 40 60 80 100

PB MLN BALF

*

**
Supplementary Figure E5

Proportions Th17 and Th17.1 cells were determined in total memory CD4\(^+\) T cells from sarcoidosis patient that donated either both PB and MLN (n=17) or PB and BALF (n=15). A-B. Proportions of Th17 and Th17.1 cells of total memory CD4\(^+\) T cells in PB and MLN of 17 sarcoidosis patients. C-D. Proportions of Th17 and Th17.1 cells of total memory CD4\(^+\) T cells in PB and BALF of 15 sarcoidosis patients. Statistics: Significance between median values of paired samples was determined using a Wilcoxon signed rank test, * p < 0.05  ** p<0.01  *** p < 0.001. Abbreviations: PB: peripheral blood, MLN: mediastinal lymph nodes, BALF: broncho-alveolar lavage fluid, Th: T helper.
Supplementary Figure E6

Disease course of a subgroup (n=25) of patients was determined 2 years after study inclusion. Resolution of disease (n=7) was defined by the absence of abnormalities on the chest X-ray and clinical symptoms. Patients with residual abnormalities on chest X-ray, but without need for treatment were designated as non-progressive chronic (n=11); and patients with need for treatment as progressive chronic (n=7)[8]. A. Proportions Th17 cells of total memory CD4+ T cells at time of diagnosis. B. Ratio of proportions Th17.1 versus Th17 cells. Statistics: Data are presented as boxplots and whiskers that show the 10-90 percentile of the data. Significance was determined using a Mann-Whitney U test. Abbreviations: BALF: broncho-alveolar lavage fluid, Th: T helper, SRC: sarcoidosis.
Supplementary Figure E6.

A. Th17 cells (% of total memory T cells)

B. Ratio of %Th17.1/%Th17 cells
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