Increased IL-17A expression in granulomas and in circulating memory T cells in sarcoidosis

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Abstract

Objective. Sarcoidosis is a systemic inflammatory disorder characterized by granulomas. Although the aetiology is unknown, sarcoidosis is thought to be mediated by Th1 lymphocytes. Recently, IL-17A has been implicated in granuloma formation in various diseases, including tuberculosis. Therefore, we hypothesized that Th17 cells play a role in sarcoidosis, paralleling recent findings in autoimmune diseases such as RA. The aim of our study was to investigate the role of Th17 cells in sarcoidosis.

Methods. T cells were investigated by intracellular flow cytometry and immunohistochemistry, in blood, bronchoalveolar lavages (BALs) and bronchial mucosal biopsies from a cohort of newly diagnosed sarcoidosis patients and healthy controls.

Results. Circulating memory CD4+ T-cell populations of sarcoidosis patients contained significantly increased proportions of IL-17A+ cells when compared with healthy controls. Interestingly, proportions of IL-17A/IFN-γ and IL-17A/IL-4 double-producing cells were significantly increased in blood of sarcoidosis patients and were present in substantial numbers in BAL. In granuloma-containing, but not in non-granulomatous sarcoidosis biopsies, we found significantly increased numbers of IL-17A+ T cells, located in and around granulomas throughout the lamina propria. IL-22+ T cells were increased in the subepithelial layer.

Conclusions. Enhanced IL-17A expression in granulomas and the presence of IL-17A+, IL-17A/IFN-γ+ and IL-17A/IL-4+memory Th cells in the circulation and BAL indicate Th17 cell involvement in granuloma induction or maintenance in sarcoidosis. Therefore, neutralization of IL-17A activity may be a novel strategy to treat sarcoidosis.

Key words: flow cytometry, granulomas, human, immunohistochemistry, IL-17, IL-22, lung, sarcoidosis, T helper-17 cells

Introduction

Sarcoidosis is a systemic inflammatory disease characterized by the presence of non-caseating granulomas in various organs with pulmonary involvement in >90% of patients [1].

These granulomas are compact, organized collections of macrophages and epithelioid cells, surrounded by and infiltrated with CD45RO+ memory T lymphocytes. Besides granulomas, pulmonary alveolitis and peripheral blood lymphopenia are typically present in sarcoidosis [1]. The pathological processes that result in granulomatous inflammation are largely unknown. Nevertheless, the findings of CD4+ T-cell accumulation, oligoclonal TCR-αβ+ expansions and production of IFN-γ and Th1-promoting cytokines, including IL-12, chemokines and chemokine receptors at sites of inflammation provide evidence for a pathological antigen-driven Th1 response [2, 3].

Recently, the pro-inflammatory cytokine IL-17A has been implicated in the pathogenesis of various granulomatous diseases, in particular in the formation of a
mycobacterial infection-induced granuloma in the lung [4]. Although IL-17A can be produced by various cell types, it is the main cytokine produced by the novel subset of Th17 cells, which is distinct from the Th1 and Th2 subsets. Th17 cells were shown to be crucially involved in many autoimmune diseases, including RA, IBD, multiple sclerosis, autoimmune uveitis and in allergic lung disease [5–8]. Th17 cells have the capacity to confer protection against extracellular bacterial and fungal pathogens such as Klebsiella pneumoniae, Citrobacter rodentium and Candida albicans, although accumulating evidence demonstrates that Th17 cells also provide protective effects during infection with more traditional intracellular pathogens [4, 9]. Interestingly, there are many reports of sarcoidosis co-existing with or mimicking rheumatic diseases such as SLE; RA and AS [10]. In this context, the observation that IL-17A is highly expressed in synovium of RA patients and that the cellular source is mainly CD4+ cells is particularly important [11–14]. Differentiation and maintenance of Th17 cells in human is critically dependent on IL-12, IL-6, TGF-β and IL-23 [15]. The finding in sarcoidosis of increased IL-12p40 (which is not only part of the Th1-inducing cytokine IL-12 but also of the Th17-inducing cytokine IL-23), together with increased IL-12p40 mRNA expression in lymph nodes [16–18], may therefore also point to a role of Th17 cells.

Human Th17 cells comprise heterogeneous subsets. Next to IL-17A, these cells produce various other pro-inflammatory cytokines, including IL-17F, IL-22 and, in some conditions, IFN-γ. IL-22 is a cytokine involved in mucosal immunity against extracellular pathogens and can also be produced independently of IL-17A, as was recently found in the context of psoriasis [19–21]. Also IL-17A/IFN-γ double-producing cells have been described [22–24], which could possibly be more pathogenic, since, for example, these cells preferentially cross the blood-brain barrier in patients with multiple sclerosis.

Recently, sarcoidosis was suggested as a Th1/Th17 multi-system disorder [25], based on the presence of IL-17+ CD4 T cells in sarcoid lung tissue and their ability to respond to the chemotactic stimulus chemokine (C-C motif) ligand 20. Moreover, IL-17A was expressed by macrophages infiltrating sarcoid tissue. However, the involvement of IL-22 or the recently identified pathogenic populations of IL-17A/IFN-γ and IL-17A/IL-4 double-producing Th cells [26, 27] in sarcoidosis pathogenesis remains unknown.

Therefore, in this report we studied the presence of double-producing Th cells. Moreover, we investigated whether there would be granuloma-dependent differences in the presence of IL-22 and IL-17A+ cells in granuloma containing compared with non-granulomatous lung mucosa biopsies obtained from sarcoidosis patients. Because of the heterogeneous cytokine profile of the Th17 subset, we analysed peripheral blood, bronchoalveolar lavage (BAL) cells and lung mucosal biopsies of newly diagnosed immunosuppressive drug-free Stage 1 or 2 sarcoidosis patients. Flow cytometric (FACS) analyses were performed for the expression of the main Th17 cytokines IL-17A, IL-17F and IL-22, along with IFN-γ, TNF-α and IL-4 and immunohistochemistry for IL-17A and IL-22.

Materials and methods

Supplementary data are available at Rheumatology Online with more details regarding bronchoscopy procedure, peripheral blood mononuclear cell (PBMC) processing, flow cytometric analysis and immunohistochemical staining of lung mucosa biopsies.

Patients and healthy control subjects

After informed consent (according to the Declaration of Helsinki), 33 patients and 33 healthy volunteers underwent fibre-optic bronchoscopy. The study was approved by the Medical Ethical Committee of the Erasmus MC Rotterdam. The patient group consisted of 15 males and 18 females with newly diagnosed sarcoidosis (mean age: 37.3 years). The diagnosis of sarcoidosis was made to conform to the guidelines of the American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders statement on sarcoidosis [1]. All patients were newly diagnosed with Stage 1 (19 patients) or Stage 2 (14 patients) sarcoidosis. None of the patients was on CS or immunosuppressive drugs at the time of diagnosis and sampling. Healthy volunteers had a male/female ratio of 13/20 and the mean age was 24.2 years. BAL was performed with a flexible fibre-optic bronchoscope (Olympus) according to standard procedures (see supplementary data available at Rheumatology Online).

Immunohistochemical analysis of lung mucosa biopsies and cytospins

Immunohistochemical stainings are detailed in supplementary data available at Rheumatology Online. All biopsy sections were stained in one session to reduce inter-staining variation and analysed in a blinded fashion by two different researchers. Sections from lung mucosal biopsies and cytospins of sarcoidosis patients. The entire lamina propria region was analysed in all 48 biopsies and cell numbers were expressed as cells per millimetre length basal membrane. The statistical analysis was performed with Pearson’s rank correlations.

Statistical analysis

For statistical evaluations the Kruskal–Wallis one-way analysis of variance between groups and the Mann–Whitney U test were performed. A P < 0.05 indicated statistically significant differences. Associations between cells were assessed with Pearson’s rank correlations.
Results

Increased Th17 profile in circulating memory CD4+ T cells from sarcoidosis patients

To investigate the involvement of Th17 cells in the pathogenesis of sarcoidosis, we analysed peripheral blood samples of 9 recently diagnosed sarcoidosis patients and 10 healthy controls by flow cytometry. Surface stainings for cluster of differentiation (CD)3, CD4, CD8 and CD45RO revealed a specific CD4+ T-cell lymphopenia in the sarcoidosis patients (Fig. S1A, available as supplementary data at Rheumatology Online). The proportions of CD3+ T cells in the mononuclear cell fractions were significantly reduced ($P = 0.02$) in sarcoidosis patients (median: 45%; range: 8–56%), when compared with healthy controls (median: 58%; range: 35–74%; Fig. S1A, available as supplementary data at Rheumatology Online). Likewise, the proportions of CD4+ T cells of the total CD3+ populations were significantly reduced ($P = 0.001$) in sarcoidosis patients (median: 33%; range 11–61%; and healthy controls: median: 63%; range 51–90%) (see Fig. S1B, available as supplementary data at Rheumatology Online). The proportions of CD8+ T cells in the mononuclear cell fractions were not different between sarcoidosis patients and healthy controls (see Fig. S1A, available as supplementary data at Rheumatology Online).

We did not detect significant differences in the fractions of antigen-experienced memory CD45RO+ CD4+ T cells between sarcoidosis patients (median: 51%; range: 31–92%) and healthy controls (median: 41%; range: 14–64%) (Fig. 1A). We used intracellular flow cytometry to determine the expression profiles for IL-17A, IL-17F, IL-22, IFN-γ, IL-4 and TNF-α in total mononuclear cell fractions upon 4 h of stimulation with phorbol myristate acetate (PMA) and ionomycin (see Fig. S1C for gating strategy, available as supplementary data at Rheumatology Online). Intracellular expression of these

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**Fig. 1** Cytokine profiles of circulating memory Th cells in sarcoidosis patients and healthy controls. (A) Proportions of antigen-experienced memory CD45RO+ cells within the populations of CD4+ T cells in PBMC fractions. (B) Proportions of CD45RO+CD4+CD3+ cells expressing the indicated cytokines, as determined by intracellular flow cytometry. (C, D) Proportions of CD45RO+CD4+CD3+ cells that were double positive for the indicated cytokines, as determined by intracellular flow cytometry. Symbols represent individual healthy controls (HCs, filled circles), sarcoidosis patients (SRC, filled squares) and lines indicate median values. *$P < 0.05$; **$P < 0.01$. 

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cytokines was almost completely confined to the CD45RO+ memory T-cell fractions (data not shown). Importantly, the proportions of IL-17A+ cells within the CD45RO+CD4+ memory T-cell population were significantly higher \((P = 0.009)\) in sarcoidosis patients (median: 3.7%; range: 3.0–6.6%) than in healthy controls (median: 2.4%; range: 1.4–5.8%; Fig. 1B). We did not detect differences between patients and healthy controls for the other two Th17 cytokines tested, IL-17F and IL-22 (Fig. 1B). The frequencies of IFN-γ-expressing cells tended to be higher in sarcoidosis patients (median: 30% of CD45RO+CD4+ T cells; range: 10–38%), when compared with healthy controls (median: 19%; range: 16–34%), but this difference did not reach significance (Fig. 1B). Also, the proportions of IL-4+ cells were significantly higher \((P = 0.05)\) in sarcoidosis patients (median: 1.8% of CD45RO+CD4+ T cells; range: 0.7–3.8%; healthy controls: median: 1.2%; range: 0.8–2.3%; Fig. 1B). No differences were observed for TNF-α between sarcoidosis patients and healthy controls (Fig. 1B).

Because IL-17A/IL-22 and IL-17A/IFN-γ double-producing cells have been described, whereby particularly IL-17A/IFN-γ+ cells might be more pathogenic [21–22], we next analysed co-expression of Th17 cytokines. We observed significantly higher proportions of CD45RO+CD4+ T cells expressing IL-17A, together with IL-22, IFN-γ or IL-4 in sarcoidosis patients than in healthy controls (Fig. 1C). No differences were observed for the frequencies of IL-17A/TNF-α or IL-17A/IL-17F (Fig. 1C and data not shown) or IL-17F/IFN-γ double producers (Fig. 1D).

Taken together, these findings show that in recently diagnosed sarcoidosis patients the peripheral blood memory Th-cell compartment contained increased proportions of IL-17+ T cells, indicating enhanced Th17 differentiation. Moreover, the increased frequencies of IL-17A/IL-22 and particularly IL-17A/IFN-γ double-producing T cells would indicate an active state of the disease. The increased frequency of IL-4+ single and IL-17A/IL-4+ double-positive memory Th cells point to a possible involvement of IL-4 in sarcoidosis, as previously suggested by Hauber et al. [28].

Increased IL-17A, but not IL-22, in BAL memory CD4+ T cells from sarcoidosis patients

The majority of CD4+ T cells in the alveolar space of sarcoidosis patients were CD45RO+ memory T cells, as determined by analysis of BAL cells (median: 94%; range 91–96%; Fig. 2A). Stimulation of BAL cells by PMA/ionomycin, similar to PBMC, indicated the abundant presence of cells expressing IL-17A (median: 25%; range: 11–33%), IFN-γ (median 54%; range: 33–70%), and TNF-α (median: 60%; range: 33–72%) within the CD45RO+CD3+CD4+ memory helper T-cell population. IL-17F, IL-22 or IL-4+ Th cells were present at very low frequencies (Fig. 2B). Importantly, large proportions of IL-17A+ memory Th cells in BAL were also IFN-γ+. The frequency of IL-17A/IFN-γ double-producing memory Th cells was therefore remarkably higher in BAL (~15%) than in peripheral blood (~1%) of sarcoidosis patients (Fig. 2C).

IL-17A/IL-4 double-producing cells were present at low, but detectable, proportions, representing a novel subpopulation (Fig. 2C). In contrast, IL-17+IL-22+ cells were almost completely absent and proportions of IL-17A/TNF-α or IL-17A/IL-17F double producers were present in the same ranges in BAL and peripheral blood from sarcoidosis patients (Fig. 2C and data not shown). IL-17F/IFN-γ double producers were virtually absent in BAL of sarcoidosis patients (Fig. 2D). The proportions of IL-17A+, IL-17A+IFN-γ+ and IL-17A+IL-4+ memory Th cells in BAL varied between patients and did not show a positive correlation with the T-cell alveolitis in BAL (data not shown).

In summary, the observation of high proportions in BAL of IL-17A+ memory Th cells, and particularly of IL-17A/IFN-γ double-producing cells, which are thought to be more pathogenic [22], clearly point to the involvement of Th17 cells in sarcoid inflammation.

Increased IFN-γ and TNF-α, but not IL-17A, in CD4+ T cells in sarcoidosis

Little is known about CD8+ and γδ T cells (the CD4+CD3+ T-cell population) in sarcoidosis, although the general observation is that these cells show a similar or a less pronounced cytokine pattern as CD4+ T cells do [29, 30]. Here, we observed that a substantial fraction of circulating CD8+ and γδ T cells produced IFN-γ, whereby the proportions were significantly higher \((P = 0.01)\) in sarcoidosis patients compared with healthy controls (see Fig. S2, available as supplementary data at Rheumatology Online). Frequencies of IL-17A, IL-17F, IL-4 or TNF-α-expressing CD8+ and γδ cells T cells were not different between sarcoidosis patients and healthy controls. When we measured IL-22 expression in circulating CD8+CD3− cells, we found fewer IL-22+ cells in sarcoidosis patients when compared with healthy controls (see Fig. S2, available as supplementary data at Rheumatology Online). BAL CD8+ and γδ T-cell populations of sarcoidosis patients contained significant proportions of IL-22+, IFN-γ+ and TNF-α+ cells and limited proportions of cells expressing IL-17A, IL-17F or IL-4. Taken together, these findings show that IL-17A is not a prominent cytokine produced by CD8+ and γδ T cells in sarcoidosis. Nevertheless, a substantial fraction of CD8+ and γδ T cells in the BAL produced IL-22.

Increased IL-17A+ cells in sarcoidosis lung biopsies containing granulomas

The presence of Th17 cytokines in memory Th cells in the BAL, representing the alveolar space of the lungs, prompted us to analyse T-cell cytokine expression in lung mucosal tissue of sarcoidosis patients. We investigated the presence of IL-17+ cells in lung mucosal biopsies from 27 sarcoidosis patients and 22 healthy controls (Fig. 3). From the 27 biopsies from sarcoidosis patients, 10 contained clear granulomas (Fig. 3C) and 17 were non-granulomatous, showing diffuse cellular infiltrates...
only (Fig. 3B). IL-17A-expressing cells were particularly found in sarcoidosis lung biopsies containing granulomas. Low magnifications of immunohistochemical stainings revealed that IL-17+ cells were specifically present in areas of inflammatory cells surrounding granulomas, as well as within the granulomas (Fig. 3C). In addition, we observed diffuse IL-17A staining within the granuloma areas (Fig. 3C). IL-17+ cells were not detected in the epithelium. Quantification of IL-17-expressing cells in biopsies from healthy controls and non-granulomatous and granuloma-containing biopsies from sarcoidosis patients showed that in all three groups the subepithelial area contained very few IL-17+ cells, although a trend of higher numbers was observed in granuloma-containing biopsies (P = 0.059, Kruskal–Wallis test; Fig. 3D). By immunohistochemical double stainings for and CD3, the IL-17+ cells were characterized as T cells (Fig. 3E).

In summary, we observed increased numbers of IL-17A+ T cells in sarcoidosis in association with granulomas. Together with the presence of diffuse IL-17A staining in the granulomas, these findings suggest a role for IL-17A in granuloma formation or maintenance.

Increased subepithelial IL-22+ T cells in sarcoidosis lung biopsies containing granulomas

Finally, we investigated the presence of IL-22+ cells in the epithelium, subepithelium and lamina propria (Fig. 4). The

Fig. 2 Cytokine profiles of circulating and BAL memory Th cells in sarcoidosis patients. (A) Proportions of antigen-experienced memory CD45RO+ cells within the populations of CD4+ T cells in PBMC and BAL. (B) Proportions of CD45RO+CD4+CD3+ cells expressing the indicated cytokines, as determined by intracellular flow cytometry. (C, D) Proportions of CD45RO+CD4+CD3+ cells that were double positive for the indicated cytokines, as determined by intracellular flow cytometry. Symbols represent PBMC (filled circles) and BAL (filled squares) from individual patients and lines indicate median values.
FIG. 3 Increased numbers of IL-17A+ cells in sarcoidosis lung biopsies containing granulomas. (A-C) Haematoxylin nucleus staining and IL-17A staining of lung mucosal frozen sections from a healthy control biopsy (A), a non-granulomatous sarcoidosis biopsy (B) and a granuloma-containing sarcoidosis biopsy (C) at 100×, 200× and 400× magnification. IL-17A+ cells as well as diffuse IL-17A staining are observed in red. (C) The high magnification photograph (1000×, far right) illustrates cytoplasmic staining with anti-IL-17A antibodies (arrows). (D) Quantifications of the numbers of IL-17+ cells in the subepithelium (left) and entire lamina propria area (right) in the indicated groups. Symbols represent biopsies from individual healthy controls (HCs, filled squares), sarcoidosis patients without (SRC, filled triangles) and sarcoidosis patients with granuloma (SRC gr, filled inverted triangles); lines indicate median values. *P < 0.05.

(E) Co-localization of an IL-17+ (in blue) and CD3+ (in red) T cell in a lung mucosal biopsy.
epithelium could be evaluated in 32 biopsies. IL-22+ cells were detected in nine of them, but no significant differences were found between sarcoidosis patients and healthy controls. Importantly, granuloma-containing biopsies showed significantly more IL-22+ cells in the subepithelial area (median: 15 cells/mm basal membrane; range: 5–27 cells/mm; Fig. 4B and C) than non-granulomatous biopsies (6 cells/mm; range: 0–13 cells/mm; P = 0.004, Mann-Whitney U-test) or healthy control biopsies (7 cells/mm; range: 0–14 cells/mm; Fig. 4A and C). When we quantified the numbers of IL-22+ cells in the total lamina propria areas, we did not detect significant differences between the three groups of biopsies (Fig. 4D).

Analyses of alveolar biopsies demonstrated the incidental presence of IL-22+ cells in healthy controls as well as in sarcoidosis patients, without significant differences between these groups. Immunohistochemical double stainings showed that IL-22+ cells were mainly CD3+ T cells, as illustrated in Fig. 4E. In those biopsies where IL-17A+ cells were detected in the lamina propria, we found an association with the presence of IL-22+ cells in the subepithelial area (r = 0.185; P = 0.05, Pearson’s test, see Fig. S3, available as supplementary data at Rheumatology Online).

In summary, our findings show that sarcoidosis patients have a significant increase in IL-22-producing cells in the subepithelial area in the lung. Thus, their main localization is different from IL-17-producing cells, which are mainly localized around and within granulomas.

**Discussion**

The pathological mechanisms that control granulomatous inflammation in sarcoidosis are only poorly understood, but it is clear that cytokines play an important role in granuloma formation. To date, sarcoidosis pathogenesis has mainly been related to increased Th1 cytokines. In this report, we provide several lines of evidence for the involvement of pro-inflammatory Th17-lineage cytokines. First, we found increased proportions of circulating IL-17A+ memory Th cells. Secondly, IL-17A+ cells, and in particular IL-17A/IFN-γ and IL-17A/IL-4 double-producing cells, which are normally very rare, were also present in BAL samples of sarcoidosis patients. Thirdly, we observed an increase in IL-17A-expressing T cells in the lamina propria of the lung in sarcoidosis patients, specifically in granuloma-containing biopsies, where IL-17A+ cells were present around and inside granulomas. Fourthly, we identified an increase in IL-22+ cells, in particular in subepithelial regions in granuloma-containing biopsies. To the best of our knowledge, we show for the first time differential distribution of IL-17A+ and IL-22+ T cells in local granulomas, BAL and the circulation in sarcoidosis. Our data particularly point at a possible role for IL-17A/IFN-γ and IL-17A/IL-4 double-producing CD4+ T cells, while we did not find evidence for increased IL-17A production by other T-cell subsets, such as γδ or CD8+ T cells.
Until now sarcoidosis has been considered a Th1-mediated multi-system disease and there has been convincing evidence reported for the role of Th1 cells in the pathogenesis of sarcoidosis over the past few years [1]. Thus, Th17 cells are clearly not the only effector cells capable of inducing or regulating granuloma pathogenesis. This would parallel findings in autoimmune uveitis and in experimental autoimmune encephalomyelitis, in which both Th1 and Th17 cells can drive tissue damage [6, 31]. Cooperation of IL-17A and IFN-γ is particularly of interest, since we observed high frequencies of IL-17A+IFN-γ+ memory Th cells in blood and BAL of sarcoidosis patients. Furthermore, it has recently been reported that IL-17A/IFN-γ double-producing CD4+ T cells can become single IFN-γ+ cells or single IL-17A-producing cells [22]. IL-17A+IFN-γ+ cells are thought to be more pathogenic and have also been identified in Crohn’s disease [32] and in coronary atherosclerosis [33].

Also our identification of IL-17A/IL-4 double-producing cells in sarcoidosis is of interest. Very few of these cells are present in the circulating memory T-cell populations in healthy individuals, but their proportions were reported to be significantly increased in patients with chronic asthma [34]. In this regard, it is very possible that these cells contribute to IL-4-induced pro-fibrotic features, such as fibroblast growth and collagen production, which are often observed in later stages of sarcoidosis [35]. In contrast to the IL-17A+IFN-γ+ and IL-17A+IL-4+ memory Th cells present in the circulation as well as BAL of sarcoidosis patients, we detected only few IL-17A+IL-22+ cells. On the contrary, our findings of: (i) high proportions of IL-17A+ and very low proportions of IL-22+ Th cells in BAL; and (ii) different locations of IL-17A+ and IL-22+ Th cells in mucosal biopsies of sarcoidosis patients, support the hypothesis that IL-22 can be produced in a IL-17-independent fashion by Th22 cells [19, 21].

IL-17A has previously been implicated in various conditions characterized by granuloma formation. In a Mycobacterium bovis infection model, IL-17A expression was detected early after pulmonary infection and IL-17A-deficient mice showed impaired granuloma formation [36]. In humans living in regions with high prevalence of Mycobacterium tuberculosis infection, peripheral blood contains high frequencies of IL-17A+ and IL-22+ memory Th cells, which may have protective properties against tuberculosis [37]. In mouse models and in humans with active pulmonary tuberculosis, both IL-17A+ and IL-22-producing CD4+ T cells and IL-17A+ γδ T cells were shown to contribute to the anti-mycobacterial immune response in human [7, 38]. Lung injury in a mouse model for chronic granulomatous disease with lethal aspergillosis was shown to involve unrestrained γδ T-cell reactivity and dominant production of IL-17A [39]. In Langerhans cell histiocytosis, which is accompanied by aggressive chronic granuloma formation, yet another cell population, dendritic cells, was shown to synthesize IL-17A [6]. An IL-17A-dependent pathway for dendritic cell fusion was identified, which was potentiated by IFN-γ and led to giant cell formation. In this context, interesting parallels between Langerhans cell histiocytosis and sarcoidosis further include the presence of multinucleated giant cells [2, 6, 40].

The aetiology of sarcoidosis is still unknown. Epidemiological and histopathological data have been suggestive of occupational airborne antigens or infectious antigens underlying this disease, but until now attempts to link sarcoidosis to a causative pathogen have been difficult and remain controversial. It is therefore presently unclear which mechanisms would initiate a Th17 response in sarcoidosis. It is conceivable that the involvement of Th17 cells in sarcoidosis points to an autoimmune process that is comparable with various other IL-17A-driven autoimmunity disorders, such as autoimmune uveitis, RA, IBD or psoriasis. Future studies are required to determine putative genetic components that enhance IL-17A synthesis, e.g. IL-23R polymorphisms, which have also been associated with autoimmunity. As we observed diffuse IL-17A staining, it is very well possible that Th17-effector cells within granulomas are required to achieve local IL-17A concentrations that can activate various myeloid cell populations. Such a model would parallel the proposed role of local Th17 cells in the bone marrow, which may regulate myeloid development [41]. An alternative explanation for the observed diffuse IL-17A staining in granuloma may be that granuloma cells are a source for IL-17A, analogous to Langerhans cells in Langerhans cell histiocytosis [40].

In summary, we provide evidence for the involvement of the Th17 lineage in sarcoidosis: IL-17A+expressing T cells were present in and around the granuloma and IL-22-expressing T cells were found in the subepithelial lamina propria in mucosal biopsies of sarcoidosis patients. This was accompanied by the presence of IL-17A+, IL-17A+IFN-γ+ and IL-17A+IL-4+ memory Th cells in BAL and by a significant increase in the proportions of these cells in the circulation. Therefore, IL-17A and IL-22 represent targets that may have clinical value in the treatment of sarcoidosis. In this context, it is promising that clinical trials of the fully human antibody, AIN457, in RA, psoriasis and non-infectious uveitis, show that targeting IL-17A interrupts inflammation and reduces disease activity [42].

Rheumatology key messages

- Th17 subset in sarcoidosis integrates previous findings of the involvement of Th1 and Th2 cells.
- IL-17A+IFN-γ+ and IL-17A+IL-4+CD4+ T cells were present in substantial numbers in blood and BAL samples of sarcoidosis patients.

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Supplementary data
Supplementary data are available at Rheumatology Online.

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