



Moving target: shifting the focus to pulmonary sarcoidosis as an autoimmune spectrum disorder

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This review highlights the emphasis of recent years on the clinical and immunological heterogeneity of sarcoidosis, the role of immune hyperactivity and autoimmunity in disease pathogenesis, and newly proposed disease-triggering antigens <http://ow.ly/tfFi30oo9yB>

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ABSTRACT Despite more than a century of research, the causative agent(s) in sarcoidosis, a heterogeneous granulomatous disorder mainly affecting the lungs, remain(s) elusive. Following identification of genetic factors underlying different clinical phenotypes, increased understanding of CD4⁺ T-cell immunology, which is believed to be central to sarcoid pathogenesis, as well as the role of B-cells and other cells bridging innate and adaptive immunity, contributes to novel insights into the mechanistic pathways influencing disease resolution or chronicity. Hopefully, new perspectives and state-of-the-art technology will help to shed light on the still-elusive enigma of sarcoid aetiology. This perspective article highlights a number of recent advances in the search for antigenic targets in sarcoidosis, as well as the main arguments for sarcoidosis as a spectrum of autoimmune conditions, either as a result of an external (microbial) trigger and/or due to defective control mechanisms regulating the balance between T-cell activation and inhibition.

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Introduction

Sarcoidosis is a granulomatous multisystem disorder of unknown aetiology, which primarily manifests in the lungs [1, 2]. Over the years, postulated causes of disease have included infectious agents [3], e.g. *Mycobacterium tuberculosis* [4, 5] and *Propionibacterium acnes* [6–8], or remnants thereof, exposure to environmental irritants [9], protein misfolding [10] or, most recently, autoimmunity [11, 12]. Still, more than 140 years after its first observation, and decades of clinical, epidemiological, genetic and immunological research, the causative agent(s) have yet to be identified.

In recent years, our group and others have contributed to increased understanding of CD4⁺ T-cell immunology in sarcoidosis, which has been shown to be far more complex than initially appreciated. Moreover, other cell types previously more or less neglected in sarcoidosis research, such as B-cells, CD8⁺ cytotoxic T-cells (CTLs), dendritic cells (DCs), natural killer cells and natural killer T-cells, are gradually becoming focal points in unravelling the mechanistic pathways influencing granuloma resolution and persistence. In parallel, steady progress has been made in the continuously ongoing, and thus far inconclusive, quest for the “sarcoidosis antigen(s)”. Hopefully, state-of-the-art technology and emerging insights into T-cell and B-cell receptor repertoires will shed light on the still-elusive enigma of sarcoid pathogenesis.

Clinical and immunological features of sarcoidosis disease phenotypes

Identification of disease-triggering factors is complicated by the heterogeneity in sarcoidosis presentation; on one hand, the acute form Löfgren’s syndrome (LS) is characterised by sudden onset, fever and distinct clinical symptoms such as bilateral hilar lymphadenopathy, erythema nodosum and/or ankle arthritis, but also a usually self-limiting disease course. On the other hand, so-called “non-LS” sarcoidosis comprises a much more diverse group, commonly distinguished from LS by insidious onset, slow progression and a higher risk of developing chronic disease and pulmonary fibrosis. Importantly, as diagnostic assessment is made primarily on clinical criteria, the molecular mechanisms underlying development of different disease phenotypes remain unknown.

The Th-1/Th-17 paradigm in sarcoidosis

While initially considered to be primarily a T-helper (Th) type 1-driven disorder, due to the high levels of interferon (IFN)- γ , interleukin (IL)-2 and tumour necrosis factor- α produced by bronchoalveolar lavage fluid (BALF) T-cells and alveolar macrophages [13, 14], presence of Th-1-skewing cytokines IL-12 and IL-18 [15–17], as well as the high T-cell expression of chemokine receptors CXCR3 and CCR5 [18], recent years have seen a shift in the understanding of sarcoidosis as a Th-1/Th-17-mediated disease. In several individual studies [19–23], a majority of CD4⁺ T-cells in sarcoidosis patients and healthy individuals have been found to express both canonical Th-1 chemokine receptor CXCR3 and Th-17-associated CCR6 in BALF, but not in peripheral blood. Moreover, simultaneous expression of transcriptional regulators T-bet (Th-1) and ROR γ T (Th-17) has been demonstrated in the lung, with “Th-1/Th-17” hybrid cells constituting ~25% in healthy subjects [19]. In the sarcoid, and especially the LS lung, this percentage was markedly increased, and patients with non-chronic disease were found to present with higher frequencies of T-bet⁺ROR γ T⁺ CD4⁺ T-cells compared to patients with chronic disease [19]. The discrepancy between cellular phenotypes in the lung and the circulation, respectively, suggests that the pulmonary microenvironment actively promotes a Th-1/Th-17 phenotype, even under homeostatic conditions. Furthermore, due to their presence in significant numbers in healthy individuals, Th-1/Th-17 cells do not appear to be inherently “pathogenic” in themselves. For instance, LEXBERG *et al.* [24] argued that the combined effector repertoires of Th-1 and Th-17 cells might confer a physiological advantage through more efficient orchestration of disease resolution (figure 1). In LS patients, where these cells were most frequent, cytokine production was found to be more varied, with secretion of IL-17A, IL-10, IL-2 and IL-22 in addition to IFN- γ , which was by far the predominant cytokine in non-LS patients. This finding emphasises that not only the phenotype, but also the functionality of the cell at any given moment is crucial in influencing the outcome of the inflammatory reaction. In Crohn’s disease, a granulomatous disorder of the gut, inhibition of IL-17A has been shown not to ameliorate, but rather to exacerbate inflammation [25], suggesting a protective effect of this cytokine and an overlap of Th-1, Th-17 and regulatory T-cell (Treg) functionality of T-cells in mucosal tissues. Both IL-17A and IL-22 have demonstrated protective effects during *M. tuberculosis*-induced granulomatous inflammation [26], and reduced IL-22 levels have been observed in patients with chronic sarcoidosis and idiopathic pulmonary fibrosis (IPF) [27]. ROR γ T⁺ CD4⁺ T-cells are also known to produce IL-10 and act to restrain inflammation [28], implying that the higher IL-10, IL-22 and IL-17A production observed in LS relates to immune regulation and mucosal homeostasis. The recent discovery that so-called “Th-17.1” cells, *i.e.* cells that have converted from a classical Th-17 phenotype to express features of both Th-1 and Th-17 cells, including production of either both IL-17A and IFN- γ , or IFN- γ alone [20], are enriched in patients with

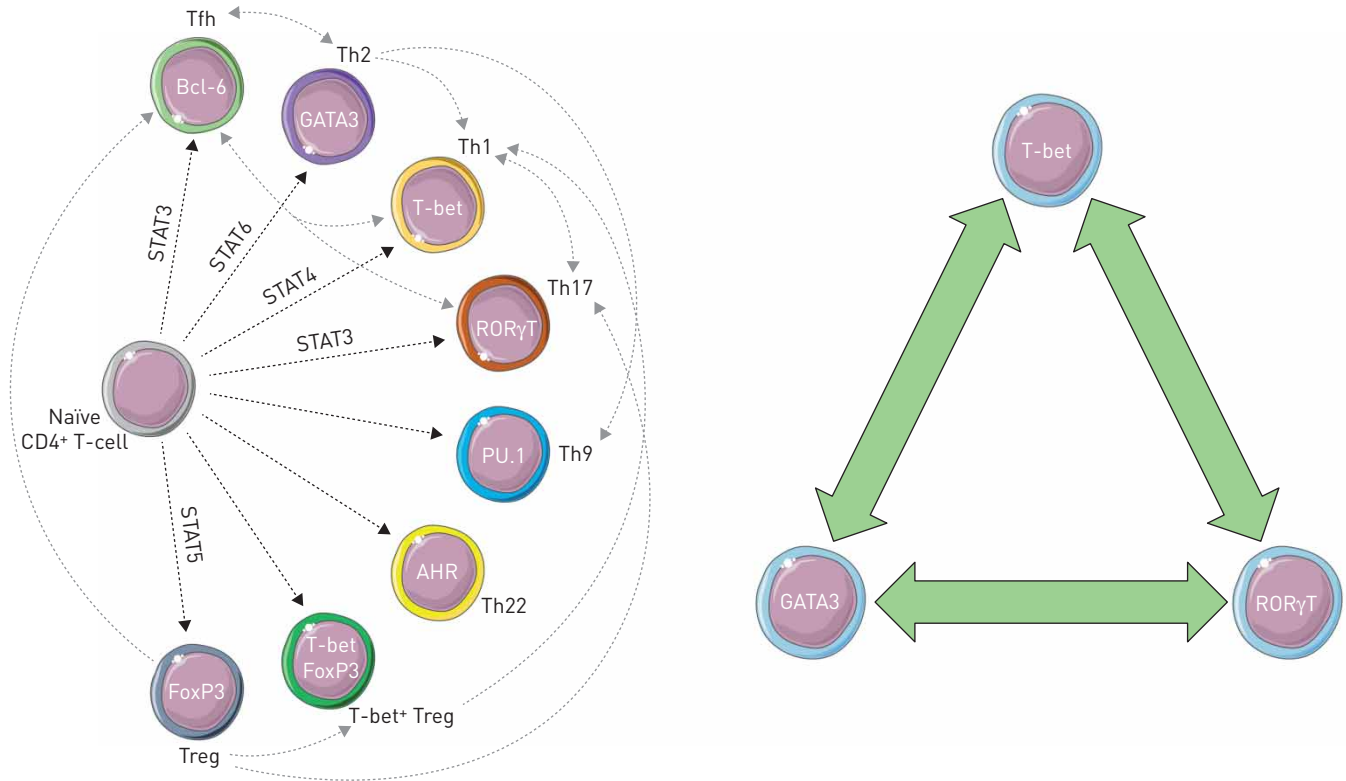


FIGURE 1 CD4⁺ T-cell plasticity. Schematic outline of documented T-helper (Th)-1 subsets, along with their “master” transcriptional regulators and postulated pathways of inter-differentiation. The concept of T-cell plasticity infers that rather than entering a state of terminal differentiation, a delicate balance of transcription factor expression (green arrows) ultimately dictates cell fate at a certain, compartment-specific time point. The cell is thereby able to respond to various stimuli and adapt its functionality accordingly depending on the triggering antigen, the tissue microenvironment and timing during the disease course. In the context of sarcoidosis, Th-1/Th-17 hybrid cells are likely to have a tissue-specific surveillance role in the lung under homeostatic conditions, but the presence of disease-associated antigens, combined with underlying genetic factors, drive their functional differentiation into either a more T-bet/ROR γ T-balanced (LS) or a T-bet-dominated (non-LS) phenotype that will significantly influence disease outcome. Tfh: follicular T-helper cell.

chronic disease [21] further suggests that IFN- γ , rather than IL-17A, may be the primary culprit in driving pathogenic processes in the lung [29]. These findings indicate that a combined IL-17A/IFN- γ reaction is central to pulmonary immunology, and that this response is enhanced in LS patients, but somehow skewed towards a more aggressive “IFN- γ only” phenotype in non-LS patients. Accordingly, it is entirely possible that the Th-1/Th-17-mediated pathogenicity observed in rheumatoid arthritis (RA), multiple sclerosis (MS) and psoriasis, for example [30–34], is primarily driven by excessive IFN- γ secretion, and that pro- or anti-inflammatory functions of IL-17A strongly depend on the local tissue microenvironment.

Granulomatous structures and signalling

Oddly, few, if any, studies have been dedicated explicitly to identifying differences between sarcoidosis phenotypes with regards to the structure or composition of the actual granuloma. As LS is characterised to a large extent by spontaneous resolution, any subtle variability in granuloma formation and maintenance could be of clinical relevance. Using mass cytometry, expression of CD44 on CD4⁺ T-cells was found to be significantly reduced in LS patients compared to non-LS patients [35]. CD44 mediates adhesion to the endothelium and extracellular matrix and its expression on lymphocytes in pulmonary sarcoidosis, as well as other mucosal disorders such as Crohn’s disease, has been speculated to influence their homing and activation, in addition to stimulating macrophage differentiation [36]. Furthermore, elevated levels of soluble CD44 have been demonstrated in conditions with an autoimmune background, including sarcoidosis [37, 38], suggesting involvement in cell–cell communication. Recently, LINKE *et al.* [39] discovered increased activity of mechanistic target of rapamycin (mTOR) complexes in sarcoid granulomas of patients with active progressive disease, compared to patients undergoing remission. Intriguingly, mTOR complexes can be inhibited by CD44 blockade [40], suggesting a dual role of CD44 in adhesion as well as signal transduction during granuloma formation and persistence. Consistently reduced CD44 expression on CD4⁺ T-cells in LS may thus contribute to an increased tendency of these granulomas to resolve spontaneously.

Human leukocyte antigen association

While the *HLA-DRB1*03* allele has long been known to associate strongly with LS and a favourable clinical outcome [41], recent genetic studies have revealed striking differences between LS and non-LS, with greater genetic homogeneity among LS patients, and presence of genetic associations not observed in non-LS, and *vice versa* [42]. The distinction between LS and non-LS is critical in the effort to understand the genetic, immunological and environmental factors contributing to development of chronic disease and resolution. Due to its characteristic clinical presentation and commonly favourable outcome, LS may, in time, even be considered as a separate disease [41].

Sarcoidosis results from immune hyperactivity

The similarity of sarcoid pathology to that of tuberculosis, characterised by granuloma formation in the lung, initially suggested a common origin and spurred the hypothesis of an infectious agent as a cause of disease. Opinions differ on the exact mechanism, but it has been suggested that sarcoidosis is caused by immune reactivity against incompletely degraded and persisting bacterial antigens, rather than a conventional infection [43]. Mycobacterial and propionibacterial DNA and protein have been identified in sarcoidosis granulomas, and several studies show reactivity of lung-derived T-cells to mycobacterial proteins [4], most frequently mycobacterial catalase-peroxidase (mKatG), the 6-kDa early secretory antigenic target (ESAT-6) and heat-shock proteins [44, 45]. Broad-spectrum anti-mycobacterial therapy has demonstrated positive effects on lung function in chronic sarcoidosis patients [46], although this was primarily attributed to anti-inflammatory effects rather than specific pathogen eradication [47]. In addition, *P. acnes* has been associated particularly with certain manifestations of sarcoidosis such as ocular, cardiac and neurological involvement [48–51], and more recent studies show alterations in the pulmonary microbiome in sarcoid compared to healthy lungs [52]. However, as presence of bacterial DNA [53] and protein [5] have been reported in ~50% of investigated sarcoidosis patients, and as live mycobacteria have never been identified in or cultured from sarcoid lungs, these findings cannot account for all patients. Likewise, favourable responses to long-term immunosuppressive treatment without emergence of infectious disease argue against intact microbial organisms in sarcoidosis [54]. While a seasonal variation in disease onset [55], particularly for *HLA-DRB1*03*⁺ LS patients, points to involvement of an infectious agent (or another, non-invasive environmentally derived triggering factor), the accessibility of which fluctuates with the seasons, there is little evidence of an active, ongoing infection in sarcoidosis patients, particularly due to the lack of continuity between studies in which microbial agents are implicated as drivers of disease. Rather, these data together suggest that several forms of pulmonary infection hold the potential to trigger a sarcoid reaction in a genetically susceptible individual, and that ensuing disease is attributed primarily to inherent defects in immune regulation. As discussed later, interference with regulatory mechanisms through checkpoint inhibition, for example, could facilitate a similar setting, serving to shift immune balance in favour of pathogenic processes.

Malfunctions in the innate immune system that result in chronic tissue inflammation are often referred to as “autoinflammatory” conditions, and are typically related to mutations in genes involved in inflammasome activation. In contrast, “autoimmunity” is attributed to a dysregulated adaptive immune response and a disease susceptibility strongly influenced by human leukocyte antigen (HLA)-related genes. Accordingly, activation of the adaptive immune system through recognition of one or several specific extracellular antigenic targets results in a response dominated by T- and/or B-cells [56]. However, a known antigen is not required for the definition of a disease as autoimmune, and although the situation in sarcoidosis has yet to be strictly defined, the documented presence of clonally expanded CD4⁺ T-cells strongly implies autoimmunity rather than autoinflammation.

The concept of sarcoidosis as an autoimmune disease is further supported by the strong HLA connection in LS patients. Moreover, a reported higher prevalence of disease in first-degree relatives [57], as well as a markedly higher risk of developing disease in monozygotic compared to dizygotic twins [58], are suggestive of an imperative hereditary component. In addition, LS patients are generally young, previously healthy and nonsmokers [59]. Self-limiting autoimmune conditions are uncommon, but while LS patients usually resolve spontaneously, it has been shown that in a small group of patients (<5%), the disease can relapse later in life [60, 61], similar to an autoimmune flare.

In 1941, the Norwegian pathologist Morten Ansgar Kveim first reported the diagnostic use of a skin test based on intradermal injection of a pasteurised saline suspension of sarcoid lymph node tissue [62]. Over the following weeks, most patients with active sarcoidosis, but not healthy controls, would develop granulomatous structures in the skin at the site of injection, which would be verified as sarcoid tissue upon histological examination [63, 64]. This reaction suggests reactivity to one or several of the contents of the Kveim reagent to be central to the disease process [65–67]. One such recently identified component is vimentin, a type III intermediate filament protein expressed in mesenchymal cells and an important

constituent of the cellular cytoskeleton [68]. Previous studies utilising mass spectrometry have, on separate occasions, identified a vimentin-derived peptide as bound to HLA-DR on alveolar macrophages in BALF of HLA-DRB1*03⁺ sarcoidosis patients [11, 12, 69]. Intriguingly, the *HLA-DRB1*03* allele has also been shown to influence the propensity and outcome of autoimmune disease systemic lupus erythematosus (SLE) [70], as well as Langerhans cell histiocytosis [71] and idiopathic inflammatory myopathies [72, 73]. In SLE, elevated levels of anti-vimentin antibodies have been found in patients with the severe kidney manifestation tubulointerstitial nephritis [74], implicating vimentin in the pathogenesis of several autoimmune disorders. Following citrullination, vimentin also acts as an antigenic target in RA [75, 76], with antibodies against citrullinated vimentin being found in the lungs prior to clinical onset of disease [77].

The disease may also stem from a combination of the aforementioned factors, as in the case of transient autoimmunity [78, 79]. Alternatively, dual reactivity to several antigens of different origin can arise due to molecular mimicry. T-cell receptor (TCR) recognition of a microbial antigen followed by cross-reactivity of activated T-cells to a structurally similar self-peptide can result in autoimmune responses, which abate as the pathogen is cleared [80]. A third possibility is that of an autoimmune reaction following allergic hypersensitivity [81], *e.g.* to certain metals [82] or inorganic compounds. Recent years have seen the successful exploration of chronic beryllium disease (CBD), another granulomatous lung disorder with a strong HLA association and notable clinical similarities to sarcoidosis. It was found that all affected patients were positive for *HLA-DQB1*02* and had previously been exposed to beryllium (Be), allowing Be²⁺ ions to interact with TCR recognition of HLA complexes presenting self-peptides in the lung. Upon Be²⁺ binding, T-cell epitopes would be altered, subsequently causing recognition of a self-peptide and initiation of immune responses [81, 83, 84]. The association of the Be²⁺ ion with the peptide-binding cleft of the HLA molecule also stabilises the TCR–HLA complex, ensuring continuous antigen presentation and constant presence of an autoreactive CD4⁺ T-cell pool in the lung [81, 85, 86]. Thus, CBD patients generally experience a chronic disease, as beryllium is not readily degraded or eradicated once it has entered the lung.

Similar to CBD, where populations of clonal CD4⁺ T-cells are observed in BALF, CD4⁺ T-cells expressing the TCR α -chain variable segment V α 2.3 accumulate in the lungs of HLA-DRB1*03⁺ sarcoidosis patients. These expansions of TCR-specific cells are found more or less exclusively in BALF of HLA-DRB1*03⁺ patients with active disease, and not in HLA-DRB1*03⁺ healthy individuals or patients with other pulmonary disorders [87]. They are not overrepresented in regional lymph nodes, nor in peripheral blood [88], and BALF V α 2.3⁺ T-cell counts decrease upon clinical resolution [89]. Moreover, the higher the level of V α 2.3⁺ T-cells at the time of lavage, the more rapid the resolution and better the prognosis [90]. More recently, an accumulation of CD4⁺ T-cells expressing the V β 22 TCR chain segment was identified in BALF of HLA-DRB1*03⁺ patients [91], which prompted further investigation of HLA-DRB1*03, the V α 2.3 and V β 22 TCR segments in combination and the recognition of potential (auto)-antigenic peptides in this complex [92]. The following sections cover further arguments for the re-evaluation of sarcoidosis, and particularly LS, as an autoimmune condition and the role of vimentin as a potential target of adaptive immune responses.

Disease outcome depends on CD4⁺ T-cell balance

As previously alluded to, disrupted balance between immune activation and regulation is a hallmark trait of autoimmune disease, and CD4⁺ T-cells are not only responsible for propagating inflammation, but also for mediating resolution, most prominently through secretion of inhibitory cytokines and expression of regulatory co-receptors. While T-cell subset patterns differ between LS and non-LS sarcoidosis, current studies clearly show that the Th-1/Th-17 paradigm alone cannot fully explain the course of disease. In one study using mass cytometry, comprehensive cluster analysis outlined the pulmonary CD4⁺ T-cell compartment as highly dynamic, and revealed concomitant expression of several markers that may be of critical importance for disease outcome. Most prominently, CD4⁺ T-cell populations in LS patients were defined by higher expression of regulatory receptors CTLA-4 and PD-1, as well as inducible co-stimulator (ICOS), while non-LS cells consistently showed higher expression of effector and activation markers [35]. BROOS *et al.* [93] have reported on reduced expression of CTLA-4 on CD4⁺ T-cells in sarcoidosis patients compared to healthy individuals, and while this trend was consistent in the current study, a significant further reduction was observed in non-LS patients compared to LS. Retained CTLA-4 and PD-1 expression in LS patients, who are known to have a particularly good prognosis, expands upon the notion that the balance between immune activation and regulation is central to sarcoid inflammation. This hypothesis is further supported by the observed development of sarcoid-like granulomatous disease in patients administered IFN- α , *e.g.* as a treatment for hepatitis C infection [94–96]. Conversely, the same phenomenon has been reported repeatedly in patients receiving anti-CTLA-4 or anti-PD-1 therapy (*e.g.* for metastatic melanoma) [93, 97–102], suggesting that abolishing this immune-dampening function in itself propagates sarcoid inflammation, seemingly regardless of antigen specificity. While some studies have argued for increased PD-1 expression to associate with reduced CD4⁺ T-cell proliferation and to

contribute to the pathogenicity of sarcoidosis in the context of an infection [103, 104], the view of sarcoidosis as an autoimmune disorder suggests that PD-1 is probably upregulated in an effort to control adaptive immune responses to persistent tissue (self)-antigens that the patient is unable to clear. In such case, therapeutic PD-1 blockade in sarcoidosis would be counterproductive, if not directly harmful [105].

Considering the entire CD4⁺ T-cell repertoire as a whole also provides further incentive for reassessment of the role of Tregs in sarcoidosis, which has been the subject of contradictory reports. Alternatively, either a reduction in FoxP3⁺ CD4⁺ T-cells [106, 107] or an increase in numbers, but with defective functional capacity, has been observed. In the latter case, reduced IL-10 production [108] and CTLA-4 expression [93], impaired survival [109] and deficient suppression of effector cell proliferation and cytokine secretion [109–112] have all been reported. In patients with CBD, antigen persistence has been observed to diminish the requirement for CD28 signalling in response to TCR engagement, eventually resulting in dysfunctional suppression mediated by the CTLA-4 and PD-1 pathways [105, 113], which could potentially parallel the situation in chronic sarcoidosis. Prior studies have reported elevated ICOS expression [107], as well as IL-10 production [108], in Tregs from LS patients, suggestive of a more self-restrictive response. In contrast, a shift towards a purely effector-driven response seems to occur in non-LS, exemplified by, *e.g.* a decrease in T-cell immunoglobulin mucin domain molecules that have been proposed to negatively regulate Th-1 responses [114]. More recently, a study showed that while no differences were observed between patient groups or healthy individuals in terms of FoxP3 expression, almost all FoxP3⁺ CD4⁺ T-cells in BALF, comprising both activated Th-cells and conventional Tregs, were also positive for T-bet [19], indicating a selective regulation of Th-1-driven inflammation [115, 116]. Thus, this actively proliferating group of cells may be involved in lung-specific immune regulation and protection against harmful (auto)immune reactions, suggesting that tissue-resident CD4⁺ T-cells may have the ability to perform certain regulatory functions without being classified as “true” Tregs; they may not even express FoxP3, and thereby be overlooked by traditional analysis methods. This, again, emphasises the difficulty of stringent subset definition and stresses the importance of distinguishing cells characterised in the circulation from cells active in their respective target organs, especially in the context of tissue-specific autoimmunity. Moreover, the critical role of CTLA-4 and PD-1 in maintaining homeostasis and preventing immune hyperactivity call for more extensive use of these markers as surrogates for regulatory capacity of CD4⁺ T-cells.

CD4⁺ T-cells are not the sole drivers of sarcoid inflammation

Repeated studies have firmly established sarcoidosis as a primarily CD4⁺ T-cell-driven disease [1, 117]. However, emerging evidence argue for more prominent involvement of other cellular and humoral responses than previously acknowledged [35, 118]. By mass cytometry, a surprisingly active proliferation was observed in the CD8⁺ T-cell compartment [35]. Given the observation that cytotoxic activity of CD8⁺ T-cells is augmented in non-LS patients [119], it is tempting to speculate that expression of regulatory receptors and secretion of inhibitory cytokines by CD4⁺ T-cells also act to restrict cytotoxic CD8⁺ T-cell activity in LS lungs, while unrestrained CTL responses, including high IFN- γ production, in non-LS effectively lead to chronic inflammation and persistent tissue damage. Indeed, it is possible that CD8⁺ T-cells constitute a more important contributor to progressive non-LS inflammation than CD4⁺ T-cells, as suggested also by consistently lower CD4/CD8 ratios in non-LS compared to LS patients [19, 92, 118, 120].

In addition to CD8⁺ T-cells, B-cells have received scant attention throughout the history of sarcoidosis research, despite being observed to undergo somatic hypermutation in the lungs and to localise around sarcoid granulomas [121], as well as to directly correlate with the percentage of T-cells in BALF [122]. However, their exceptional capacity for antigen uptake and presentation make them exceedingly interesting in the context of antigen discovery, as well as in development of novel diagnostic and prognostic tools. B-cell proliferation was recently shown to be particularly pronounced in HLA-DRB1*03⁺ lungs, and to coincide with CD4⁺ T-cell activity, indicating differences in the organisation of tertiary lymphoid structures between patient groups. Moreover, while the total immunoglobulin content in BALF was higher in HLA-DRB1*03⁻ patients, the concentration of antibodies targeting specific autoantigenic epitopes was higher in HLA-DRB1*03⁺ individuals [118], in line with a more antigen-driven, efficient and less aggressive immune response in these patients. The involvement of B-cells and particularly class-switched, antigen-specific antibodies in sarcoid inflammation may be suggestive of memory development, although more detailed studies on B-cell clonality, epitope specificity and surface markers are required to validate such statements. In addition, it was recently demonstrated that the glycosylation status of IgG antibodies differed between sarcoidosis patients and healthy controls, as well as between LS and non-LS. Non-LS patients presented with the highest ratio of agalactosylated-to-digalactosylated IgG, reflecting a more pro-inflammatory antibody repertoire, while LS patients more closely resembled the healthy control group [123]. Intriguingly, in LS patients, disease recurrence years after complete clinical remission has only been observed in HLA-DRB1*03⁺ individuals [41], pointing to both a superior capacity for antigen clearance, but also possibly development of long-lived (self)-memory responses in these patients, such as have been demonstrated in experimental models of SLE [124, 125].

Vimentin is a potential autoantigenic target of T- and B-cell inflammation in the lung

In 2015, a molecular three-dimensional model of the TCR $V\alpha 2.3/V\beta 22$ -HLA-DRB1*03 complex was generated based on TCR sequencing data. This revealed the ideal fit of a peptide derived from the C-terminal end of cytoskeletal protein vimentin into the peptide-binding cleft, with connecting points towards all four HLA binding pockets, as well as to the CDR3 loop of the $V\beta 22$ chain, thus implicating vimentin as a potential autoantigen in sarcoidosis [92]. The same peptide has previously been isolated from HLA-DR molecules on alveolar macrophages from sarcoidosis patients [11, 69], and shown potential to stimulate $IFN-\gamma$ production by T-cells [12]. In addition, vimentin-specific antibodies were identified in BALF of sarcoidosis patients and particularly HLA-DRB1*03⁺ individuals, with a switch from vimentin N-terminal specificity in healthy individuals to C-terminal anti-vimentin antibodies (AVAs) in HLA-DRB1*03⁺ sarcoidosis patients [118]. Moreover, the percentage of $V\alpha 2.3^+V\beta 22^+ CD4^+$ T-cells in BALF correlated with specific AVA production [118], supporting the likely recognition of this peptide by $V\alpha 2.3^+V\beta 22^+ CD4^+$ T-cells when presented on HLA-DRB1*03, as demonstrated by molecular modelling [92]. Overlapping or partially overlapping T- and B-cell epitopes have been identified, e.g. in coeliac disease [126], indicative of ongoing T-/B-cell interaction, such as could be observed as marked T- and B-cell co-localisation and proliferation in the inflamed sarcoid tissue [118]. Efficient antigen processing and presentation by B-cells may contribute to activation and differentiation of T-cells within and surrounding the granuloma, while T-cell help to B-cells in turn can drive somatic hypermutation and affinity maturation, ultimately resulting in the development of memory cells (figure 2).

In the context of sarcoidosis, it remains to be determined whether vimentin is a cause or effect of inflammation. Vimentin is known to be secreted from activated macrophages [127], which are abundant in the granulomatous environment, and mass spectrometry analysis verified that vimentin is readily available for uptake and processing in the lung [118]. Its release could either result from cell damage by

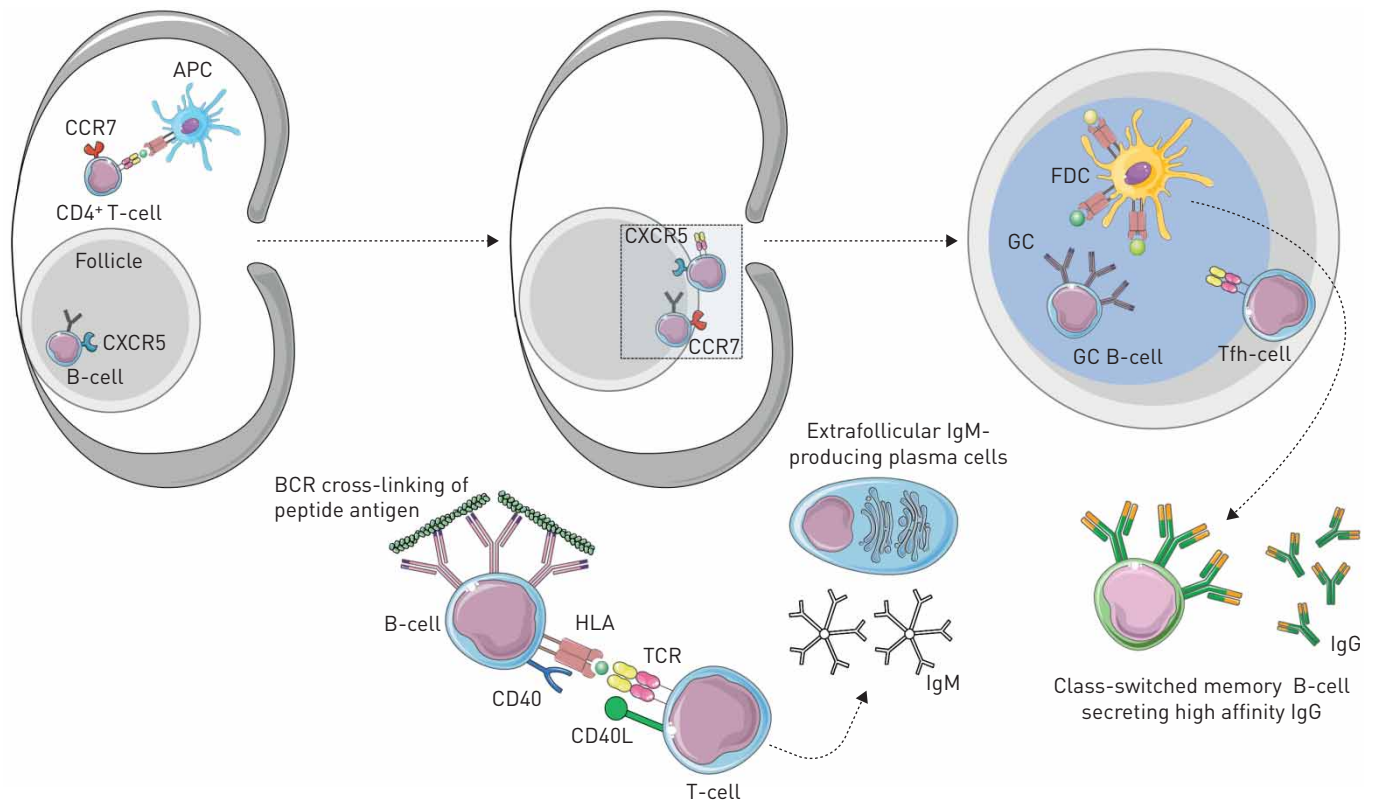


FIGURE 2 T-/B-cell interaction. Graphic representation of the initial stages of T-/B-cell interaction in secondary lymphoid organs, depicting the opposing switch in expression of chemokine receptors CXCR5 and CCR7 that allows for synchronised migration to the follicular border, subsequent interaction of T- and B-cells activated by the same antigen and differentiation of B-cells into antibody-producing plasma cells. Continuous antigen presentation by follicular dendritic cells (FDCs) and survival signals provided by follicular T-helper (Tfh) cells are central to the germinal centre (GC) reaction and generation of class-switched, high-affinity B-cells. In sarcoidosis, it is suggested that such B-cells, activated by a C-terminal vimentin epitope and receiving help from T-cell receptor (TCR)-specific CD4⁺ T-cells recognising a C-terminal vimentin peptide, will then continue to produce antigen-specific IgG and IgA directed to the vimentin C-terminal, and possibly other structurally similar antigenic targets. APC: antigen-presenting cell; BCR: B-cell receptor; HLA: human leukocyte antigen.

intrusion of an external factor, or as secondary reaction to a triggering event driving granuloma formation and macrophage differentiation. In favour of vimentin being a primary antigen is the identification of vimentin as a component of the Kveim reagent by EBERHARDT *et al.* [68], and the demonstrated capacity of this vimentin to promote IFN- γ production by T-cells. In contrast, reports of anti-vimentin reactivity in other (auto)inflammatory conditions, such as in lupus nephritis [74], RA [75] and IPF [128], suggest that AVA reactivity is not disease-specific, but rather a sign of tissue autoimmunity, and possibly related to disease severity. This was exemplified by a weak, but detectable association between reduced lung function and higher AVA titres [118], similar to that observed in IPF [128] and with kidney pathology in SLE [74]. Alternatively, it is possible that anti-vimentin responses are a result of molecular mimicry following infection or exposure to environmental or occupational antigens. Further studies are required to elucidate the true role of vimentin in sarcoidosis pathogenesis, but these data strongly suggest its involvement to be central to both T- and B-cell responses.

Functionally heterogeneous $V\alpha 2.3^+V\beta 22^+ CD4^+$ T-cells are key mediators of disease outcome

The expansion of $V\alpha 2.3^+V\beta 22^+ CD4^+$ T-cells specifically in HLA-DRB1*03⁺ patients [92, 129] implicates these cells as central to the process of antigen recognition. Previous studies on $V\alpha 2.3^+$ T-cells, prior to the discovery of joint expression with $V\beta 22$, have clearly demonstrated that this expansion, which ranges up to 10-fold during active disease, unfailingly normalises after clinical recovery [89], which is consistent with an antigen-specific response that ebbs out following antigen clearance. With high expression of CD69, T-bet, ROR γ T, CXCR3, CCR6, but not FoxP3 and only low levels of CD27, this population also demonstrates an advanced state of differentiation [19, 92]. Interestingly, a less dramatic, but significant, expansion of $V\alpha 2.3^+ CD4^+$ T-cells was observed in patients negative for HLA-DRB1*03, but positive for HLA-DRB3*01. These two HLA molecules are similar in structure and function, and able to present a highly similar peptide antigen repertoire [130]. Accordingly, molecular modelling visualised the connection between TCR and HLA-DRB1*03 as primarily mediated by the $V\alpha 2.3$ segment, while $V\beta 22$ mainly interacted with the HLA-bound peptide, but also with $V\alpha 2.3$ [92].

However, as carriage of *HLA-DRB1*03* is most frequent in LS patients, who have a favourable prognosis, and LS is now proposed to be an autoimmune condition, the intriguing, and seemingly contradictory, question remains as to how autoreactive cells can contribute to disease resolution. One hypothesis is that with an intracellular antigen such as vimentin, antigen-specific cells might aid in its clearance from the extracellular environment, where its components may drive unwanted inflammatory reactions. Differentiated $CD4^+$ T-cells with the capacity to produce a broad range of cytokines and orchestrate a coordinated immune response could thereby cause an initially more intense reaction, represented by the acute symptomatic onset observed in LS, but also force resolution of granulomas and thereby disease. Along these lines, the T-/B-cell interaction implicated especially in HLA-DRB1*03⁺ patients would further suggest that AVAs may act to opsonise or neutralise extracellular vimentin, possibly participating in its removal. Consequently, in the absence of HLA-DRB1*03 and $V\alpha 2.3^+V\beta 22^+$ T-cells, as in most non-LS patients, neither clearance nor immune regulation would be as efficient, resulting in prolonged IFN- γ production, less-specific humoral responses, unrestricted cytotoxic $CD8^+$ T-cell activity and eventually, due to lack of regulatory mechanisms, irreversible tissue damage that may progress to pulmonary fibrosis (figure 3). As vimentin is a vital component of mesenchymal cells, it is also intriguing to speculate on the role of fibroblasts and their contribution to the granulomatous environment and in resolving *versus* chronic disease. Excessive fibroblast activation and remodelling within the granuloma may contribute to driving a secondary autoimmune reaction, where less efficient clearance of free vimentin from the extracellular environment and other factors such as CD44 upregulation propagates a chronic pro-inflammatory response, particularly in HLA-DRB1*03⁻ patients. Notably, a recent publication suggested involvement of PD-1 signalling in fibrotic development [131], inferring that the disrupted regulatory pathways observed in sarcoidosis may over time have far-reaching consequences if inflammation is allowed to persist.

Concluding remarks and future perspectives

A series of consecutive studies spanning several years delineate a critical role for TCR-specific $V\alpha 2.3^+V\beta 22^+ CD4^+$ T-cells in antigen recognition and possibly clearance, resulting in an active, but transient, inflammatory response. The high differentiation state of these cells, exemplified by expression of multiple transcription factors, chemokine receptors, activation and regulatory markers, coupled with the remarkable degree of T-cell clonality observed within and between patients positive for the *HLA-DRB1*03* allele, are all strongly indicative of specific antigen exposure. Moreover, correlation of percentage of $V\alpha 2.3^+V\beta 22^+$ T-cells with titres of specific antibodies suggest interaction between $CD4^+$ T-cells and B-cells in the inflamed lung, with potential implications for improved prognostic assessment. While this review

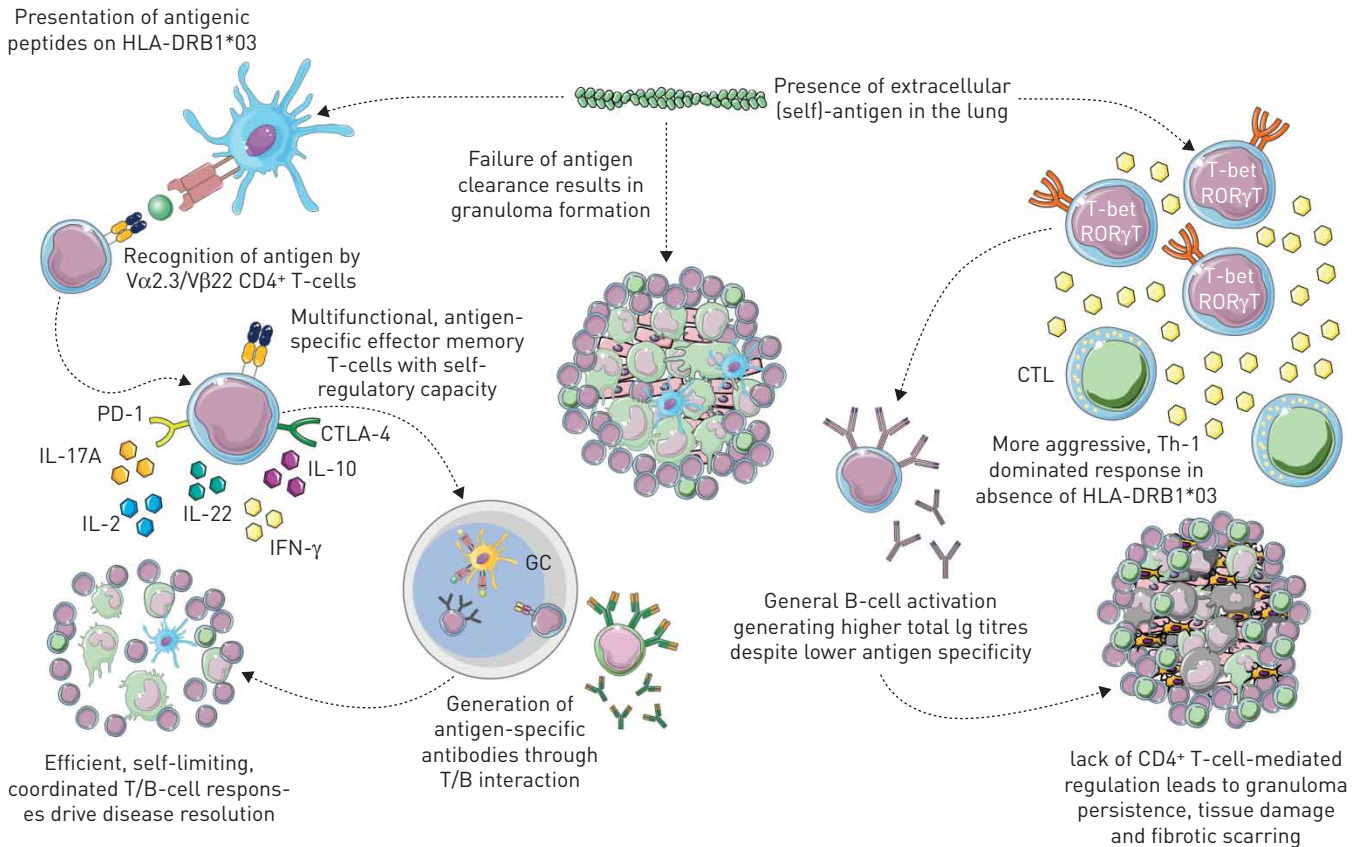


FIGURE 3 Proposed mechanism of disease in T-bet/ROR γ T-balanced (LS) and T-bet-dominated (non-LS) sarcoidosis. Graphic summary of the proposed immunological mechanisms underlying LS and non-LS. The presence of a still unknown (self)-antigen in the pulmonary compartment triggers recognition and uptake by alveolar macrophages and dendritic cells. In both disease forms, inability to completely clear the antigenic source results in its encapsulation and formation of characteristic granulomas. In LS, and particularly human leukocyte antigen (HLA)-DRB1*03⁺ patients, T-cell receptor (TCR)-specific CD4⁺ T-cells are able to recognise peptide antigens and respond with a broad range of cytokine mediators. At the same time, these differentiated effector cells, expressing both T-bet and ROR γ T, appear able to restrict immune activity, as noted by maintained CD4⁺ T-cell expression of regulatory receptors CTLA-4 and PD-1. CD4⁺ T-cells in HLA-DRB1*03⁺ patients are further believed to interact with B-cells, giving rise to antigen-specific antibodies. Ultimately, an efficient antigen-driven T/B-cell response contributes to spontaneous dissolution of granulomas and resolution of disease. In contrast, non-LS CD4⁺ T-cells demonstrate a more aggressive, T-helper (Th)-1-dominant phenotype primarily expressing T-bet and producing high levels of interferon (IFN)- γ . Moreover, higher expression of adhesion marker CD44 may contribute to granuloma persistence, while lack of regulatory capacity in CD4⁺ T-cells allows for unrestrained cytotoxic T-cell (CTL) proliferation, resulting in tissue damage. B-cell activation occurs in absence of germinal centre (GC) formation, resulting in higher total Ig concentration, but a lower degree of antigen specificity. Together, these processes of potent but less antigen-targeted lymphocytic activity produce a state of chronic inflammation and tissue disruption, with a risk of developing permanent fibrotic scarring.

has focused particularly on vimentin as a candidate target of both T-cells and B-cells in the lung, the involvement of vimentin in several other autoimmune diseases questions whether this is an occurrence specific for sarcoidosis, or common for tissue manifestations of several chronic inflammatory conditions. While the triggering incident, together with genetic background, are decisive for the emergence of a certain disease phenotype, the studies highlighted here also indicate that the order of events implicated in sarcoid pathogenesis, such as antigen recognition by T-cells, vimentin release, loss of regulatory balance and development of humoral responses, among others, may differ between patients, who will thus have different immunological prerequisites for management of the insult. Importantly, as indicated by the studies covered in this review, the traditional depiction of sarcoidosis as a solely CD4⁺ T-cell- and mainly Th-1-driven disease is now under dispute, and not only should regulatory features of T-cells be further explored, but also the role of B-cells, CD8⁺ T-cells, DCs and other tissue-resident immune cells with potential impact on antigen processing, presentation and downstream effector functions.

Future studies should also aim for more stringent and consistent subclassification of patients, not merely by LS and non-LS, which are readily distinguished based on characteristic symptoms but still frequently grouped together, but also by HLA type, sex, presence of extrapulmonary symptoms and organ involvement. This would be of most benefit to the larger group of non-LS patients, where prognosis is poorer and even less is known with regards to antigenic triggers. The disparity in symptomatic

presentation between different ethnic groups, HLA backgrounds or sexes indicate sarcoidosis to be not a single disease entity, but rather a spectrum of disorders, and proper classification of these conditions is essential for correct clinical management as well as further research. In this review, several examples show LS and non-LS to harbour similar, but significantly different immunological traits, which are likely to be instrumental in disease progression and outcome. The seemingly less aggressive, more antigen-driven, and more self-limiting immune response in LS relates well to the active inflammation and often spontaneous resolution observed in this patient group. Ultimately, the long-term goal of both antigen discovery and detailed immunological studies should be to improve diagnostic and especially prognostic procedures, create more precise and individualised therapeutic options, and if possible, to develop preventive measures. For this reason, understanding not only the cause of disease, which may well differ between LS and non-LS, but also the immunological response to this initial pathogenic event is critical. Data gathered from human samples offer a mere snapshot of a complex disease course, and as exemplified by an increasing number of studies, the cellular composition in the lung markedly differs from that of peripheral blood, wherewith sampling from the periphery is insufficient for proper assessment of the present immunological state of the patient. Despite the invasive nature of bronchoscopy, repeated investigations during follow-up and after clinical remission would be highly desirable from a research perspective, and provide information that could prove essential in unravelling the mechanisms that govern disease resolution and progression. Furthermore, systematic exploration into the affected tissue should be prioritised in order to gain insight into differences in granuloma formation and persistence between clinical phenotypes, which may prove useful in the development of new treatments. Despite being highly anticipated from a clinical perspective, proper understanding of vital immune reactions in the target organ is likely to be required prior to successful identification of any diagnostic and/or prognostic marker readily available through blood sampling.

As Oscar Wilde once wrote, “The truth is rarely pure and never simple”, and it is indeed unlikely to be the case in the unresolved mystery that is sarcoid pathogenesis; perhaps we have only begun to scratch at the surface of a far larger group of related, but not identical, diseases. Hopefully, however, ongoing research is helping us to find the missing pieces of the sarcoidosis puzzle.

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