Immunology of sarcoidosis: old companions, new relationships

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Purpose of review
The immune determinants of granuloma formation and disease progression in sarcoidosis have not been completely disclosed, and the role of both innate and the adaptive immunity is still under investigation.

Recent findings
M2 macrophage polarization, previously thought to be a specific feature of a progressing and fibrosing disease, has been related to the initial steps of granuloma formation both in animal and in-vitro models. The dysregulation of specific metabolic pathways and autophagy has been associated with disease activity and progression. T cells have been reported to be strongly influenced by a macrophage-driven microenvironment and more dangerous when acquiring hybrid phenotypes (e.g. Th17.1) or even becoming anergic, leading to disease chronicization. Locally released serum amyloid A was suggested to induce a more pro-inflammatory Th17 transcription program. The possible role of in-situ humoral immunity and bone marrow-derived mesenchymal stromal cells has also been highlighted.

Summary
Evidence points at microenvironment and cell functional features rather than cell polarization or differentiation as determinants of pathogenesis. In terms of therapeutic implications, future advances will rely on molecular disease profiling, aiming at personalized and combined therapeutic approaches.

Keywords
adaptive immunity, autophagy, granuloma, impaired antigen clearance, innate immunity

INTRODUCTION

Sarcoidosis is a multisystemic granulomatous disease of unknown etiology occurring in individuals of any age, sex, and ethnicity. Both genetic and environmental background may influence disease incidence, phenotype, and severity. Lung involvement occurs in more than 90% of diagnosed patients but the clinical phenotype and disease behavior may be highly variable [1–3].

The pathological hallmark of sarcoidosis is the epithelioid noncaseating granuloma, a nondisease-specific entity arising from the recruitment of monocytes/macrophages organized in multinucleated giant cells, T and B lymphocytes, fibroblasts, and other matrix-associated cells. Thus, both adaptive and innate branches of the immune system appear to be strongly involved in the granulomatous process [1,4]. The determinants of disease pathogenesis, extensively investigated for decades, however, remain uncertain, and immunological heterogeneity has been highlighted [5,6].

Considerable progress has been made in innate and adaptive immune profiling of sarcoidosis in recent year, and it has now been proposed that sarcoidosis is an autoimmune spectrum disorder [6]. Recently described immunologic hallmarks include:

(1) enhanced local expression of Th1 and Th17 cytokines and chemokines [7,8]
(2) Distinct lung CD4+ T-cell patterns in Lofgren's syndrome and non-Lofgren's syndrome sarcoidosis [9]
(3) oligoclonal expansion of CD4+ T cells within the lungs of HLA-DRB1*03 sarcoidosis patients,

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KEY POINTS

- M2 macrophages have been shown to be strongly involved in disease progression, but in-vitro and in-vivo models also highlight their presence during initial granuloma formation.
- Metabolic adaptation to the environmental and inflammatory milieu deeply impacts on autophagy regulation, leading to impaired antigen clearance and promoting granuloma persistence/progression.
- The idea of a specific T-helper cell differentiation responsible for granuloma initiation and/or chronicization might be put aside, in light of the increasing evidence of high plasticity of Th cells (including Th17).
- Cell functional features like T-cell exhaustion might be as relevant and cytokine secretion profile in driving sarcoidosis progression.
- Macrophages–T cells interplay and their relationships with the surrounding microenvironment, rather than single mediators, should be the main targets of disease profiling, driving future personalized therapeutic approaches.

consistent with an antigen-induced disease process [10,11]
(4) dysfunctional Treg cells [12,13]
(5) peripheral T-cell exhaustion [14,15]
(6) anergic dendritic cells responses [16,17]
(7) dysregulated Toll-like receptor (TLR) signalling in highly activated alveolar macrophages (AMs), [18–22] and
(8) granulomatous response shaping by metabolic and environmental triggers [4,20]

Together these findings suggest that a strong but noneffective innate immune reaction to an unknown antigen(s), possibly favoured by specific HLA genes and conditioned by the environmental-driven metabolic adaptation, leads to chronic adaptive immune stimulation, which is unable to clear the triggering factor(s). The failure to eliminate the antigen(s), is considered the main event resulting in granuloma formation in sarcoidosis [5]. Similarities and differences have been highlighted with other granulomatous diseases, including mycobacterial infections; in mycobacterial infection, the clearance of intracellular pathogens represent a great challenge for the immune system. One of the key mechanisms for elimination of Mycobacteria (including *Mycobacterium tuberculosis*) by the innate immune system is represented by autophagy, a process designed to promote cellular senescence and cell-surface antigen presentation, protecting against genome instability and preventing necrosis and its consequences [23].

INNATE OR ADAPTIVE IMMUNITY? LESSONS FROM REAL LIFE

Recent findings have advanced understanding of both innate and the adaptive immunity in sarcoidosis, suggesting that integrating mechanisms acting in a nondichotomic fashion are determinants of disease progression (and putative therapeutic targets). We will, therefore, discuss recent evidence as a fluent narration rather than a step by step process, focusing on the interplay and not on peculiar role of single cells or mediators.

Traditionally, Sarcoidosis has been defined as a Th1 and, more recently, as a Th1/Th17-mediated disorder [7]. Undoubtedly CD4+ T cells have been investigated as the key characters in the play. More recently, researchers have been focusing not on T-cell subset-specific determinants, but on plasticity, exhaustion/senescence, and other functional features that may not be simply described by dual distinctions (e.g. Treg and Th17 cells) or univocal definition (e.g. Th17.1 cells). Moreover, by using different animal models, it has been shown that well developed epithelioid granulomas can still be generated in the complete absence of adaptive immunity: severe combined immunodeficiency (SCID) mice and Zebrafish embryos (lacking T cells in the first days of life) can develop defined and organized granulomatous lesions when infected with Mycobacterium strains [24–27]. Even in humans, the occurrence of granulomatous disease in patients with defects in adaptive immunity, like common variable immune deficiency (CVID) [28] or RAG deficiency [29], supports a key contribution by innate immunity in driving the critical steps to form a mature granuloma. It has also been shown that primary monocytes from peripheral blood of CVID patients present an elevated in-vitro tendency to fuse and form giant cells [30]. On the other hand, no granulomatous diseases have been reported in X-linked agammaglobulinemia patients, where a Bruton Tyrosine Kinase defect prevent the development of mature B cells [31].

Although the morphological changes of the different maturation stages of granuloma have been well described, the characterization of the critical mechanisms involved in the granulomatous immune response at molecular level have only recently begun to be dissected. Macrophages, in particular, seem to be central in this process as they form the core of granuloma since the beginning. Macrophages (both tissue resident and monocytes-derived) are involved in tissue homeostasis and

repair but they also carry out an essential guarding function towards environmental warning cues [32]. Thanks to their broad pathogen recognition receptors (PRRs) repertoire (e.g. TLRs, scavenger receptors, and NOD-like receptors) and their phagocytic activity, they can detect danger signals coming from infectious pathogens, tissue injury, foreign substances or dead/dying cells, become active, and following interaction with T cells, aggregate to assemble granuloma. Depending on the eliciting antigen and the surrounding environmental trigger, such cellular aggregate may resolve or not. In case of persistent stimulation, macrophages first undergo to epithelioid differentiation and subsequently develop multinuclear giant cells fusion while recruiting other cellular partners as T and B lymphocytes, granulocytes, monocytes, dendritic cells, and fibroblasts [33]. It is precisely about dissecting the initial steps of granuloma formation and characterizing the immune-metabolic signature of macrophages that significant advances have been recently provided in understanding sarcoidosis. It is also worth highlighting that most findings in humans are from peripheral blood, lymph nodes or lung tissue/BALF cells, and so potentially may not represent the disease mechanisms occurring in other organs involved in multisystemic sarcoidosis.

Two follows one, or not?

It has long been proposed in sarcoidosis that the local pro-inflammatory milieu generates an initial M1 macrophages polarization, as M1 macrophages are typically induced by sarcoidosis signature inflammatory cytokines like interferon-gamma (IFN-γ) and tumor necrosis factor alfa (TNF-a), in combination with TLR engagement [31]. This leads to NF-κB signalling and the activation of several interferon-regulatory factors (IRFs) [32], which are signature genes of the M1 phenotype. In 2011, however, M2 polarization of macrophages and multinucleated giant cells from granulomas of patients with systemic neuromuscular sarcoidosis was identified. Furthermore, these alternatively activated M2 macrophages were shown to express high levels of CCL18, to induce myofibrosis and to be significantly insensitive to conversion by Th1 cytokines to M1 state [34]. More recently, tissue M2 macrophage activation in skin biopsies from patients with systemic sarcoidosis [35▪] and in lung and lymph node samples from pulmonary sarcoidosis was identified by immunohistochemistry [36]. Although all these findings may come from chronic sarcoidosis patients, thus potentially not being representative of primary events in granulomatous inflammation, a recently described in-vitro sarcoid granuloma model led to the identification of IL-13-regulated M2 macrophage polarization during granuloma genesis [37▪▪]. Accordingly, previous data from animal model of spontaneous granuloma formation revealed M2-like macrophage phenotype within granulomas in lung, liver, and lymph nodes [4].

To explain these apparent contradictory results, it is important to consider that, at present, most of the reported studies rely on the 'dual nature' of macrophages, defined as classical activated M1 and alternative activated M2. Accordingly, sarcoidosis pathogenesis has been thought to transit from an initial prominently M1 phase (promoting granuloma formation) to a prominently M2 phase during disease progression (promoting fibrosis) [5].

However, through in-vivo and human studies, it is increasingly recognized that macrophage biology in far more complex, with M1/M2 polarization used *in vitro* as a simplified model to approximate the plasticity and the broad spectrum of responses, which characterize this cell type *in vivo* [38,39]. A number of recent studies may serve as paradigmatic explanations. Peroxisome proliferator-activated receptor gamma (PPAR-γ), whose deficiency was observed in alveolar macrophages from pulmonary sarcoidosis patients, is known to promote M2 polarization in response to different stimuli, including physical exercise [40,41]. By using a murine model of conditional deletion of PPAR-γ in macrophages, however, Silveira *et al*. [42▪] demonstrated that moderate aerobic training was still able to induce a M2 functional macrophage pattern in spite of a M1 phenotypic surface profile in PPAR-γ KO mice. In addition, TLR2 is capable of promoting both M1 and M2 immune responses, depending on the conditions [43] and even the environmental nutrient supply (e.g. arginine) is a relevant determinant of macrophage activation fate [44▪▪].

Metabolism matters!

As mentioned above regarding PPAR-γ and arginine, a topic that has gained increasing momentum in recent years is a deeper understanding of how macrophage metabolic pathways are involved in sarcoid granuloma formation and disease progression. Indeed, metabolic triggers including serum starvation, amino acids and growth factor deprivation, hypoxia, and exposure to chemicals and toxins may all induce intracellular signals leading to an upregulation of autophagy. Mammalian target of rapamicyn complex 1 (mTORC1) is a well known coordinator of anabolic and catabolic cell processes, acting as inhibitor of autophagy [45]. One of the main achievements in understanding the role of metabolic pathways in sarcoidosis was the demonstration of an active

involvement of the mTOR pathway in granuloma formation in a murine model, and notably, chronic mTORC1 activation has been identified as a feature of clinical disease progression in human sarcoidosis [4]. Authors selectively inactivated tuberous sclerosis complex 2 (TSC2) in myeloid cells, which resulted in chronic mTORC1 activation and a murine phenotype characterized by spontaneous noncaseating granulomatous aggregates in lung, liver, and lymph nodes. Sustained activation of mTORC1 was sufficient to initiate and maintain granulomas and bone marrow transplantation from TSC2-deleted mice recapitulated granuloma formation in wild type recipients. Moreover, a 3-week treatment with the mTORC1 inhibitor everolimus completely resolved granulomas in TSC-2-deleted mice. Molecularly, authors found that mTORC1 inhibited apoptosis, induced M2-like macrophage proliferation, and metabolic reprogramming toward increased glycolysis and mitochondrial respiration by inducing the expression of CDK4. In the same study, lung samples from sarcoidosis patients with self-limiting and progressive disease were analyzed and gene set enrichment analysis showed that, like in the murine model, the hallmark genes for the mTORC1 pathway were significantly enriched in the subset of progressive patients, as well as E2F targets genes and a glycolysis gene set, thus suggesting that macrophage proliferation and glycolysis might contribute to human disease progression or resolution.

Furthermore, clinical relevance of mTORC1 has since been substantiated by its recent identification in CD68 macrophages from cutaneous sarcoidosis patient who had a complete disease remission following treatment with the Janus kinase 1 (JAK1) inhibitor tofacitinib [46▪], as well as by whole-exome sequencing and pathogenicity network analysis of familial cases of non-Löfgren syndrome sarcoidosis [47]. As mentioned above, mTORC1 is a negative regulator of autophagy, whereas JAK-1 inhibition activates autophagy; this further underscores the contribution of aberrant autophagy processes to the chronic sarcoid granulomatous inflammation (Fig. 1c) [48▪].

Moving forward, sustained activation of mTORC1 pathways has been shown to promote glycolytic metabolism through the production of the downstream key glycolytic regulator hypoxia inducible Factor-1a (HIF-1α), irrespective of oxygen concentrations [49,50]. HIF-1a is an oxygen-sensitive transcription factor acting as a key transcriptional regulator of hypoxia-associated genes. These genes are in turn involved in metabolism, cell differentiation, proliferation, and angiogenesis and are functional to adapt tissues to a decreased availability of O_2 [51].

As a further confirmation of the central role of metabolic switch towards glycolysis during granulomatous response, a recent study demonstrated elevated protein levels of HIF-1α, as well as HIF-1α signaling pathway components, such as HIF-1β, HIF-2α, and p300, in alveolar macrophages from sarcoidosis patients cultured under normoxic conditions [52▪]. Of note, HIF-1α protein expression was found in granulomatous lung tissue from sarcoidosis patients, localized in AMs and multinucleated giant cells [52▪]. As described previously, HIF-1α ultimately promotes pro-inflammatory macrophage functions by activating glycolytic enzymes and stimulating the production of IL-1β [53]. Indeed, Talreja *et al.* [52▪] demonstrated that, in Alveolar Macrophages (AMs) from sarcoidosis patients, increased levels of HIF-1α correlated with elevated levels of IL-1β (Fig. 1c). Furthermore, HIF-1α inhibition by small interfering RNA (siRNA) in patients' Peripheral Blood Mononuclear Cells (PBMCs) significantly diminished the anti-CD3-induced production of IL-1β, IL-17, and IL-6 [52▪]. Thus, the mTORC1-driven HIF-1α overactivity appears to set the ground for the pro-inflammatory milieu observed in sarcoidosis patients. As a consequence, in light of the role of IL-1β and IL-6 in Th17 differentiation of CD4+ T cells, a key population in the pathogenesis of sarcoidosis and in its chronicization, HIF-1a appears to be one of the links between innate and adaptive immunity in the genesis of this complex granulomatous disease.

THE LINKS/THE OTHER PILLARS

IL-17-producing Th17 cells are not a harmful T-cell population by definition. Physiologically they act as a critical regulator of the defense against pathogenic bacteria and fungi at mucosal surfaces while favoring the homeostasis between resident organisms constituting the microbiota [54]. Of note, IL-17A has been shown to induce or prevent autophagy in tuberculosis patients' monocytes, according to the level of dysregulation of MAPK1/3-signaling pathway. IFNγ has also been shown to induce autophagy in this setting, identifying autophagy as an effective mechanism of mycobacterium killing [55]. Dysregulation of MAPK pathway has also been reported in macrophages from BALF of sarcoidosis patients [56].

When implicated in autoimmune diseases, compared with their 'benign' counterpart, pathogenic Th17 cells have been suggested to present a distinct transcriptional program, requiring IL-23 and IL-1β, together with IL-6 or IL-21. It has recently been demonstrated that the other key cytokine for Th17 differentiation, TGF-β, may be replaced by the acute phase response proteins Serum Amyloid A 1 and 2, within pro-inflammatory microenvironments. SAAs can in this case engage right that distinct signaling pathway leading to a more pro-

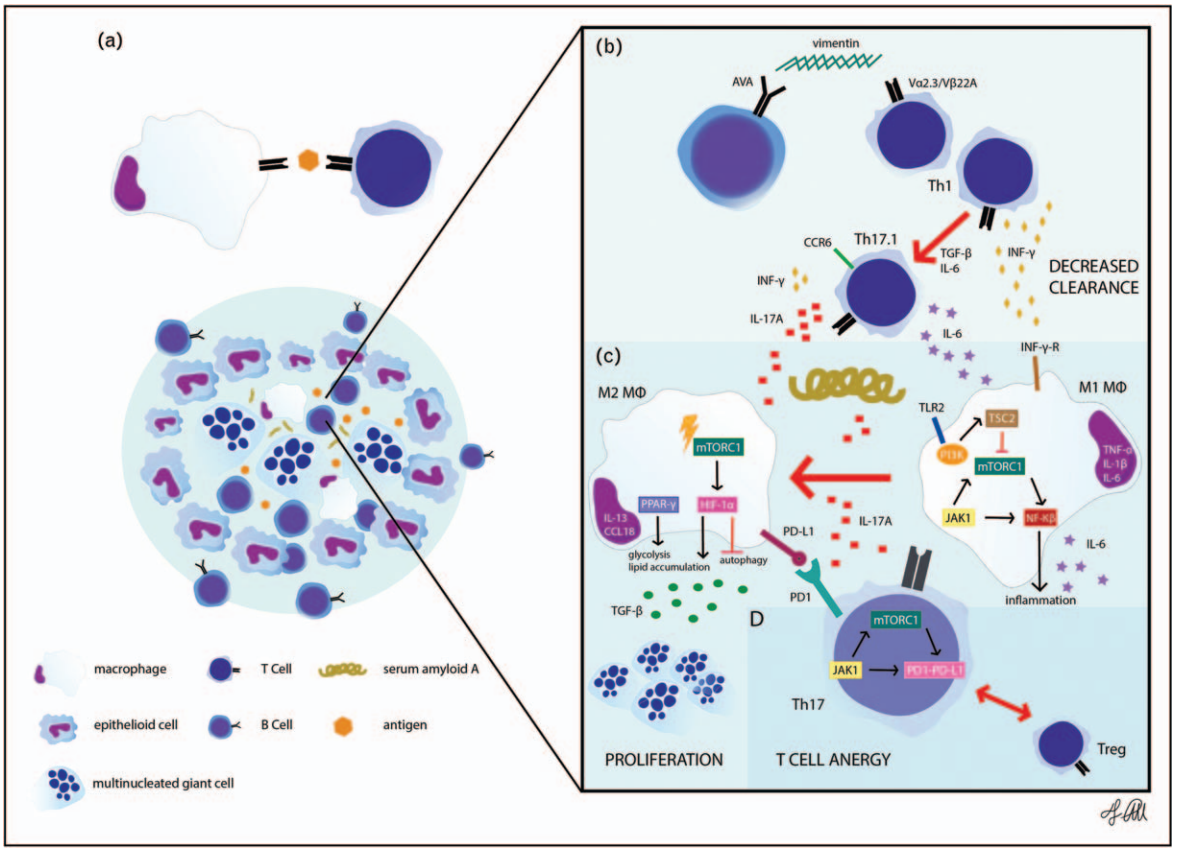

FIGURE 1. Innate and adaptive immunity interplay in the initiation and persistence of sarcoidosis granuloma. (a) Putative antigen presentation to CD4+ T cells might be the initial step that induces the formation of a granuloma. (b–d) Recently described immunological hallmarks sustaining the formation and impairing the resolution of granuloma in sarcoidosis. These integrating mechanisms may not act as subsequent steps but simultaneously. (b) The enhanced local expression of IL-6 and IL-1β from activated macrophages, may sustain the Th17 differentiation of CD4+ T cells, a key population in the pathogenesis of sarcoidosis. In this pro-inflammatory microenvironment, different cell types (including macrophages) secrete SAA contributing to a Th17 cell shift through a specific, pro-inflammatory, transcriptional program and leading to a further impairment in antigen clearance and to disease chronicization. Th17 plasticity is also testified by a particular subset of CCR6+ Th17 cells, named Th17.1, sharing Th1 (IFN-γ production) and Th17 features (IL-17A production). AVAs producing perigranuloma B cells found in inflamed lungs together with CD4+ T cells recognizing a similar epitope through the Vα2.3/Vβ22 TCR sustain the hypothesis of an in situ T-cell-dependent B-cell response. (c) The local pro-inflammatory milieu has been thought to generate an initial M1 polarization of macrophages, induced by sarcoidosis-related inflammatory cytokines, such as IFN-γ and TNF-α, in combination with TLR engagement (e.g. TLR-2) leading to the activation of NF-κβ signaling. However, IL-13 and IL-17A together with hypoxia and amino acid deprivation promote M2 polarization and a metabolic switch towards glycolysis and lipid accumulation (PPAR-γ deficiency). mTORC1 hyperactivation sustains M2 macrophages features that have also been described in models of initial granuloma formation. The chronic mTORC1-driven HIF-1α overactivity leads to aberrant autophagy, resulting in antigen persistence together with epithelioid transformation and subsequent multinuclear giant cells proliferation. Moreover, the JAK/STAT signaling also seems to contribute in impairing antigen clearance, by transducing signals from IL-17 receptor and regulating autophagy in macrophages, as demonstrated by the successful use of the JAK1 inhibitor tofacitinib in cutaneous sarcoidosis. Metabolic dysregulation and autophagy inhibition may promote sarcoidosis progression. (d) T cells present signs of exhaustion, particularly in progressing disease, with increased expression of the receptor PD1. The activation of PD-L1–PD1 axis may promote a tolerogenic microenvironment with M2 macrophage polarization, Th17-like phenotype differentiation, expansion of dysfunctional Treg cells and adaptive immune anergy. All these may contribute to disease chronicization/progression. AVAs, antivimentin antibodies; HIF-1α, hypoxia inducible factor-1a; IFN-γ, interferon-gamma; IL-1β, interleukin-1β; IL-13, interleukin-13; IL-17A, interleukin-17A; IL-6, interleukin-6; JAK1, janus kinase 1; MΦ, macrophage; mTORC1, mammalian target of rapamicyn complex 1; NF-κβ, nuclear factor kappa-light-chain-enhancer of activated B cells; PD1, programmed death 1; PD-L1, programmed death-ligand 1; PI3K, phosphoinositide 3-kinase; PPAR-γ, peroxisome proliferator-activated receptor gamma; SAA, serum amyloid A; TCR, T-cell receptor; TGF-β, transforming growth factor–β; Th, CD4+ T helper; TLR, toll-like receptor; TNF-α, tumor necrosis factor α; Treg, regulatory T cell; TSC, tuberous sclerosis complex 2.

inflammatory program of Th17 differentiation [57▪▪]. Importantly, SAA may be secreted by different tissues and cell types, including macrophages, and it had already been associated to promotion and maintenance of chronic inflammation in sarcoidosis, progressively aggregating within granulomas together with the putative sequestered antigen [20,58]. Thus, a deeper understanding of the mechanisms leading to the SAA-driven 'bad' Th17 phenotype would likely offer further therapeutic options in Th17 mediated diseases.

T-CELL PLASTICITY AND FUNCTIONAL FEATURES

The role of imbalance between Th17 and Treg cells and the expansion of dysfunctional Treg cells in sarcoidosis have been extensively discussed and reviewed, and the plasticity of Th17 cells has become much clearer over the last few years [12,13,59,60].

This plasticity have been further confirmed when starting from the initial concept of a Th1/Th17- mediated disorder, a specific subset of CCR6+ Th17 has been reported as the major source of IFN-γ in bronchoalveolar lavage fluid (BALF) from sarcoidosis patients, compared with the classical $CCR6^-$ Th1 cells [7,61]. This particular subset of CCR6+ Th17 cells, named Th17.1, shares Th1 (IFN-γ-production) and Th17 features. Th17.1 cells have been involved in the pathogenesis of several immune-mediated diseases and their higher proportion in lungs, when compared with mediastinal lymph nodes and peripheral blood, was found to correlate with chronicization of sarcoidosis (Fig. 1b) [62▪]. Thus, Th17.1 cells have been proposed as a possible diagnostic and/or prognostic marker as a well as a putative therapeutic target in patients experiencing disease progression [62▪].

Another functional concept regarding T cell, exhaustion, has been recently strengthened in sarcoidosis by the evidence that CD4+ T cells present signatures of exhaustion particularly in patients with progressing disease [63]. Exhausted T cells are functionally defined by a reduced response to TCR activation in terms of proliferation and cytokine production, while increasing in apoptosis rate and upregulating inhibitory immune receptors [64]. Increased expression of the receptor Programmed Death 1 (PD-1), in particular, when occurring together with the loss of multiple effector function, is a well known marker of T-cell exhaustion [65]. The exhausted CD4+ T cell phenotype has been correlated with immune dysfunction in sarcoidosis, reported as greater at site of disease progression (lungs) than in peripheral blood-derived cells; moreover, disease resolution has been shown to correlate with the reversal of the exhausted cell phenotype. Thus, it has been suggested that exhaustion-related T-cell dysfunction might play a role in the pathogenesis of sarcoidosis (Fig. 1d). Of note, in a similar way, *M. tuberculosis* cell wall glycolipid, mannose-capped lipoarabinomannan (ManLAM) has been shown to induce T-cell anergy as a possible mechanism of antagonism towards antigen clearance [66]. As the persistence of poorly degradable microbial antigens (including mycobacterial) has been associated with sarcoidosis granulomas, it has been reasonably hypothesized that antigen persistence itself may induce T-cell exhaustion that might in turn favor a microenvironment less permissive for antigen clearance, in a sort of vicious circle [63].

Interestingly, recent evidence from cancer immunology suggest that activation of PD-1 axis via PD-ligand-1(PD-L1) promotes a tolerogenic microenvironment with M2 macrophage polarization, T-cell differentiation to a tumor-permissive Th17-like phenotype and adaptive immune anergy (Fig. 1d). Thus, it is plausible that PD-1–PD-L1 might also play a role in the context of sarcoidosis in the cross-talk between T cells and macrophages, possibly impairing antigen clearance [67]. Furthermore, it has been reported that $PD\text{-}1^+CD4+$ T cells from systemic sarcoidosis patients are a source of both transforming growth factor-β (TGF-β) and IL-17A, whose production is mediated by signal transducer and activator of transcription 3 (STAT3). $PD\text{-}1^+CD4+$ T cells from sarcoidosis patients experiencing disease progression also presented increased IL-6 production and RORC expression; all these data support, again, an unbalance towards a Th17 cell phenotype. Co-culture of these $PD\text{-}1^+CD4+$ + T cells has also been demonstrated to induce collagen-1 production by human lung fibroblasts; subsequent PD-1 pathway blockade has been shown to significantly decrease STAT3, RORC, TGF-β, and IL-17A expression, finally leading to a reduction in collagen-1 secretion [68▪]. These findings suggest that targeting the PD-1 pathway might prevent progression and fibrotic evolution of sarcoidosis [68▪]. Surprisingly (or not), anti-PD1 monoclonal antibodies have instead been reported as potential inducers of granulomatous diseases [69,70]. Of note, hampered proliferative capacity of exhausted CD4+ T cells from sarcoidosis patients was shown to be secondary to reduced expression of key mediators of cell cycle progression, including the PI3K/AKT/mTOR pathway; PD-1 blockade induced the recovery of PI3K/AKT/mTOR expression [15]. This suggests that, while mTORC1 hyperactivation in macrophages sustains granuloma formation and promotes chronic granulomatous disease, as described above, reduction of mTOR expression in CD4+ T lymphocyte correlates with cell exhaustion and disease progression. This may explain why PD-1 blockade has been reported both as a

promising treatment in progressing sarcoidosis and as a possible cause of granulomatous disease. The same context-dependent janus bifrons behaviour has been reported for anti-TNFα treatment: able to induce granulomatous disease in nonsarcoidosis patients, while a valuable therapeutic option for the treatment of refractory sarcoidosis [71,72].

T LOVES B AND REVERSE

A further important concept is the interplay between T and B cells, with B cell also implicated in the pathogenesis of sarcoidosis. This might explain anecdotal results reported on the use of anti-CD20 monoclonal antibodies in refractory disease, although definitive data are awaited [73]. In 2013, Kamphuis *et al.* reported a peri-granuloma localization of IgA-producing plasma cells and of numerous B cells, with a significant reduction of CD27+ memory B cells and a significant increase of $CD27^{-}$IgA+ B cells in peripheral blood. These peripheral blood findings were reversed by anti-TNF-α treatment. No impairment in serum immunoglobulin levels and antigen-specific immunoglobulin responses was reported; however, high frequency of somatic hypermutations and increased usage of downstream IgG subclasses were suggestive for prolonged or repetitive antigen responses [74]. More recently, starting from the previous finding that HLA-DRB1∗03^{+} sarcoidosis patients present an in situ expansion of $V\alpha2.3^{+}V\beta22^{+}$ T cells that recognize vimentin peptides in the context of that specific HLA, Kinloch *et al.* [75▪] reported a selective in situ humoral immune response to the vimentin C-terminus, in the lung of HLA-DRB1∗03+ sarcoidosis patients. The authors found that Vimentin was more abundant in the more cellular and inflamed lung, containing both CD4+ and CD20+ cells with features of tertiary lymphoid neogenesis. Antivimentin antibodies (AVA) against the vimentin C-terminus were more frequent in BALF of HLA-DRB1∗03+ patients and AVA titers in BALF correlated with the percentage of BALF CD4+ T cells expressing the Vα2.3/Vβ22 TCR (Fig. 1b) [75▪]. Whilst requiring further investigation, these findings support the concept of an in situ adaptive immune response, consisting in a T-cell dependent B-cell response to a defined antigen, occurring in the lung of sarcoidosis patients.

WHAT ABOUT MESENCHYMAL CELLS?

Finally, we have not considered mesenchymal cells involved in granuloma formation and the relationship between inflammation and fibrosis. This will be discussed in another article of the present series. However, it has recently been demonstrated that bone marrow-derived mesenchymal stromal cells (MSCs) are able to modulate the inflammatory character of alveolar macrophages from sarcoidosis patients [76▪]. In particular, it has been shown that in-vitro co-culture of BALF-derived monocytes/macrophages from sarcoidosis patients with MSCs may induce a shift from 'pro-inflammatory' M1 to 'anti-inflammatory' M2 polarization, thus suggesting a possible therapeutic application of MSCs in sarcoidosis treatment [76▪]. First of all, these data confirm that mesenchymal cells may indeed be involved in the modulation of granulomatous microenvironment, adding a further character to this complex pathogenic mechanism. On the other hand, in light of what discussed above about the difficult in-vivo application of strict in-vitro paradigms of alternative (and mutually exclusive) polarization of immune cells, and considering the emerging role of M2 macrophages in the pathogenesis of chronic sarcoidosis, once more we need to think that in-vivo results of an in-vitro effective approach may not be so easily applicable to real-life sarcoid granulomatous inflammation, so highlighting the importance of developing models, which effectively recapitulate human disease.

CONCLUSION

In conclusion, the increasing understanding of cell-specific roles in sarcoidosis pathogenesis challenges the integration of all findings into a unifying synthesis. The evidence suggests that we are facing a disease (better, a spectrum of disorders) with a fascinating immunologic background where no single pathway or cell type presents a univocal behavior in granuloma initiation and progression. Things may vary according to clinical phenotype, previous and ongoing treatment, genetic background, and environmental factors, and we cannot exclude that the involved organ-specific microenvironment and its metabolic consequences may also affect cells behavior and disease chronicization/progression. It is, therefore, plausible that we should also consider cell functional features rather than simply polarization or differentiation. Thus, in terms of therapeutic implications, we may not expect to develop univocal strategies working for all patients, all organ involvements and all disease stages, but we will need to think in terms of molecular disease profiling, aiming at personalized and often combined therapeutic approaches.

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Conflicts of interest

There are no conflicts of interest.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

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