Mechanism of granuloma formation in sarcoidosis

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Purpose of review
The formation of noncaseating granuloma is a hallmark of pulmonary sarcoidosis. This review summarizes recent progress made to explain the cellular dynamics within the granuloma structure that may considerably differ between the two clinically distinct variants, that is, acute and chronic sarcoidosis.

Recent findings
Compelling evidence exists that in acute but not chronic sarcoidosis CD4\textsuperscript{+} T lymphocytes specifically recognizing the auto-antigen vimentin on human leukocyte antigen-DR3 molecules accumulate in sarcoid granuloma. These so-called TH\textsubscript{17.1} cells produce high amounts of the TH\textsubscript{17}-related cytokines interleukin-17 (IL-17) and IL-22 in addition to interferon-\gamma. Moreover, regulatory T cells from patients with acute sarcoidosis are ICOS\textsubscript{high}, providing a mechanistic link to the comparably high concentration of IL-10 exclusively found in the airways of these patients. Next to obvious differences in T effector cell and T\textsubscript{reg} subsets, alveolar macrophages harbor a functional mitochondrial system in acute sarcoidosis patients, while this system is impaired in patients with chronic disease.

Summary
We provide a comprehensive update on the cellular components and their functional implications in sarcoid granuloma formation, with special emphasis on the specific characteristics of granuloma in acute versus chronic sarcoidosis. Moreover, the specific antigens thought to be involved in both forms of the disease are discussed.

Keywords
granuloma, Löfgren syndrome, non-Löfgren syndrome, sarcoidosis

INTRODUCTION
Sarcoidosis is a heterogeneous, systemic disease characterized by noncaseating granulomatous inflammation affecting many organs. Almost 90\% of the patients develop pulmonary sarcoidosis with the lung and mediastinal lymph nodes representing the most common sites affected by a sustained and progressive inflammation [1]. The prevalence of sarcoidosis varies between different ethnic groups with the highest prevalence among Scandinavians [2] and African Americans [3], while only rarely reported among Arabians [4] and Asians [5]. Sarcoidosis occurs in younger adults, typically peaking between 20 and 39 years of age. The disease has a bimodal distribution pattern, with the second disease incidence peaking between 60 and 69 years of age, especially among women [6]. Although the exact cause of sarcoidosis remains obscure, a number of putative etiopathogenic factors have been postulated. Among them, the presence of mycobacteria [7,8] or propionibacteria DNA [9,10] has very recently been identified in a considerable proportion of clinical isolates suggesting bacterial infections as either the cause or an essential cofactor for sarcoidosis pathogenesis. Once manifested, the hallmarks of sarcoidosis are aberrant immune responses to so far ill-defined antigenic triggers as well as a distinct granuloma formation. A clinical variant of sarcoidosis, called Löfgren syndrome, usually exhibits acute and distinct clinical entity that is characterized by the triad of erythema nodosum, hilar lymphadenopathy, and arthritis. This trait of sarcoidosis has an excellent prognosis with a high remittance rate within the first 2 years following disease onset. However, a significant proportion of sarcoidosis patients who do not develop Löfgren syndrome (non-Löfgren syndrome) exhibits an
Both autoantigen and pathogen triggers are responsible for granuloma genesis in sarcoidosis. Insidious onset of disease which develops into a chronic phase with granuloma persistence and a high risk for developing pulmonary fibrosis [11]. In this review, we comprehensively summarize recently described sarcoid cellular components, their functional attributes, and newly discovered antigen(s) that provide mechanistic insights at mature granuloma site, reflecting the circumstances for the granuloma genesis and sarcoidosis pathogenesis.

SARCOID GRANULOMA FORMATION AND INTEGRITY

Tiny aggregates of highly differentiated immune cells with discrete lymphoid-like structures are known as granulomas. Sarcoid granulomas are commonly found in the lungs, lymph nodes, eyes, and skin. In general, when blood patrolling monocytes encounter foreign antigens, they differentiate into specialized antigen-presenting cells (APCs) such as macrophages or dendritic cells [12]. Upon phagocytosis and degradation of the antigens, processed proteins are presented to specialized cells of the adaptive immune system, particularly T lymphocytes. In early sarcoid granuloma, aggregating macrophages are converted into epithelioid cells, which are then organized into a cluster of cells to form immature granuloma. Under the influence of inflammatory signals, cell–cell fusion occurs between macrophages and monocytes/dendritic cells giving rise to multinucleated giant cells (MGCs) [13]. Diverse forms of macrophages that are surrounded by activated T lymphocytes tend to accumulate at sites of inflammation, which is considered to be the crucial step for the formation of mature granuloma [14]. Granuloma formation is a spatiotemporal process that occurs slowly because of the failure of antigen clearance that is constantly fended by persistent deregulated immune cells, thereby preventing antigen dissemination. The sarcoid granuloma structure is composed of two segments: core and crust. The core region consists of tight clusters of macrophages, epithelioid cells, and few MGCs. The crust region usually exhibits high numbers of T lymphocytes and very few B lymphocytes [15]. Granuloma integrity is known to be tightly regulated by a coordinated action between innate and adaptive immunity and poorly degradable antigen(s).

The lack of preclinical animal models reflecting the human sarcoid granulomatous pathogenesis is one of the major obstacles in sarcoidosis research. Currently, the only way to extend our knowledge on mechanistic issues of granuloma formation is to investigate clinical specimens from sarcoidosis patients. During initial diagnosis, a bronchoscopy is performed to aspirate the bronchoalveolar lavage fluid (BALF) whose content represents the inflammatory status in the alveolar spaces of the lungs. Such aspirate serves as a valuable immunological tool to investigate the heterogeneous pool of inflammatory cells and soluble components of an advancing granuloma [11]. Alternatively, granuloma-containing lung biopsies are also taken to examine the lesion-specific immunopathological effects [16]. Because of the limited access to BALF material and the fact that peripheral blood-derived mononuclear cells (PBMCs) constantly circulate to the sites of inflammation thus playing a potential role in granuloma formation, the PBMCs are usually being investigated [17].

MYELOID CELLS IN SARCOID GRANULOMA FORMATION

Despite being a key player in several other inflammatory diseases [18–20], the overall frequency of neutrophils appears to be normal in the sarcoid lung. Nevertheless, elevated BAL neutrophil counts were observed in some of the severe cases or aged sarcoidosis patients, indicating a prognostic value of neutrophils in estimating disease worsening with neutrophil/lymphocyte ratio determination being recently recommended for diagnosis [21,22]. This suggests that while neutrophils are not the dominant population within the granuloma, their excessive accumulation might precipitate disease severity, therefore requiring a high-dose steroid therapy. Next to neutrophils, the expansion of circulating inflammatory CD14+CD16++ monocyte populations was observed in sarcoidosis [23*], which might represent the source for the constant replenishment of granuloma with myeloid cells. Increased proportions of proinflammatory so-called M1 alveolar macrophages were found in the sarcoid airways [24]. Analysis of membrane-spanning proteins of sarcoid alveolar macrophages revealed elevation of two innate host defense signaling pathways: Fcγ-receptor mediated phagocytosis and clathrin-mediated endocytosis. This implies the involvement
of macrophage-phagocytic systems and infections in sarcoidosis pathogenesis. Of note, chronic sarcoidosis patients (non-Löfgren syndrome) exhibit an impaired oxidative phosphorylation of mitochondrial proteins [25], which interfere with the oxidative homeostasis by eliciting uncontrollable reactive oxygen species (ROS) production that subsequently mediates tissue damage.

Signaling of the TL1A and DR3 molecules, a tumor necrosis factor (TNF)-like cytokine-receptor pair belonging to the TNF superfamily, has been implicated in sarcoidosis pathogenesis. During the active disease phase, higher transmembrane levels of TL1A and DR3 were observed on sarcoid alveolar macrophages and sarcoid tissues. Such activated pulmonary macrophages yielded a substantial amount of matrix metalloproteinase (MMP)-9 under the influence of DR3 signaling. Simultaneously, the levels of tissue inhibitor of metalloproteinases (TIMP)-1 were found to be declining in these activated alveolar macrophages [16]. Furthermore, serum circulating levels of CD163, a scavenger receptor for hemoglobin–haptoglobin complexes, were elevated in sarcoidosis patients. In line with this, soluble CD163 production was enhanced during the in-vitro induced formation of MGCs, providing a mechanistic link between activated macrophages and the sarcoid granuloma formation [26]. The MMP-9-sufficient microenvironment [16] close to granuloma might be the cause for ectodomain shedding of CD163 from the monocyte/macrophage membrane. The sarcoid macrophage-dependent inflammation was also assessed by elevated exhaled breath condensate TNF-α levels, a major candidate mediator to drive the granulomatous reaction [27].

Sarcoid tissue displays enhanced toll-like receptor (TLR)-9 expression, the only DNA-sensing TLR especially in alveolar macrophages and giant cells, presuming the involvement of infectious triggers in disease pathogenesis. In addition, alveolar macrophages exhibited increased TLR-9 expression and released high levels of CXCL10 (a CXCR3 chemokine) upon TLR-9 stimulation with Cytosine-phosphate–Guanine (CpG) oligonucleotides [28]. Dissection of the molecular mechanisms that is being active in sarcoid alveolar macrophages and PBMCs revealed a significantly increased expression of the intracellular signaling molecules IRAK-1 (interleukin-1 receptor associated kinases) and Rip-2 (receptor interacting protein), resulting in elevated production of interleukin-1β (IL-1β), IL-6, and interferon-γ (IFNγ) [29]. These alveolar macrophage-derived cytokines are needed for the differentiation of TH1 and TH17 cells, thus providing an underlying mechanistic link between innate immune cell signaling and adaptive immune responses involved in granuloma formation.

**LYMPHOID CELLS IN SARCOID GRANULOMA FORMATION**

T lymphocytes and especially CD4+ T helper cells (TH1) are the predominant cell type present at the sites of disease activity and play a pivotal role in granuloma formation, maintenance, and the overall disease outcome [30]. Activated sarcoid macrophages and dendritic cells secrete the key cytokines IL-12 and IL-18, which foster TH1 differentiation at the granulomatous site. These TH1 lymphocytes amplify immune responses by secreting high-levels of the TH1 hallmark cytokines IFNγ and IL-2 in conjunction with the TH1-polarizing transcription factor T-bet as well as CXCR3 [14]. Despite the well described TH1 biased immunity in sarcoidosis, fractions of IL-17A+ producing CD45RO+ memory T lymphocytes were recently found to be substantially increased and deposited in and around the areas of the pulmonary granuloma in sarcoidosis lungs [31]. Recently, high-level IFNγ-secreting TH17 effector cells, termed TH17,1 cells, were shown to be enriched in the lung of patients with chronic sarcoidosis [32]. Although it remains unclear whether these TH17,1 cells were derived from plastic TH17 cells, it was conceived that IFNγ that is ubiquitously present in the sarcoid lung might influence the migration and eventual transformation of circulating TH17 cells into hybrid TH17,1 cells within the granuloma. Another study has shown that sarcoid TH17 cells exhibit diminished expression of the co-inhibitory molecule CTLA-4 [33], which provides a negative signal and thereby contributes to the termination of T lymphocyte activation. Thus, impaired CTLA-4-mediated control of T cell activation might be mechanistically involved in the detrimental role of TH17 cells in granulomatous pathogenesis. Furthermore, although increased proportions of circulating activated CD45RO+ regulatory T cells (Tregs) were found in patients with chronic sarcoidosis, the functionality of these Tregs was severely impaired. Multiple mechanisms are considered to be involved in the altered immune suppressive qualities of sarcoid Tregs. One possible explanation might be the increased expression of the cell apoptotic marker CD95 in Tregs. CD95+ sarcoid Tregs failed to resist CD95L-mediated apoptosis and thereby exhibited reduced survival [34]. Furthermore, sarcoid Tregs were shown to express a lower level of cell-bound CTLA-4 [33] which is pivotal for efficient Treg-mediated suppression [35]. Sarcoid Tregs failed to efficiently suppress proliferation and cytokine production by autologous T helper cells [34]. Thus,
FIGURE 1. Mechanisms of granuloma formation in sub-variants of sarcoidosis. (a) Chronic sarcoidosis (non-Löfgren syndrome). Phagocytic alveolar macrophages present antigen in the context of Major Histocompatibility Complex (MHCII) molecules to CD4+ T lymphocytes (TH1 cells) thus stimulating their activation and secretion of IFNγ. Migration of CD4+ T lymphocytes to the site of inflammation is guided through the chemokine receptor CXCR3. In addition to CXCR3 these cells express the TLA1 cytokine like protein to interact with its receptor DR3 and produce high amounts of MMP-9, which cleaves CD163 expressed on the surface of macrophages. Shed soluble (s) CD163 circulates in the blood stream as a representative marker for macrophage activation. The activated macrophages express high levels of the DNA sensing TLR9. TLR9 activation boosts the secretion of CXCL10, the ligand for CXCR3, thereby attracting more TH1 lymphocytes to the granuloma. Furthermore, the activated macrophages upregulate IRAK-1 and Rip2 expression resulting in enhanced production of IL-1β and IL-6. These cytokines are needed for TH17 lymphocytes differentiation. As a consequence, accumulated TH17 cells produce enormous amounts of IL-17 and IL-22. Interestingly, the oxidative phosphorylation (OXPHOS) pathway is impaired in alveolar macrophages, which might result in disproportionate production of reactive oxygen species (ROS) causing further tissue damage. (b) Acute sarcoidosis (Löfgren syndrome). Phagocytic alveolar macrophages present the sarcoid-specific antigen...
the fatal combination of the accumulation of TH17/TH1 cells on the one hand and functionally impaired T cells on the other hand will further support the stabilization of granulomatous structures and eventually promote the pathogenesis into chronic sarcoidosis.

In acute sarcoidosis, T lymphocyte bearing the T cell receptor (TCR) restricted Vα2.3/Vβ22 chain accumulate in the lungs of human leukocyte antigen (HLA) DRB1*03 Löfgren syndrome patients [36]. The HLA-DRB1*03 allele was shown to present antigen(s) in a way that effectively boosted the activation of specific T lymphocytes resulting in antigen clearance and subsequent resolution of the granulomatous lesions. Recently, a higher frequency of hybrid TH1/TH17-like T-bet+RORγT+CD4+ T lymphocytes that coexpress the chemokine receptors CXCR3/CCR6 was found in the lungs of Löfgren syndrome patients. These cells generally exhibited a high proliferative capacity and produced high amounts of IFNγ and IL-17A. Of note, reduced IFNγ and elevated levels of the TH17-related cytokines IL-17A, IL-22, and IL-2 were detected in the BALF of Löfgren syndrome patients when compared with chronic sarcoidosis patients. Intriguingly, lung-restricted TCR Vα2.3+Vβ22+CD4+ T lymphocytes from Löfgren syndrome patients expressed higher levels of T-bet and RORγT as well as CXCR3/CCR6 [11], indicating immune surveillance by highly plastic and qualified effector T lymphocytes armed for clearing the antigen from the granuloma. Although the frequency of lung-resident Tregs was reduced in Löfgren syndrome patients, these Tregs displayed elevated surface expression of the inducible costimulator (ICOS) [23*]. ICOS is a costimulatory molecule expressed on activated T lymphocytes as well as on Tregs. ICOS activation on Tregs is well known to boost Tregs to super-synthesize IL-10, thus creating an immunosuppressive micro-environment [37]. This notion was supported by the detection of increased BALF IL-10 concentrations from Löfgren syndrome patients compared with non-Löfgren syndrome patients [11]. Moreover, the involvement of B lymphocytes in sarcoid granuloma formation cannot be neglected as Löfgren syndrome patients exhibited higher levels of Propionibacterium acnes (P. acnes)-specific IgA antibodies [38]. These data cumulatively point to the existence of highly variable mechanisms in the formation of Löfgren syndrome granulomatous lesions, as shown in Fig. 1.

**POSTULATED ANTIGEN(S) IN SARCOID GRANULOMA FORMATION**

Unsuccessful elimination of the antigens by host immunity is considered to build the basis for the granuloma formation in sarcoidosis. Several of the residual insoluble mycobacterial antigens, such as catalase-peroxidase (mKatG), superoxide dismutase A (Sod A), ESAT6, and M. tuberculosis heat shock proteins (Mtb-hsp) [9,39], were associated with the elicitation of type IV immune responses being responsible for the sarcoïd granuloma formation and disease chronication [8]. Very recently, a Swedish research team reported an autoimmune trait behind the onset of Löfgren syndrome in a Scandinavian patient cohort. Here, the filament protein vimentin was determined to be the antigenic factor driving clonal expansion of lung-restricted Vα2.3+Vβ22+CD4+ T lymphocytes in the granulomatous inflammation [40**]. Such responses are readily achievable in HLA-DR3+ sarcoidosis patients with Löfgren syndrome, leading to the resolution of persisting granuloma and eventually the disease. A mechanistic study has revealed that the expression of vimentin on macrophages could be modulated by an active mycobacterial infection. Here, the infection evades the host defense by reducing the production of ROS, which in turn decreases the ectopic expression of vimentin [41*]. However, these effects were reversed by treatment with anti-vimentin antibodies. One might assume mechanisms taking place at the Löfgren syndrome granulomatous lesions, in which the Vα2.3+Vβ22+ T lymphocytes recognizing the vimentin might induce a reverse signaling inside vimentin+APCs resulting in increased ROS production and...
improved killing of intracellular pathogens. Interestingly, blood cells isolated from Löfgren syndrome patients produced higher levels of peroxynitrite, a stealth biological product of nitric oxide and superoxide radical reaction, in response to the stimulation with Mtb-hsp [39]. This may explain the ability of the cellular machinery to produce balanced oxidants for efficient antigen clearance. Beyond this, one may speculate that Löfgren syndrome patients harbor a dormant form of Mycobacterium and therefore demonstrate an increased expression of Mtb-hsp16 [39]. These unsolved issues certainly warrant further investigation concerning the presence of pathogens, particularly in vimentin+ APCs, in the lung of Löfgren syndrome patients.

Besides mycobacteria, the P. acnes was localized in sarcoid granulomas and therefore was suggested to be another potential factor driving sarcoidosis pathogenesis [9]. Recently, P. acnes was isolated from the sarcoid mediastinal lymph nodes [42]. Screening of several putative propionibacterial antigens by proteomic approaches revealed that the catalase (KAT) component of P. acnes was present in high amounts in sarcoidosis patients. This was functionally proven by stimulating sarcoid blood cells with P. acnes KAT resulting in IFNγ production by antigen-specific TH1 cells [43]. Moreover, upon stimulation with the entire P. acnes bacteria, the sarcoid BAL cells produced high quantities of TNF-α and GM-CSF (granulocyte-macrophage colony stimulating factors) [38]. In the sarcoid lung, these cytokines derived by P. acnes-specific TH1 cells might amplify myeloid cell differentiation and promote fusion between the cells to generate MGCs for sarcoid granuloma formation.

CONCLUSION

This review summarizes current knowledge on the involvement of myeloid and lymphoid immune cells in granuloma formation in pulmonary sarcoidosis. We discussed the specific differences observed in sarcoid granuloma compositions in patients with acute (Löfgren syndrome) versus chronic (non-Löfgren syndrome) disease manifestation. However, to date we cannot define generalizable mechanistic principles underlying sarcoid granuloma formation because this would certainly require considering additional variables such as different ethnic groups, geographical locations, yet undefined triggers, granuloma-sequestrated components, young versus aged patients, and genetic predisposing factors. Future research should take into account these issues to ultimately build the basis for the rational design of targeted sarcoid-specific therapies.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- of special interest
- of outstanding interest


This is the first study to describe the lung accumulated ICOS<sup>hi</sup> T<sub>reg</sub> phenotype in sarcoidosis patients, with marked increase in Löfgren syndrome patients. Likewise, ICOS-L<sup>hi</sup> monocytes were also demonstrated in sarcoidosis, with marked increase in Löfgren syndrome patients. Thus, the study emphasizes the involvement of ICOS/ICOS-L axis in disease course and perhaps in recovery.


This study questions the long-supported Th1 paradigm in sarcoidosis pathogenesis. Here, increased percentage of Th17.1 phenotypes were seen, which secreted high concentration of IFNγ in the lung of sarcoidosis patients.

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