Pathogenesis of sarcoidosis

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Summary

Sarcoidosis is a systemic granulomatous disorder of unknown origin. Recent research uncovered underlying immunological and genetic mechanisms, which will pave the way for more effective pharmaceutical studies. At present some of this knowledge is clinically exploited to monitor therapy and expected genetic progress will allow the development of prognostic genetic patterns or molecular signatures. Moreover, it has become obvious that several etiologic agents and cofactors will exist. These will be of animate and inanimate nature and their interplay with host mechanisms discussed in this review determines disease phenotypes.

Sarcoidosis is a multi-organ disorder of unknown origin, characterised in the affected organs by a T-lymphocyte - mononuclear phagocyte infiltration, granuloma formation, and distortion of the normal micro-architecture [1]. The lung is the most commonly involved organ, and studies with lung inflammatory cells recovered by bronchoalveolar lavage (BAL) revealed detailed concepts of the immunopathogenesis of the disease, which will be in the focus of this review. A genetic predisposition to acquire sarcoidosis has been assumed over a long time. However, not until recently genes predisposing for sarcoidosis have been unequivocally identified. The assumption of a genetic contribution to the aetiology of sarcoidosis is mainly based on the observations that prevalence and incidence rates of sarcoidosis are different between ethnic groups and races [2,3], and that the disease tends to cluster in families [4,5]. Consequently, family studies were the first to demonstrate unequivocally a genetic predisposition to be discussed in this review.

Definition

Sarcoidosis is best defined in histopathological terms as a disease characterized by the presence of non-necrotizing epitheloid cell granulomas in all of several affected organs, proceeding either to resolution or to conversion into hyaline connective tissue. The clinical diagnosis, however, can only be supported by typical histopathological findings. Pathognomic criteria or a diagnostic
“golden standard” are absent. Most experts thus include several clinical, radiological, immunological, and histologic features into their diagnostic criteria since other disease processes can simulate sarcoidosis in many ways. Occasionally, all of these features may suggest the diagnosis of sarcoidosis in patients later proven to have other diseases. Therefore rigorous efforts have to be made to exclude alternative diagnoses, e.g. tuberculosis, lymphoma, berylliosis, Blau syndrome, Wegner’s disease etc., and patients diagnosed as suffering from sarcoidosis must regularly be subjected to review and further testing.

Epidemiology

Since many individuals with sarcoidosis are asymptomatic, estimates of incidence and prevalence depend mainly on the way generating epidemiological data in national health systems. Confounders are differences in case definition, disease presentation and heterogeneity, intensity of diagnostic work-up, patients’ socioeconomic status and race. In Europe, sarcoidosis is the most frequently observed interstitial lung disease of unknown aetiology. The prevalence rates range from 64 patients per 100,000 population in Sweden to 0.2 per 100,000 population in Portugal with in-between numbers observed in Denmark, (53 per 100,000); Germany (43), Ireland (40), Norway (27), The Netherlands (22), the United Kingdom (20), Switzerland (16), France (10), Hungary (5) and Spain (1.2). The prevalence for the Caucasian population of North America is 3 and for Afro-Americans 47 per 100,000 [8,9]. Sarcoidosis is found in all races affecting slightly more women than men. Most commonly it manifests in adults of 20 to 45 years, although all ages can be affected. Interestingly, a second incidence peak for the female gender near age 60 has been observed in Denmark, Japan, and Singapore, and around age 70 in Israel [10–13]. Sarcoidosis incidence rates seem to be stable over time while those of idiopathic pulmonary fibrosis are increasing [14]. However, the overall incidence of sarcoidosis is most likely underestimated since its incidence at autopsy is higher than in epidemiologic studies [15,16].

Most interestingly, also differences in the pattern of organ involvement and the severity of the disease in relation to race and ethnic background point to a genetic susceptibility of sarcoidosis. Erythema nodosum associated with acute disease and good prognosis is most frequently seen in young Cauca- sians, as originally described by Löfgren [17]. A recent study from Sweden demonstrates that the good prognosis of Löfgren’s syndrome is associated with certain HLA alleles and that patients missing these alleles suffer from a higher frequency of non-resolving disease [18]. In Japan Löfgren’s syndrome is uncommon but cardiac and eye involvement are more frequent than in Europe [11]. Lupus pernio and other cutaneous manifestations of sarcoidosis, considered to be stigmata of chronic disease [19], appear more frequently in African Americans than in Caucasians [20,21]. Only rough estimates of the mortality rates of untreated sarcoidosis are available. Overall mortality from sarcoidosis is estimated to be ≈ 5% [1]. In an epidemiological study from Denmark with a median follow-up of 27 years, an excess mortality from sarcoidosis and sarcoidosis-related diseases was perceived in the first 20 years in patients with advanced radiological findings and deteriorated lung function. Although the mortality of the sarcoid cohort was higher than that of the general population the difference was not statistically signifi- cant [22,23]. A significant increase in mortality in aged indi- viduals of male gender, which is approximately double that of a comparison cohort has to be attributed to sarcoidosis in a study from the United Kingdom [14]. Over two decades a recent study from the United States demonstrates – in contrast to the afore mentioned study from the UK – an increase in saroid death in patients 55 years or older regardless of sex or race [24,25]. The highest increase was seen in African American females and younger age and black race are risk factors for cardiac or fibrotic manifestations causing deaths [24] which is in accordance with the observation by a French group showing that most of sarcoidosis associated deaths occurred in patients suffering from fibrotic lesions in the lung [26].

Etiology

Many agents have been implicated as putative stimuli that elicit the granulomatous response of sarcoidosis. As early as 1905, C. Boeck described sarcoidosis as “a bacillary infectious disease, which is either identical to tuberculosis or closely related to it”, however, proof of this hypothesis remains elusive and there is
an ongoing discussion about this question. Epidemiological data and similarities with other infectious diseases support the hypothesis that sarcoidosis is induced by an infectious organism or its remnants. Seasonal clustering of sarcoidosis in the months of June and July [27], change of prevalence over climate zones [9], time and space clusters [28,29], an increased incidence in health workers [30], an association of environmental exposure with sarcoidosis [31] or specific sarcoidosis phenotypes [32] and the transmission or recurrence of sarcoidosis by or in transplants [33,34] have been observed and support the hypothesis of transmissible animate sarcoidosis-inducing agents. Numerous case series, case reports, and epidemiologic studies demonstrated an association between sarcoidosis with uptake of silica [31], talc [35,36], man-made fibres [37] or other inanimate agents [38,39] by inhalation or ingestion at workplace [28,32,40–42], at home [32,43], from the environment [32] or as components of pharmaceutical products [44]. Exposure to crystalline silica is associated with a number of chronic pulmonary and extrapulmonary disorders next to sarcoidosis but in contrast to the above mentioned study from Iceland [31] an association with sarcoidosis could not be identified using data from a occupational mortality surveillance program [45]. Nevertheless, there is considerable although indirect evidence that sarcoidosis is elicited by an inanimate etiological agent or inanimate cofactors which can be found in the environment, at the workplace and at home. These factors might be common environmental agents which require further cofactors and a susceptible host. This view is supported by observations of sarcoidosis clusters in cohorts of New York fire fighters before and after the terror attack of the world trade centre. A study in 1999 estimated the incidence of sarcoidosis in New York fire fighters to be 13–15/100,000, which provides a baseline before the disaster [46]. After the terror attack the incidence in fire fighters rose to 86/100,000 in the first year and sunk thereafter to 22/100,000 over the next four years [47] which suggests a release of one or several sarcoidosis eliciting cofactors from the debris of the world trade centre. Of note, many of these patients had also extrapulmonary manifestations. Whether working in the debris or just the exposure at the time point of the disaster is sufficient for an increased risk is an unresolved question [48–50]. In support of this notion, exposures to metal dusts and organic agents in fumes or aerosols can cause granulomatous disorders of the lung that are indistinguishable from sarcoidosis as identified by the ACCESS study which used questionnaires for a retrospective assessment of occupational exposure [40,41]. More stringent diagnostic workup in an occupational setting leads to a decrease of sarcoidosis incidence and increase in other granulomatous disorders [42,51].

Seasonal and geographic variations with a predominance of the diagnosis in the springtime and an increased incidence in moderate to subpolar climate zones or areas near the coastline point to an animate etiological agent in the environment [9,52,53]. In aggregate, there is mounting epidemiologic evidence of inanimate or animate agents in the environment capable of inducing sarcoidosis or being an etiologic cofactor.

Genetics

Genes predisposing for sarcoidosis have been identified in the recent past and rapid progress in molecular technologies will aid the discovery of further risk loci and variants. Several new susceptibility genes have been identified and the roles of those previously known were further defined over the last decade. Nevertheless, clinical genetics of sarcoidosis is still in its infancy. As outlined above in the yet unknown aetiology of sarcoidosis, environmental exposures are believed to interact with genetic factors in determining the pattern of sarcoidosis presentation, progression, and prognosis [1,54,55]. The majority of sarcoidosis genetic studies have been candidate gene studies using a case control design. In general, the quality of these and other genetic studies depends on precise definitions of disease itself along with the phenotypes of cohorts and the ability to make comparisons with appropriate controls. While numerous paradigms have been used to phenotype sarcoidosis, it is often difficult to allocate a given patient to a distinct phenotype using clinical or immunological criteria beyond acute and chronic disease. In addition, phenotyping of patients often requires a long-term follow-up because a considerable percentage of patients presenting initially with acute disease and apparent good prognosis might subsequently develop chronic sarcoidosis with severe organ damage later on [56,57]. Moreover, the fact that environmental [51] and occupational [58] exposures may cause phenocopies of sarcoidosis such as life guard lung and chronic beryllium disease complicates any genetic analysis. Nonetheless, subgrouping of patients according to specific clinical features and phenotypes of disease will in future studies most likely increase the ability to identify new genes of importance for sarcoidosis.

The contribution of an inherited predisposition to the aetiology of sarcoidosis is well documented by an increased risk of sarcoidosis in close relatives of patients. A positive family history of sarcoidosis ranges from 2.7% of subjects in Spain to 17% in African Americans [59–61]. A familial relative risk of 4.7 was calculated in the Case Control Etiologic Study in Sarcoidosis (ACCESS) study, and this risk was higher in US Caucasians than in African Americans [4]. In all of these surveys the familial risk usually includes two patients, and affected parent and offspring pairs are roughly as common as affected sibling pairs. This constellation is indicative of multiple small or moderate genetic effects. This notion is supported by results of genome-wide association scans in familial and sporadic sarcoidosis, which identified numerous chromosomal regions contributing to sarcoidosis risk [62–64] and new susceptibility genes [65–68], respectively. In a multi-centre sarcoidosis
The recent development of extremely high density genotyping chips allows genome-wide association studies based on hundreds of thousands of single nucleotide polymorphisms (SNPs) in several thousand of cases and controls. This offers the possibility to create an extensive genetic map and to identify previously unsuspected predisposing genes without the need to gather and genotype extended series of multiplex families. Due to the importance of human leukocyte antigens (HLA) in antigen presentation and immunoregulation the highly polymorphic major histocompatibility complex (MHC) locus has been investigated intensively by association studies. Studies on HLA-DRB1 associations with sarcoidosis dominate the literature, reporting gene variations affecting susceptibility, phenotype, and prognosis, in many cases with different associations noted in different populations. Differences in the distribution of HLA-DRB1 genes between subjects with sarcoidosis and matched controls showed a highly significant P value \((P < 0.0001)\) in the ACCESS study [72]. HLA-DRB1*1101, -0402, -1201 and -1501 were the specific DR alleles most associated with sarcoidosis. Associations of specific HLA-DRB1 alleles with specific phenotypes of sarcoidosis have also been observed; for example, eye disease was associated with DRB1*0401 while abnormal calcium metabolism was associated with DPB1*0101 [72]. Some results have been demonstrated across studies and different racial and ethnic groups. For example, HLA-DRB1*03 has been consistently associated with spontaneous resolution and mild disease as demonstrated in Swedish [56], Polish [73], Croatian [74], Czech [75], and Finish populations [76]. A striking influence of DRB1*03 on the disease course has been shown in a distinct subgroup of sarcoidosis patients, with Löfgren’s syndrome [18]. Also, chronic disease has been associated with HLA-DRB1*15 in several Caucasian populations [56,73,77]. Interestingly, in the ACCESS study, the DRB1*15 was protective for African Americans [72]. Because of strong linkage disequilibrium within the MHC region, e.g. between DRB1*0301 and DQB1*0201 in white populations, correlations with resolving disease and DQB1*0201 are equally strong [56,77,78]. Also, the strong linkage between DRB1*1501 and DQB1*0602 makes it difficult to separate the risk for chronic disease-associated with DRB1*1501 or DQB1*0602, as reported in Swedish [56], British [78] and Dutch [78,79] populations. DQB1*0602 has, moreover, been associated with specific clinical manifestations such as small fiber neuropathy [80] and in Japan with splenomegaly [81]. The finding of HLA class II associations supports the notion that certain HLA alleles are more efficient in presenting distinct antigens, thereby influencing the immune response and subsequent inflammation. In line with this concept, T-cells expressing the T-cell antigen receptor chain AV253 accumulate in the lungs of HLA-DRB1*0301 positive patients [82,83]. This leads to the hypothesis that in these patients, who have a good prognosis, sarcoid-antigen presentation takes place in the context of HLA-DRB1*0301, generating an efficient AV253 T-cell immune response which eliminates the antigen and induces spontaneous resolution. In detail, within the HLA-DRB antigen-binding groove, distinct “pockets” are important for binding of the antigen-peptide and pockets number four of DR and nine of DQ, and the specific amino acids within those respective pockets, were shown to be important for associations with sarcoidosis [84] and its prognosis [76]. Pocket 4 of DR was also associated with sarcoidosis risk in the ACCESS study [72], while HLA-DRB1*F47, which affects pocket 7, was associated with sarcoidosis in whites [72]. In another study, the protective HLA-DR alleles DRB1 and DRB4 both were shown to have small hydrophobic amino acids in another pocket (pocket 6), which also could influence antigen-peptide binding [85]. Further support for the importance of specific antigen-peptides in sarcoidosis is provided by the identification of potential auto-antigens in antigen-binding grooves of HLA-DR molecules of HLA-DRB1*0301 positive patients [86]. Next to HLA antigens, co-stimulatory molecules of the immunoglobulin superfamily are necessary for orchestrated activation of T-cells. One of these is the butyrophilin-like 2 gene (BTN2L2), which was identified as a sarcoidosis susceptibility gene [65] in a genome-wide single nucleotide polymorphism (SNP) association study and replicated in independent studies [87,88]. Its primary disease-associated splicing-site (rs 276530) introduces a premature stop. The resulting truncated protein product encoded by this splice form cannot be inserted in the cell membrane needed for its proper function of down-regulating activated T-cells [89]. Thus, the gene product of the A-allele of a bi-allelic variant has an impeded immune-dampening function and there is evidence for its influence on disease phenotype [87]. Analyses of the cytokines released by activated immune cells of the lower respiratory tract demonstrate a predominance of Th1 cytokines and cytokine patterns heralding spontaneous resolution or progressing disease have been identified [90]. Many of these cytokines and their receptors disclose functional polymorphisms, which suggest a genetic basis for their release in health and disease. As a result, numerous candidate gene association studies have been conducted in sarcoidosis evaluating these Th1 cytokines. Polymorphisms of TNF and its receptors were the first to be under scrutiny since TNF activity

is pivotal in the pathogenesis of sarcoidosis (see below). Associations of promoter polymorphisms with different disease phenotypes have been identified with discrepant results in different ethnic groups [91–97]. Chemokines likely important in immune cell trafficking, such as CCL2 and CCL5, and their receptors have been analyzed with mixed results noted, some associated with disease, some with disease phenotypes and others with no significant associations [71,98–104]. Molecules of innate immunity such as Toll-like receptors (TLR) are involved in the activation of immune cells in the course of sarcoidosis, in particular TLR2 [105]. TLR10, TLR1, and TLR6 serve as co-receptors for TLR2 and are found in a cluster on chromosome 4. Interestingly, different haplotypes of the TLR10–TLR1–TLR6 gene cluster associate with self-remitting, classical Löfgren’s syndrome or chronic sarcoidosis [106]. Conflicting results have been obtained for TLR4 gene variants in Caucasian and Japanese cohorts [107,108], which point to a causative variation in the vicinity of the TLR4 gene [109]. Nevertheless, an increased expression of TLR2, TLR4, and TLR9 and their signal transduction is observed in sarcoidosis [110–112]. TLRs are members of the pattern recognition receptors involved in microbial defence. Mannose receptor type C-1 (MRC1) is another member of this category sensing glycan structures and it is expressed on macrophages and dendritic cells. Glycan structures containing mannose, fucose and N-acetylgalactosamine are commonly found on cell walls of mycobacteria and related genera, which are implicated in the pathogenesis of sarcoidosis. The MRC1 gene is found on chromosome 10p12 in the vicinity of other genes associated with sarcoidosis and Crohn’s disease [66]. A polymorphism of MRC1 with a remarkable odds ratio of 2.53 for sarcoidosis has been identified in a Japanese study, which is, however, also involved in other inflammatory disorders [113]. The extent of serum angiotensin converting enzyme (sACE) increase appears to reflect the total body granuloma load [114]. The ACE gene carries a polymorphism generated by the insertion of 287 base pairs into intron 16 that does not alter the enzyme structure but affects its production in health and disease, which is also true for sarcoidosis. Moreover, it is the most powerful factor determining ACE-production. Therefore, the use of genotype-adjusted reference values for sACE increases the sensitivity and specificity of this test [115–117]. This can be regarded as a first step to personalized medicine in sarcoidosis but unfortunately, its clinical usefulness is limited to populations of Caucasian origin. Of note, this polymorphism does not influence disease susceptibility [97]. The increasing use of genome-wide association studies (GWAS) defines genes associated with disease without any prior knowledge of disease pathogenesis. These studies will likely continue to find application in sarcoidosis genetics to define new genes associated with disease and disease phenotypes. In fact, SNPs in two genes on chromosome 10 have been found associated with sarcoidosis in two GWAs studies, including Annexin A11 [67], known to be important in apoptosis and proliferation, and another gene with yet unknown function named C10ORF6, which was also associated with Crohn’s disease [66]. The function of these genes in sarcoidosis is not yet known [118], however, the association of Annexin A11 has been reproduced by several groups [119,120]. Other GWAS defined numerous chromosomal regions most likely harboring further susceptibility genes [64,68]. These findings demonstrate the power and importance of the use of genome-wide studies in hypothesis generation to define genes with unknown function in sarcoidosis risk. In addition, although only few epigenetic and transcriptomic studies have been conducted in sarcoidosis to date, this is likely a fruitful area for future research studies to delineate disease pathogenesis and risk in sarcoidosis [121]. Genetic mapping in rare diseases turns hypothesis-driven research upside down since it is based on the assumption that causal genes can be localized by systemic genome-wide analysis of DNA variation. Stricto sensu it represents a new category of linkage analysis, which implicates a genomic region in the susceptibility to a disorder or one of its phenotypes. The mechanisms whereby polymorphic genes influence disease are not obvious from our pathomechanistic concepts or the genes in the identified chromosomal regions. This is exemplified by the finding of Annexin A11 as a sarcoidosis susceptibility gene [67,118,120], and the associating SNPs themselves are only in rare cases the causal variants, as it is the case in BTN2 [65,122]. SNPs identified in association studies are reproducible but their relative risk ranges only around 1.5. The relative sarcoidosis risk of 1.68 observed for the A-allele of BTN2 and the odds ratio of 2.53 for MRC1 are extraordinary high [65]. Other sarcoidosis susceptibility SNPs identified on chromosome 6 outside the major histocompatibility complex disclose odd ratios around 1.5 [68]. For a univocal identification of those low relative risks the statistical fluctuations encountered need to be compensated by an high statistical significance in large cohorts [123]. A typical genome-wide scan of 1500 patients and 1500 controls for a variant with a frequency of 20% has a power of only 13% if the risk per allele is 1.3, which demonstrates the need of very large cohorts in rare disorders. This approach is further complicated by the fact that we expect hundreds of variants with low relative risks to constitute the genetic architecture of the inherited component of a given disorder. The frequent variants will most likely be of small effect and only very few with larger effects can be expected [122,124]. Thus, the known genetic associations in sarcoidosis are not yet sufficient to calculate genetic risk profiles to allow personalized medicine of sarcoidosis.

Immunopathogenesis

The human organism generates granulomata whenever an antigen cannot be degraded and completely eliminated by
its macrophages. In these cases multinucleated giant cells and epitheloid cells emerge which are the building blocks of non-necrotizing granulomata characterizing sarcoidosis. An immune stimulation is required for these processes which is delivered by activated mononuclear cells accumulating around a granuloma [125]. Non-necrotizing granulomata, the histological hallmark of sarcoidosis, are found in any involved organ. However, they are not pathognomonic feature of the disease. Of note, also necrotizing granulomata are observed in small number in sarcoidosis, which broadens the spectrum of differential diagnosis. In general, granulomata caused by *Mycobacterium tuberculosis*, Wegener’s granulomatosis or silicosis can easily separated by histological examination. In lymph nodes, however, cat scratch disease and brucellosis have to be considered as differential diagnoses in the presence of non-necrotizing granulomata. Sarcoid-like lesions can also be observed in lymph nodes draining areas harboring neoplastic disorders and in lymph nodes involved in a reconstitution syndrome of acquired immunodeficiency syndrome (AIDS) or common variable immunodeficiency (CVID) [126,127].

Next to non-necrotizing granuloma, activated T-cells and macrophages can be found in affected tissues. These inflammatory processes are compartmentalized with exaggerated immune cell activation in organs involved in sarcoid inflammation but quiescent or only modestly activated cells in the peripheral blood. For example interleukin (IL)-2 is secreted by activated cells from bronchoalveolar lavage showing clonal expansion but not from peripheral blood T-cells without clonal expansion [128,129]. Sarcoid alveolar macrophages exhibit characteristics known from dendritic cells. Thus, in contrast to normal alveolar macrophages they are capable to present antigen and to stimulate T-cells. Moreover, they secrete cytokines which are involved in the T-helper cell (Th)1 immune response and are chemotactic for Th1-lymphocytes such as IL-1, IL-6, IL-12, interferon-inducible protein (IP)-10, and tumour necrosis factor (TNF) [125]. Also for macrophages/monocytes a compartmentalization of activation has been shown since peripheral blood monocytes from sarcoidosis patients do not release TNF but alveolar macrophages release high amounts, which correlates with disease phenotypes [130–132].

T-lymphocytes at sarcoid lesions expose activation characteristics usually found in cells activated by a nominal antigen [128,133] and their T-cell antigen receptor α, β, γ and δ chains are oligodional, which suggests an antigen-driven selection process [129,134,135]. Activated mononuclear cells accumulate in sarcoid lesions and two mechanisms contribute to this accumulation, i.e. a local proliferation [136,137] and an immigration along a chemokine gradient [138,139]. These mechanisms are regulated by positive and negative feed back loops involving Th1 and Th2 cells promoting cellular and fibrotic processes respectively and involving Th17 cells and regulatory T-cells (Treg) stimulating and down-regulating extracellular pathogen response respectively [140–145]. Imbalances in these regulatory mechanisms in sarcoidosis result in Th1 cytokine patterns, an increase in Th17 and a partial inactivity of Treg [141,143,146] summing up to an increased proliferation and activation of T-cells. Noteworthy, IL-23 is an important mediator stimulating Th17 cells and in sarcoidosis a polymorphism of yet unknown relevance in its receptor is overrepresented [147].

A long list of potential infectious causes of sarcoidosis emerged from histological and serological studies. Among others mycobacteria, fungi, rickettsia and borrelia have been implicated but these reports could not be reproduced [148–150]. The Kveim-Siltzbach reagent is a preparation from human sarcoid spleen, which was formerly used for the diagnosis of sarcoidosis [151]. Due to its granuloma-inducing capacity in sarcoidosis patients, it was frequently used to search for the causative agent of sarcoidosis. Although mycobacteria are often discussed as causative agent in sarcoidosis, mycobacterial DNA could not be detected in the Kveim-Siltzbach reagent [152]. However, using a proteomic approach analysing the Kveim-Siltzbach reagent researchers found a poorly soluble antigen in sarcoid lesions derived from mycobacteria, the so called catalase-peroxidase G (mKatG). In a subsequent study they could demonstrate in patients from the US and Sweden that about 50% of sarcoidosis patients mounted a cellular and humoral immune response against this antigen [153,154]. Along this line other investigators demonstrated recognition of several mycobacterial antigens by sarcoidosis CD4 cells expressing distinct HLA-DR epitopes [155]. Propioni bacteria have been isolated by culture from sarcoid tissue [156] suggesting this commensal organism is of relevance in sarcoidosis aetiology. Using polymerase chain reaction propionibacterial DNA was detected in 98% of tissue samples from Japanese and European sarcoidosis patients versus 60% of the controls [155,157]. Nevertheless, it is assumed that sarcoidosis is not of infectious aetiology but is based on an exaggerated immune response of an individual with a susceptible genetic background against pathogen-associated molecular patterns (PAM) of harmless commensals. Serum amyloid A (SAA) and other proteins accumulate in the granuloma and serve as a trap for the etiological agent. Hereby a nidus is established which may give rise to chronicity. Moreover, SAA serves as a ligand for TLR2 and other receptors of innate immunity which stimulates macrophages and T-cells [105]. Next to PAMPs, anorganic substances like beryllium [58], crystalline silica [31] and other anorganic compounds are capable of inducing granulomatous immune responses, which cannot be distinguished from sarcoidosis. Since sarcoidosis is a very heterogeneous disorder, it can be assumed that not only one but several etiological agents may induce the characteristic Th1-hyperreactivity. At present, a genetic susceptibility and a hyperreactivity against PAMPs of harmless commensals seem to be key factors of sarcoid aetiology [105,158].
Along these facts, the immunopathogenesis of acute sarcoidosis is conceptualized in the following. In acute sarcoidosis, resident T-cells and macrophages, in most cases of the lung, become activated by an antigen, which is presented by alveolar macrophages. Accessory molecule interactions cause a strong stimulation and additional stimulating signals are delivered by the recognition of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) by pattern recognition receptors (PRRs) (Figure 1A). The activated macrophages increase their expression of co-stimulatory molecules and release chemokines like CXCL10 attracting additional T-cells of CD4/TH1 phenotype (Figure 1B). These T-cells become activated by macrophages with exaggerated accessory function and proliferate by autocrine IL-2 production (Figure 1D). These strong stimuli induce an extensive preponderance of Th1-lymphocytes and M1 macrophages in acute disease and the antigen contained in the granuloma is dissolved and eliminated by that strong immune response. Consecutively, transforming growth factor-β (TGF-β) released by macrophages, epithelial cells, or cells of the granuloma themselves leads to a downregulation of T-cell activation and TNF release of alveolar macrophages most likely induced by Treg (Fig. 1E; Treg: cells with white nucleus). This downregulation results in a vanishing of T-cell alveolitis and abrogates granuloma integrity. In most cases the granuloma will completely resolve or might leave only a minor scar (Figure 1F) [125]. Interestingly, spontaneous resolution is heralded by increased TGF-β release of bronchoalveolar cells [90].

In chronic sarcoidosis the same or a different antigen as in acute disease, which is presented by alveolar macrophages, activates resident T-cells and macrophages. Due to missing or only week accessory signals in chronic sarcoidosis an insufficient or only moderate T-cell stimulation is achieved (Figure 2A). As in acute disease, additional stimulating signals are delivered by the recognition of PAMPs or DAMPs by PRRs, however; the stimulatory processes are not strong enough to result in sufficient levels of TNF or other mediators. Noteworthy, in chronic disease CXCL10, IL-12 and IL-18 are only found at background levels indicating a minor activation of alveolar macrophages (Figure 2B). T-cell activation is also low due to missing co-stimulation by alveolar macrophages and T-cell recruitment is minimal due to absent chemotactic factors. In this micromilieu the immigrating cells undergo only a weak stimulation. Consequently, compared with acute disease the number of T-cells in the bronchoalveolar lavage (BAL) is reduced. Th1 commitment is incomplete because of insufficient levels of IL-12 and IL-18 (Figure 2C). Nevertheless, activation of alveolar macrophages and their release of TNF induce the generation of multinucleated giant cells and subsequent granuloma formation (Figure 2D). Corticosteroid resistant TNF release of these cells is observed in recalcitrant disease [132]. IL-10 and the contact of alveolar macrophages with fibroblasts induce their differentiation into M2 macrophages releasing profibrotic CCL18 (Figure 2E). CCL18 induces activation and matrix production by fibroblasts adjacent to granuloma leading to fibrotic remodelling of the lung. Persistence of the disease eliciting antigen or non-degradable remnants maintain inflammation with Th2 and M2 cells which leads to a fibrotic remodelling of the lower respiratory tract or any other involved tissue (Figure 2F).

**Clinical applications**

By bronchoalveolar lavage the lung is easily accessible. Therefore, the immunological changes in the context of sarcoid inflammation have been studied in great depths in this organ and there is evidence that similar mechanisms take place in other organs. Great numbers of mononuclear cells and small numbers of polymorph nuclear cells immigrate to the alveolar
space and typical changes in the differential cell count of BAL can be observed. The close interaction between alveolar macrophages and T-lymphocytes can be appreciated by enumerating rosettes consisting of macrophages surrounded by a dense ring of T-cells. The activation of these cells can be gauged by measuring their above mentioned cytokine release. Spontaneous resolutions are heralded by TGF-β release of alveolar macrophages and chronic recalcitrant disease is associated with prednisolone-resistant TNF release [90,132]. However, these immunologic phenomena are not exploited in daily routine since bronchoalveolar lavage is an invasive method not practicable for repeated measuring of inflammatory activity. BAL differential cell count can be used to estimate sarcoid activity. An increased CD4/CD8-ratio is observed in acute sarcoidosis and in patients with a ratio above 3.5 a spontaneous resolution is frequently observed. This allows a close follow-up in these cases with non-mandatory treatment indication to allow spontaneous resolution to take place. A slight increase of polymorphonuclear cells above the background of 3% is associated with progressing disease requiring treatment in the near future [159].

In particular, in acute sarcoidosis numerous alterations can be observed in peripheral blood. The immigration of CD4+ T-cells to the organ manifestations causes a depletion of these cells in the periphery, which might be accompanied by lymphocytopenia in the blood [160]. In contrast to the exaggerated Th1 response in organ manifestations, peripheral blood cells are in an immunosuppressed state which can be demonstrated by their reduced or absent response against recall-antigens. This is in accordance with the observation of increased numbers of regulatory T-cells and increased concentrations of anti-inflammatory cytokines such as IL-10 in peripheral blood [161].

In a routine setting sequential tests of T-cell function such as ex-vivo cytokine release are not practicable and serum markers are desired. Several molecules shed by activated immune cells or epitheloid cells give rise to elevated serum levels, which may be used to probe the corresponding immune processes. At present, useful parameters are available for granuloma burden, T-cell activation, and macrophage/monocyte activation; discussed in the following.

Angiotensin converting enzyme (ACE) is secreted by epitheloid cells of granulomata and its serum level indicates the total body granuloma burden [114]. Its changing over time rather than absolute levels correlate with disease activity. Unfortunately, the sensitivity, specificity, and prognostic values of serum ACE is low. However, the detection of an insertion/deletion polymorphism in the ACE gene, which influences ACE serum level of healthy individuals, enables the use of genotype-corrected normal values resulting in a greater sensitivity and specificity of this biomarker [115]. Elevated ACE levels during the course of sarcoidosis are of no relevance for systemic blood pressure. Neopterin, a small 250 (g mol⁻¹) molecular weight metabolite of the guanosine triphosphate pathway is released by activated macrophages and monocytes. Elevated serum levels are found in sarcoidosis and can be used to monitor the activity of these cells, which is usually found in patients with progressing disease. No correlations have been found between neopterin serum levels and biomarkers from BAL [159,162]. Soluble interleukin-2 receptor (sIL-2R or sCD25) can be found in BAL fluid and serum of sarcoidosis patients and it is released by activated alveolar immune cells [163]. Increased serum levels can be found in cases with active inflammatory processes,
which require a closer follow-up because this biomarker is associated with progressive organ damage requiring therapy [159,162]. The named serum markers and bronchoalveolar lavage cytology patterns can be used to monitor disease activity in the spontaneous course awaiting spontaneous resolution or to gauge the effectiveness of an initiated therapy in suppressing inflammation.

Therapeutic consequences

Personalized medicine is in its infancy. Further elucidation of the genetic background of sarcoidosis, however, will identify genetic patterns of prognostic usefulness. Those patterns may allow the prediction of spontaneous resolution or warn of recalcitrant disease or identify the risk for certain organ involvements. Moreover, genotyping for polymorphisms of drug targets will enable recruitment of small study cohorts of patients who will most likely benefit from a new pharmaceutical approach. We expect that genomic research and analysis of the transcriptome and the metabolome will yield molecular signatures, which will ease drug development in sarcoidosis and rare diseases in general.

The use of bronchoalveolar lavage has forwarded the understanding of sarcoid pathogenesis and pivotal mechanisms of recalcitrant disease have been identified. In particular, alveolar macrophage activation and their release of TNF are in the center of chronic disease [131,132]. This knowledge paved the way for pharmaceutical trials evaluating different anti-TNF principles and drug combinations. The usefulness of pentoxifyllin in tapering-off corticosteroids has been shown and this study was based on the TNF-dampening ex vivo effect of this drug [164,165]. In sarcoidosis the cytokine network is in imbalance and by the use of peptide hormones like vasoactive intestinal peptide its balance can be restored and inflammatory processes down-regulated which shows that naturally occurring peptides can be developed to anti-inflammatory drugs [141]. A study using the anti-TNF monoclonal antibody infliximab demonstrated effects on pulmonary and extrapulmonary sarcoidosis [166]. Although effective, the study endpoint was not achieved and, therefore, this drug was not approved for the treatment of sarcoidosis. At present, another anti-TNF monoclonal antibody, golimumab, is tested in a clinical trial, which also employs an antibody against IL-12, ustekinumab (NCT00955279). This study accomplished recruitment and results are expected in 2013.

In future, the detailed concepts of the immunopathogenesis of sarcoidosis will allow focused search of new pharmaceutical approaches and by the use of molecular disease signatures. It will be possible to obtain meaningful results in small but homogenous cohorts. This will reduce the costs of pharmaceuticals in rare diseases considerably and ease the approval of new drugs.

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Pathogenesis of sarcoidosis


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