

## Sarcoidosis: Unknown Etiology and Genetic Predisposition Provides Therapeutic Challenges

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### Abstract

Sarcoidosis is an idiopathic multisystem disorder characterized by noncaseating epithelioid granulomas predominately affecting lungs and lymph nodes, but with potential to affect any organ system. Sarcoidosis shares similarities in development to other infectious granulomatous diseases, such as berylliosis and tuberculosis; however, its elusive etiology and non-distinctive histology have provided many diagnostic challenges. Evidence of a transferrable antigen combined with a high incidence rate in the lungs has focused efforts primarily on antigens with airborne transmissibility. While *Mycobacterium tuberculosis* and *Propionibacterium acnes* have provided strong associations to implicate each as a contributor to sarcoidosis pathogenesis, detection challenges remain and consensus of a definitive antigen is lacking.

Uncovering common polymorphisms has added another layer to the pathophysiology of sarcoidosis. Polymorphisms involving BTNL2, NODS, Notch and Anxa11, as well as certain HLA alleles, such as DRB1\*0301 and DRB1\*1101 may confer predisposition or resistance to sarcoidosis. Additionally, polymorphisms such as BTNL2 rs2076530 and Anxa11 rs1049550 show efficacy in increasing susceptibility or have no effect in certain ethnic groups. These polymorphisms also show familial linkages and may provide markers for disease severity.

Without definitive diagnostic criteria, sarcoidosis remains a multistep diagnosis of exclusion. Therapeutics has improved clinical management of sarcoidosis while providing an avenue to further elucidate a possible antigen. While corticosteroids are often used as a first line of defense, unacceptable side effects may occur, leading to the implementation of alternative therapeutics. Alternatively, Disease Modifying Anti-Rheumatic Drugs, antimalarial drugs, Tumor Necrosis Factor Alpha (TNF- $\alpha$ ) antagonists and antimicrobial drugs have recently been implemented with beneficial results. In this review we discuss potential causative antigens, diagnostic challenges associated with sarcoidosis and review current therapeutics.

**Keywords:** Sarcoidosis; Tumor necrosis factor; Bronchoalveolar lavage fluid; Acid fast bacilli; High performance liquid chromatography; Polymerase chain reaction

### Introduction

Sarcoidosis is an idiopathic multisystem disorder characterized by noncaseating epithelioid granulomas predominately affecting lungs and lymph nodes, but with potential to affect any organ system [1]. Granuloma formation is thought to occur in response to putative organic or inorganic antigens eliciting a delayed hypersensitive immune response in susceptible individuals and ultimately segregating the poorly degradable antigen [2,3]. Sarcoidosis shares similarities in development to other infectious granulomatous diseases, such as berylliosis and tuberculosis, while exhibiting characteristic histological patterns of a Th1 immune response and more recently a Th17 immune response [4].

While a small percentage diagnosed with sarcoidosis is asymptomatic, the remainder observes a range in severity. Progression from acute to chronic sarcoidosis occurs in 10-30% diagnosed, leading to debilitation or death [1]. Variation in severity is thought to have an

epidemiological link implicating genetic predisposition, race, gender, familial clusters and environmental or occupational factors. The elusive etiology and non-discernible histology associated with sarcoidosis have provided many diagnostic challenges. Without definitive diagnostic criteria, sarcoidosis remains a multistep diagnosis of exclusion.

### Historical Perspective and Clinical Manifestations

The first person with confirmed sarcoidosis skin lesions was described by Johnathan Hutchinson in 1880 [5]. It was not appreciated until much later that the most common clinical manifestation of sarcoidosis is the lungs, involving ~90% of patients. Other common sites of involvement are the skin, of which 10% of patients with cutaneous sarcoidosis will have no lung involvement. Involvement of the eyes with uveitis being more common, the central nervous system and the heart are also noted.

Autopsy series demonstrates that ~two-thirds of sarcoidosis patients have cardiac involvement, but it is clinically significant in less than half. Sarcoidosis involvement of the kidneys, genito-urinary tract

and bones has also been reported. In fact, sarcoidosis can involve virtually any organ [6].

## Discussion

### Antigenic challenges

Evidence of a transferrable antigen eliciting an immune response was established through intracutaneous injection of sarcoidosis tissue suspension into individuals suspected of having sarcoidosis [7]. Positive or negative detection of granulomas after a 4 to 6 week period became the basis for the Kveim Siltzbach test [7]. In favor of a transmissible antigen between humans is donor acquired sarcoidosis development, in which naive recipients receive transplant tissue from a donor suspected of having active sarcoidosis, with a subsequent diagnosis of sarcoidosis in the recipient [8,9]. A case control study providing evidence of possible airborne transmissibility conducted with a cohort on the Isle of Man concluded that 40% of newly diagnosed sarcoidosis patients had previous contact with a sarcoidosis patient, whereas 1% of newly diagnosed had no contact with sarcoidosis patients [10,11]. Utilizing animal models, additional support for a transmissible antigen was provided by generation of sarcoidosis in mice after intraperitoneal, intravenous, or injection of human sarcoidosis homogenates [12-17], as well as development of granulomas in rabbits after injection of Bronchoalveolar Lavage Fluid (BAL) from sarcoidosis positive patients [18]. Many putative antigens have been proposed to induce sarcoidosis granuloma formation; however, consensus of a definitive antigen is lacking. Rather, efforts have served to narrow the field of potential causative antigens while converging efforts on the most probable. With lungs observing the highest incidence rate, efforts have primarily focused on antigens with airborne transmissibility [19,20].

### Mycobacterium

Mycobacteria are one of the most widely researched etiological agents predicted to induce or stimulate progression of granuloma formation in sarcoidosis. Driving these investigations is similarities that arise in histological and clinical presentation between sarcoidosis and tuberculosis [21]. Utilized since 1882, microscopy and Acid Fast Bacilli (AFB) staining is a widely used inexpensive method to detect Mycobacteria [22]. Two types of stains are commonly used. A basic fuchsin stain combined with light microscopy allows detection in 15 minutes, but requires viewing many fields before a definitive judgment is made [23]. Alternatively, auramine-rhodamine fluorochrome staining improves sensitivity and detection time, but requires costly fluorescent microscopes [22,23]. Detection of cell wall deficient *Mycobacterium*, or L-form, is problematic because a cell wall rich in mycolic acid is needed for detection by AFB stain [24]. Although AFB and culturing methods are widely used they suffer from low sensitivity, requiring a minimum of  $5 \times 10^3$  bacilli per mL of sample, and have yet to detect *Mycobacterium* in sarcoidosis lesions [23].

Culturing *Mycobacterium* provides an avenue of replication to increase bacterium copy number. Challenges arising from liquid and solid medium culturing are delayed diagnosis by three to six weeks, or longer if isolated *Mycobacterium* is dormant, and contamination or false positives from *Mycobacterium* species other than tuberculosis [25,26]. Liquid medium culturing improves diagnostic time, but is prone to contamination [22,25]. Growth time is improved by implementing automated or semi-automated rapid culture techniques [27]. Automated detection has extinct semi-automated detection due

to exhausting disposal of radioisotopes and the relative ease of measuring gas pressure, carbon dioxide production, or oxygen consumption in automated systems [22,27].

Improvements in specificity proceeding detection of *Mycobacterium* growth allow for identification of *Mycobacterium* species. Such detection methods include probing, High Performance Liquid Chromatography (HPLC) and Polymerase Chain Reaction (PCR) analysis [22,27]. A limited number of probes for ribosomal RNA can aid in differentiating *Mycobacterium* species with specificity cresting 90% [28]. HPLC provides rapid detection of mycolic acid variations between *Mycobacterium* species while improving versatility over probing methods [27] Initial costs of equipment hamper widespread use of HPLC for *Mycobacterium* detection. PCR analysis of extracted material from serial diluted specimens of a known concentration puts the assays sensitivity at five microorganisms [29]. Isolation of genomic material from *Mycobacterium* is problematic and includes a multistep process of eliminating PCR inhibitors and limiting false negatives [30]. DNA extraction is also complicated by the lysing process of mycobacterial cell walls [31]. The methods used to increase specificity must be preceded by isolation of intact *Mycobacterium* and positive detection through staining or culturing. Detection challenges also arise when whole bacterium are absent, as culturing of *Mycobacterium* from sarcoidosis lesions has not been successful.

Utilizing molecular mechanisms of detection increases sensitivity and specificity while diminishing the duration of diagnosis. Initial Quantitative Polymerase Chain Reaction (qPCR) analysis detected multiple *Mycobacterium* species as causative antigens using slightly different methods and various primers [32]. Direct sequence analysis by Li, et al. [33] showed sequence overlap with *Mycobacterium* species (*tuberculosis*, *avium*, *kansasii* and *marinum*), whereas Popper, et al, detected *Mycobacterium avium* IS1110 sequence and Drake, et al. [34] predominately detected *Mycobacterium tuberculosis* using primers for 16s rRNA, as well as rpoB, but also detected *gordoniae*, *kansasii* and *avium* in sarcoidosis patients [32-34].

Advances in serological assays have pinpointed immunomodulatory antigens that elicit immune responses and may be useful for diagnosis of sarcoidosis [35]. Proposed antigens driving an exaggerated Th1 response were identified using Enzyme-linked Immunospot Assay (ELISPOT) analysis to detect Interferon- $\gamma$  secretion from sarcoidosis peripheral blood mononuclear cells (PBMC) in response to Early Secreted Antigenic Protein (ESAT-6), Catalase-Peroxidase (KatG) or Superoxide Dismutase A (Sod-A) peptides [36,37]. Analysis of BAL cells from pulmonary sarcoidosis patients allows for perspective of cellular response at the site of active disease. Stimulating sarcoidosis BAL cells using ESAT-6 or KatG was followed by subsequent detection of IFN- $\gamma$  in 73% of CD4<sup>+</sup> T-cells as well as 61% of CD8<sup>+</sup> T-cells [38]. In addition IFN- $\gamma$  and IL-2 were detected by extracellular cytokine detection methods [39]. Localization of signals using MALDI-IMS, were consistent with the presence of ESAT-6 in sarcoidosis granulomas [40]. Using MALDI-TOF mass spectrometric peptide fingerprinting Song, et al. [41] identified KatG from *Mycobacterium tuberculosis* in sarcoidosis tissues [41]. The presence of KatG was confirmed by immunoblot analysis, with anti-mKatG monoclonal antibodies and by localization of KatG DNA using in situ hybridization [41].

Precipitating immune complexes of *Mycobacterium tuberculosis* implicated heat shock proteins as biomarkers linked to stage I and II of sarcoidosis [35]. Increases in Mtb16 were prevalent in stages I and II

over control samples, while Mtb65 showed a significant increase in stage II only [35]. While *Mycobacterium* has provided strong evidence to indict itself as a possible causative antigen of sarcoidosis, *Propionibacterium acnes* is currently the only organism that has been cultured from sarcoidosis lesions. Providing definitive evidence that *P. acnes* is a causative antigen is not without its own set of challenges.

### Propionibacterium

*Propionibacterium acnes* (*P. acnes*) is a facultative anaerobic gram positive rod shaped bacterium indigenous to mucosal surfaces and a member of the skin flora found on most healthy adults [42]. *P. acnes* typically exploit a commensal human relationship, but recent awareness of its pathogenic capacity has implications of an opportunistic pathogen [43]. An act of complicity was established when cultured lymph node biopsy homogenates tested positive for *P. acnes* in 78% of sarcoidosis tissue while only testing positive in 21% of non-sarcoidosis tissue [44,45]. Isolation of *P. acnes* was the first step in unraveling its etiological role; however, detection of *P. acnes* in 21% of non-sarcoidosis tissue samples leads to speculation about its granuloma inducing potential [45].

A minimum of 500 *P. acnes* colony forming units was detected in lung and lymph node (mediastinal, gastric and intestinal) tissues of patients with diseases other than sarcoidosis, which questions its true pathogenicity [46]. Injecting *P. acnes* into sensitized rats caused granuloma formation [47]. Additionally, at the cellular level sarcoidosis patient BAL cells exhibit evidence of a hypersensitive reaction to *P. acnes* extract denoted by increased proliferation and increased IL-2 production primarily by alveolar lymphocytes [48-51]. Implementing an ELISA system, increased IL-6 secretion from alveolar macrophages was detected in 33% of sarcoidosis patients upon stimulation with *P. acnes* [49]. Alveolar macrophages are also the primary cells which secrete IL-1 and TNF- $\alpha$  cytokines in sarcoidosis granulomas [52]. Analysis of BAL cells by qPCR detected *P. acnes* in 70% of sarcoidosis patients and in 23% of non-sarcoidosis patients; however, in situ localization detected *P. acnes* in alveolar macrophages only [53].

To combat possible contamination of cultured isolates with commensal *P. acnes* and to overcome the slow growth delay typically associated with bacteria culturing, additional immunological and

molecular mechanisms were utilized. Using quantitative PCR analysis, detection of *Propionibacterium* species yielded varying results from 0-80% detection in sarcoidosis patients to 0-30% in control patients [54-56]. Without 100% detection of *P. acnes* in sarcoidosis patients additional quantitative analysis detected *P. granulosum* in 60% of sarcoidosis patients, including those with undetectable *P. acnes* [56]. Detection of two different *Propionibacterium* species in conjunction with detection of *Mycobacterium* in three sarcoidosis patients indicates multiple bacteria may be capable of eliciting a granuloma forming response [56].

Although contamination of samples has been minimized by utilizing qPCR analysis for 16s rRNA, primer specificity may provide challenges with shared phylogeny between *P. avidium* and *P. acnes* [57]. Comparative data analysis using qPCR and in situ hybridization established a link between indigenous *P. acnes* and sarcoidosis [2]. Utilizing control and sarcoidosis lymph nodes subject to qPCR and in situ hybridization showed a correlation in increased 16s rRNA expression and in granulomatous signal localization [2]. With *P. acnes* implicated as a granuloma inducing bacterium in sarcoidosis a target antigen eliciting immune response was sought.

A Th1 inducing antigen elucidated using sarcoidosis patient's serum screened against a  $\lambda$ gt11 genomic DNA library of *P. acnes* was *P. acnes* unique recombinant protein (RP 35), which is a fragment of trigger factor protein [58]. Additionally, increases in RP35 antibodies, specifically increases in IgG and IgA of 18% and 39% respectively were seen in sarcoidosis patients BAL [58]. Continuing this momentum by comparative analysis of sarcoidosis against healthy controls was Furusawa et. al who reported *P. acnes* stimulation of PBMC increased IL-2 and IL-12 mRNA expression while decreasing IL-17 mRNA [59].

### Genetics

Although sarcoidosis shows characteristic histological patterns, its pathophysiology may be linked to genetic predisposition of specific Human Leukocyte Antigen (HLA) polymorphisms, T-cell preprogrammed and memory responses, as well as antigen specificity [60]. Efforts have focused on HLA polymorphisms that increase sarcoidosis susceptibility or provide protection; however HLA associated candidates have also drawn attention (Table 1).

Gene	Aberation Type	Aberation	Prognosis	Ethnicity
BTNL2	SNP	rs2076530	predisposition	German, Portugese, Danish and Caucasians (low level risk)
			No effect	African American and Japanese
	SNP	rs2076530+rs9268480	Predisposition	European Americans and African Americans
NOD	Deletion	6 Base Deletion Nod2	Predisposition	
NOTCH	Polymorphism	Polymprphism to Notch4	Predisposition	
Anxa11	SNP	rs1049550	Predisposition	German and African Americans
	SNP	rs1049550*T	Protective	

**Table 1:** Genetic aberrations of HLA associated genes and prognosis of ethnic groups harboring them.

An HLA associated candidate garnering significant interest is butyrophilin-like 2 (BTNL2), a member of the immunoglobulin gene

superfamily that has shown efficacy in inhibiting T-cell proliferation and cytokine production through a putative receptor [61,62]. A

genome wide search established diathesis between sarcoidosis and chromosome 6p21, which initiated Single Nucleotide Polymorphism (SNP) analysis [63,64]. The SNP rs2076530 on exon 5 of the BTNL2 gene produces a genetic variant that shows predisposition in German, Portuguese and Danish patients to sarcoidosis and when combined with the loss of at least one protective MHC class II molecule further predisposition to persistent sarcoidosis [63,65-68]. This truncated splice site mutation correlates with increased risk of sarcoidosis development independent of HLA class II genes in 23% of caucasian subjects, but failed to link with susceptibility in African American subjects, as well as Japanese subjects [63,64,69,70]. Homozygosity of rs2076530 on both alleles shown in familial studies increases the risk of sarcoidosis and increases familial genetic susceptibility due to inheritance of the SNPs [71]. Interestingly, the haplotype combining rs2076530 with another nearby SNP (rs9268480) is associated with sarcoidosis in both European Americans and African Americans [70]. The severity and persistence of pulmonary sarcoidosis has recently shown direct correlation with levels of rs2076530, implicating it as a marker for risk as well as severity [72].

NODs are a protein family of cytosolic receptors containing a nucleotide-binding oligomerization domain [73]. Nod1 is expressed in many cell types, whereas Nod2 primarily is expressed in monocytes [73]. The importance of Nod1 and especially Nod2 is exhibited in the innate immune response by recognizing cell wall components of invading microorganisms [73]. However, mutations in Nod2 have been linked with susceptibility of inflammatory granulomatous disorders [74]. A genetic aberration linked to early onset sarcoidosis is a six base deletion in the Nod2 gene [75]. Involvement of Nod2 protein in the immune response hinges on its ability to recognize invading bacterium and activate NF-κB; however, when the Nod2 gene is mutated NF-κB may be constitutively activated [76-79]. Nod2 and the functionally similar Nod1 may serve as an early factor in triggering granuloma formation.

Genome wide association studies discovered NOTCH, a novel independent locus associated with sarcoidosis susceptibility in African and European Americans [80]. Recent studies have implicated the importance of NOTCH with normal T-cell development [81]. Specifically, polymorphisms have been identified with the gene encoding NOTCH4, leading to speculation about its role in sarcoidosis susceptibility [80].

Annexin11, encoded by the Anxa11 gene, belongs to a group of calcium-dependent phospholipid-binding proteins. The function of Annexin11 has been linked to Calcium signaling, apoptosis, autoimmune disorders and terminal stage of cell cytokinesis [82-84]. Annexins have conserved C-terminal domains containing calcium-dependent phospholipid-binding sites and unique N-terminal domains. Polymorphisms occurring within or downstream of the Anxa11 gene have been implicated by genetic linkage studies as potential contributors to the risk and severity of sarcoidosis [85]. Two additional sarcoidosis associated ANXA11 SNPs were recently identified in African Americans and exhibit a strong correlation to radiographically persistent disease (stage IV) [86]. ANXA11 shares similar functionality with class II HLA-(DRB1, DRB2 and DRA) in their detectability by immune response or tolerance inducing exosomes [87,88].

Presentation of antigens by APCs is undertaken by HLA class I which present antigens to CD8 T-cells and HLA class II which present antigens to CD4 T-cells. The class I HLA-B8 antigen was the first associated with susceptibility of sarcoidosis. HLA class II has five

different isotypes designated HLA-(DM, DO, DP, DQ and DR), of which DP, DQ and DR directly present antigen to CD4 T-cells. Each isotype is composed of one α and one β chain encoded by α and β genes of the class 2 region on chromosome 6. With multiple alleles capable of generating each isotype the yield is multiple allotypes. With the ability of MHC class II to directly present antigen to CD4 T-cells, HLA class II polymorphisms are of particular importance for determining susceptibility or protection from sarcoidosis development (Table 2). A previous multicenter study (A Case Controlled Etiologic Study of sarcoidosis) performed in the United States identified patterns that indicate carriers of HLA-DRB1\*1101 and HLA-DPB1\*0101 alleles are at an increased risk for sarcoidosis development [89]. A genetic association study for familial linkages in African American patients determined HLA-DQB1 alleles could be associated with susceptibility or protection [90]. Phenotypic analysis of Swedish patients that develop Lofgren syndrome or see disease resolution may associate this risk with carrying HLA-DRB1\*03 [91,92]. While most studies have linked certain alleles to a poor prognosis, a study with Dutch and British patients conveyed good prognosis when carrying HLA-DQB1\*0201 [93]. These links clearly demonstrate a genetic predisposition to sarcoidosis when lacking or possessing certain alleles.

Allele	Association	Race	Source
HLA-DPB1*0101	susceptibility	African American	89
HLA-DQB1	susceptibility or protection	African American	90
HLA-DQB1*0201	Good Prognosis	Dutch and British	93
HLA-DQB1*0502	susceptibility	African American	89
HLA-DRB1*03	susceptibility	Swedish	91,92
HLA-DRB1*0402	susceptibility	White	89
HLA-DRB1*1101	susceptibility	African American and White	89
HLA-DRB1*1201	susceptibility	African American	89
HLA-DRB1*1401	susceptibility	White	89
HLA-DRB1*1501	susceptibility	White	89
HLA-DRB3*0101	susceptibility	African American	89

**Table 2:** HLA class II single nucleotide polymorphism effects on sarcoidosis susceptibility vary by ethnicity.

### Therapy

A diagnosis of sarcoidosis established by exclusion of other granulomatous diseases requires histological conformation as well as systemic disease evidence [60]. Diagnosis adheres to a diagnostic algorithm but is still problematic since it usually requires multiple examinations after patients have prolonged diagnosis by an average of three months [94]. A clinical presentation representative of sarcoidosis classical characteristics helps eliminate the statistical likelihood of other diseases; however, without a definitive diagnostic tool this currently serves as the best mechanism of diagnosis [60]. If symptomatic and a prognosis of spontaneous resolution is not projected a personalized therapeutic plan is formulated.

## Corticosteroids

Treating acute and chronic sarcoidosis begins with corticosteroids, the nearly unanimous first line of defense. [1,95,96]. Corticosteroids such as Prednisolone and its precursor Prednisone inhibit inflammation through stimulation of anti-inflammatory genes, as well as through repression of inflammatory genes such as Interleukin 1, Interferon-gamma (IFN- $\gamma$ ) and Tumor necrosis factor (TNF)- $\alpha$  [97,98]. Corticosteroids can also inhibit NF- $\kappa$ B activity and restore potent Th1 immune responses back to a normal Th1 and Th2 balance [99-101]. Prolonged corticosteroid use can lead to toxicity, resistance and secondary infections. Developing resistance to corticosteroids is characterized by increased (TNF)- $\alpha$  release by alveolar macrophages, implicating anti TNF- $\alpha$  as an alternative or secondary therapy [102].

## Disease Modifying Anti-Rheumatic Drugs

Methotrexate and its less toxic analog leflunomide have been billed as the preferred corticosteroid sparing agents for pulmonary sarcoidosis [103,104]. Methotrexate is an anionic bicarboxylic acid at physiological pH [105]. Sharing a chemically similar structure to folic acid, with the exception of an amino group attached to the pteridine ring and the addition of a methyl group at position N10, methotrexate blocks folic acid metabolism by competitively inhibiting dihydrofolate reductase [105]. Administering methotrexate to 50 patients for a minimum of two years demonstrated usefulness in 80% and has shown efficacy in patients with less than two years of methotrexate treatment; however, of the 50 treated for longer than 2 years toxicity was seen in 15% [106].

Leflunomide, the less toxic analog of methotrexate, acts as an anti-inflammatory by inhibiting activity of cyclooxygenase 2, as well as a pyrimidine synthesis antagonist by blocking dihydroorotate dehydrogenase activity [107]. This inhibits cell cycle progression which in turn blocks expansion of activated lymphocytes and suppresses release of TNF- $\alpha$  [108]. The first use of Leflunomide to treat sarcoidosis showed efficacy in decreasing angiotensin converting enzyme levels, returning C-reactive protein levels to normal and eventual resolution of sinonasal sarcoidosis [109]. Subsequent studies recruiting leflunomide demonstrated partial or complete resolution in 78% of patients with ocular or pulmonary sarcoidosis, complete or partial resolution in 82% with extrapulmonary sarcoidosis and regression in two patients with cutaneous sarcoidosis [110-112].

Azathioprine is a purine analog which is converted in vivo by thiopurine-S-methyltransferase to its active form 6-mercaptopurine [108]. Once activated it represses cellular immunity by inhibiting cell proliferation of predominately B and T lymphocytes through blockage of purine synthesis [108]. Recently, azathioprine was directly compared to methotrexate as a steroid sparing second line of defense alternative. Results were indicative of similar increases in pulmonary function for both drugs; however, more infections requiring antibiotics intervention were seen in the azathioprine group [113].

Mycophenolate is an immunosuppressant drug which inhibits inosine monophosphate dehydrogenase, the reversible inhibitor mycophenolate, and blocks an integral step of purine synthesis. This in turn suppresses the immune response by blocking lymphocyte proliferation. These drugs ultimately block RNA and DNA synthesis while inducing cell cycle arrest [105]. Mycophenolate is not commonly implemented; however, in small trials it has shown effectiveness in treating cutaneous, ocular and neurological sarcoidosis [114-116].

## Antimalarials

Chloroquine and its derivative hydroxychloroquine are immunomodulating agents which show efficacy in treatment of sarcoidosis by impairing antigen presentation and cytokine release, but also exhibit a lag phase of maximal effectiveness [108,117]. With a delayed maximal effectiveness of several months it is recommended to combine with corticosteroids for the initial lag phase of chloroquine and then wean the corticosteroids. Hydroxychloroquine has shown less toxicity than chloroquine; however, chloroquine appears to be more effective. With only limited or small trial results available the true efficacy of antimalarials has not fully been determined; however, they have shown effectiveness in cutaneous and pulmonary sarcoidosis [118,119].

## TNF- $\alpha$ Antagonists

A blockade of TNF- $\alpha$  with infliximab is thought to reverse the inflammatory response. Infliximab is a murine derived chimeric monoclonal antibody that has superiority over placebo in improving lung function in pulmonary sarcoidosis [120]. A blockade of TNF- $\alpha$  with infliximab is thought to reverse the inflammatory response and has shown promise in patients with FVC less than 70% [120,121]. Additionally, the presence of reticulonodular infiltrate on a chest roentgenogram [122] or detection of C-reactive proteins is associated with better treatment response [123]. A possible alternative administered in the event of allergic reaction against the murine portion of Infliximab is the fully humanized adalimumab due to potentially less frequent allergic reactions [108]. Pentoxifylline is a xanthine derivative with recent evidence of TNF- $\alpha$  synthesis inhibition by phosphodiesterase inhibition and accumulation of cyclic adenosine monophosphate [108].

## Antimicrobial

A link between sarcoidosis and fungi was established through epidemiological studies with fungi exposure correlating to increased risk of sarcoidosis [124-126]. Analyzing BAL fluid of sarcoidosis patients, Tercelj, et al were able to detect increased levels of the fungal cell wall component  $\beta$ -glucan, which has previously shown immunomodulatory effects and granuloma inducing potential [127]. While antifungal therapy shows promise treating secondary infections associated with sarcoidosis, such as *Aspergillus*, *Histoplasma capsulatum*, *Blastomycosis dermatides*, and *Cryptococcus neoformans*, it has recently shown efficacy in improving sarcoidosis. A comparative study analyzing groups treated with antifungal therapy or corticosteroid therapy resulted in improved x-ray scores for both groups, but with a greater improvement after antifungal therapy [125]. A follow-up study after treatment with antifungal therapy demonstrated persisting effects with no recurrences [125]. Antifungal therapy also shows efficacy in treating osseous sarcoidosis, determined by improvements in soft tissue swelling, as well as bone cortex density and thickness [128].

Treatment of sarcoidosis using antibacterial therapy has shown efficacy; however, both immunomodulatory and antibacterial mechanisms have been debated as the reason for improving clinical presentation. In a study with 12 patients Bachelez, et. al administered minocycline resulting in 8 patients with complete clearance of sarcoidosis, of which 7 could be completely removed from therapy, however 3 of these 7 patients subsequently required additional treatment with minocycline due to relapse [129]. Administering

minocycline to a sarcoidosis patient resulted in initial regression of lesions, but was followed by remission [130]. With detection of *Propionibacterium* in the patient an argument can be made for the antibacterial function of minocycline [130]. However, minocycline also has the ability to inhibit granuloma formation and inhibit T-lymphocyte activation and proliferation [131,132]. The rate of relapse after withdrawing minocycline points to reversal of immunomodulatory functions [130]. Previous studies have shown efficacy targeting rpoB and DNA gyrase with rifampin and levofloxacin respectively [32,133].

Concomitant levofloxacin, ethambutol, azithromycin, and rifampin (CLEAR) is a synergistic antibiotic regimen recently utilized as a clinical trial therapy for chronic pulmonary sarcoidosis exhibiting decreased levels of LCK and NF- $\kappa$ B [134,135]. Clonal exhaustion, or anergy, of T-cells is characterized by a decrease in NF- $\kappa$ B, CD3 delta and LCK [136]. In the event of LCK inactivation, no phosphorylation of CD3 tyrosine residues on activation motifs occurs which blocks CD4 T-cell response [136]. Restoration of LCK and NF- $\kappa$ B expression by antimycobacterial therapy in chronic sarcoidosis patients improves forced vital capacity while also further indicting *Mycobacterium* as a putative contributor and CLEAR as a therapeutic alternative [134].

## Conclusion

Sarcoidosis is a granulomatous disease of unknown etiology caused by antigenic stimulation of the immune system with disease susceptibility determined by genetic predisposition, race, gender, familial clustering and environmental or occupational factors. Many methods have been utilized in previous attempts to elucidate a definitive etiological antigen. At the forefront of these attempts resides *Mycobacterium tuberculosis* and *Propionibacterium acnes* with the most implicating evidence. Both elicit immune responses in individuals diagnosed with sarcoidosis; however, *Mycobacterium* has not been isolated from sarcoidosis lesions and *P. acnes* have been isolated from sarcoidosis lesions as well as negative controls. The absence of *Mycobacterium* from sarcoidosis lesions may be attributed to limited sensitivity of current detection methods. With evidence of immunological responses to *Mycobacterium* and *Propionibacterium*, future sarcoidosis analysis must include pathogenic bacteria and indigenous bacteria. Antimicrobial therapeutic regimens such as CLEAR have revealed benefits in sarcoidosis patients while providing additional evidence of a Mycobacterial burden.

Formation of granulomas serve to contain foreign antigens; however, immune system modulation by foreign antigens may allow the granuloma to serve as a protective capsule for a latent infection triggering a persistent immune response balanced by antigen stimulated pro and anti-inflammatory signals until the immune system weakens, possibly through anergy, and allows active infection. Immunoinhibition may have adverse effects not only due to immune suppression, but also through release of segregated antigens and subsequent sarcoidosis relapse or stimulation at other sites. With a waning immune response, systemic release of a segregated antigen from the granuloma is imminent which demonstrates potential metastatic capabilities of sarcoidosis antigens via blood and lymphatics leading to secondary infection. Determinants of a secondary infection may include a compromised immune system as well as genetic predisposition for which a correlation has been demonstrated between allele variation and susceptibility of sarcoidosis in various organ systems. A plausible explanation for a diagnosis of sarcoidosis in systems other than those directly encountering airborne antigens,

without involvement of other organs, is spontaneous resolution of a primary infection before diagnosis. This strongly correlates with postulates of an airborne antigen and a high incidence rate of sarcoidosis in lung tissue. Creating viable detection, prophylactic and therapeutic options for sarcoidosis will take a multifaceted approach of elucidating the causative antigen while deciphering genetic involvement in susceptibility. Signs point to the cause of sarcoidosis being multifaceted and therapeutic options must be tailored on per patient basis. Also bacterial resistance must be taken into consideration when a therapeutic plan is constructed. Sarcoidosis appears to have a strong genetic link to susceptibility with multiple antigenic determinants. Further studies are needed to fully understand the underlying mechanism and cause of sarcoidosis; however, until then it remains a multistep diagnosis of exclusion.

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