Short Conceptual Overview

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Particulate matter granulomas masquerading as sarcoidosis: a diagnostic dilemma

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Abstract: Sarcoidosis is a multiorgan disease with no single etiology. Evidence over the years points to complex interactions between environmental and genetic factors and a hypersensitive immune response to these insults. This brief overview discusses the uncertainty in the diagnosis of sarcoidosis versus other granulomatous diseases masquerading as sarcoidosis. The diagnostic dilemma is highlighted by a brief case review. The development of newer techniques in molecular biology and the identification of a panel of biomarkers in the future with appreciable specificity and sensitivity would help in the process. Future studies to determine receiver operating curves (ROC) using multiple biomarker combinations would help develop robust testing. More in-depth studies are also needed for defining the immunological basis of sarcoidosis because recent studies implicate Th17 cells in addition to the Th1 cell pathway. It is very likely that direct exposure to environmental agents and systemic distribution of these agents can elicit an exaggerated immune response leading to multiorgan granuloma formation mimicking sarcoidosis. A genetically susceptible host may be necessary to complete the granulomatous response to the particulate matter.

Keywords: particulate matter granulomas; sarcoid-like disease.

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Introduction

The diagnosis of sarcoidosis remains a challenge. No single etiology has ever been ascribed to this disease. Evidence over the years points to multiple etiologies including occupational and environmental exposure to inflammatory stimuli and a hypersensitive immune response by the host to these insults (1). Granulomatous lung disease has been historically reported in miners and construction workers exposed to dust and mineral particles (1, 2). A case report described occupational exposure to agents used in dental offices that resulted in a sarcoidosis-like presentation in a dental surgeon (3). Indoor air quality and microbial pathogens also act as triggers for an inflammatory response (4). A genetically susceptible microenvironment will actively contribute to the generation and progression of a granulomatous sarcoid-like illness. In a murine model of chronic granulomatous lung inflammation, multiwall carbon nanotubes have been shown to be responsible for the inflammatory response (5). Such a variety of causes of the development of sarcoidosis make it a difficult disease to diagnose with certainty. This conceptual overview includes a case report to illustrate this diagnostic dilemma.

Diagnosis of particulate matter granulomatous disease

The case presented here suggests that particulate matter exposure in the development of granulomatous inflammation leading to sarcoid-like disease has important consequences in defining prognosis and treatment. A 36-year-old male with a history of ‘pulmonary sarcoidosis’, chronic renal insufficiency, obstructive sleep apnea, systemic/pulmonary hypertension and Grave’s disease presented with increasing fatigue and malaise. The patient was originally diagnosed with sarcoidosis by mediastinal lymph node biopsy. On histopathological examination, the mediastinal lymph node was replaced

by non-necrotizing granulomas and fibrosis consistent with sarcoidosis. Gram, acid fast bacillus (AFB) and Congo red stains were negative. However, no examination of the tissue was performed under polarizing light at that time. Treatment was initiated with oral corticosteroids. The patient subsequently presented with dilated cardiomyopathy and malignant dysrhythmia (inducible monomorphic ventricular tachycardia) and was diagnosed by endomyocardial biopsy with infiltrative sarcoidosis of the heart. He was treated with high dose oral steroids resulting in steroid-induced diabetes mellitus. Despite treatment, he continued to deteriorate in terms of his cardiac function leading to an orthotopic heart transplant 5 years post-original presentation. Histopathological exam of the explanted heart showed poorly formed granulomata and areas of fibrosis. However, review of the biopsies of the explanted heart under polarizing light microscopy noted particulate matter in cardiac tissue. Additional particulate matter was localized by a polarizing light microscopic examination of lymph nodes and of a blood vessel from the explanted heart. Retrospectively, particulate matter was present in the earliest biopsy, changing his diagnosis from sarcoidosis to particulate-matter-induced 'sarcoid-like' granulomatous disease. Post-transplant he received standard anti-rejection medications (mycophenolate mofetil, azathioprine and prednisone). All endomyocardial biopsies subsequently showed neither granulomas nor rejection. Prior hilar adenopathy and interstitial lung disease and serum angiotensin-converting enzyme (ACE) activity were undetectable.

On retrospective analysis, cardiomyopathy in this patient that progressed despite steroid treatment was possibly the result of all of his co-morbidities. The lack of adequate definitive molecular markers and facilities to perform polarizing microscopy at the time masked the sarcoid-like granulomatous disease diagnosis. Additionally, a particulate matter disease diagnosis is favored in this case because of the clinical course that followed his cardiac transplant. His lymphadenopathy and interstitial lung disease resolved. Levels of ACE, though not specific for sarcoidosis, were undetectable, further negating diagnosis of 'true sarcoidosis'. As tissue from the explanted heart was not stored indefinitely, newer molecular markers suggested in recent literature could not be tested in this patient.

This case illustrates the diagnostic dilemma of sarcoidosis, a diagnosis of exclusion. However, routine polarizing light examination of the biopsies was not originally performed to search for the presence of particulate matter. The presence of particulate matter within non-necrotizing granulomas of a patient with sarcoidosis would have negated the diagnosis of sarcoidosis. Such particulate matter has been presumed to be calcium oxalate (6). In the Reed/Anderson study, the crystals in granulomas were visible when stained with Ziehl-Nielson but not with hematoxylin and eosin (H&E), Gomori's methenamine stain (GMS) or periodic acid-Schiff reagent (PAS). However, Visscher et al. (7) noted crystalline inclusions in lung granulomas were easily visible with H&E stain (7). These crystalline inclusions were subsequently found by spectroscopy to be mainly calcium oxalate and calcium bicarbonate. In this patient, all particles have been noted on H&E staining under polarizing light. In a prior study from our group, 57% of 58 tissue biopsies showed particulate matter in granulomas consistent with sarcoid-like disease (8). The presence of polarizable particulate matter in the lymph glands, cardiac tissue and blood vessels point to a systemic nature of particulate matter disease leading to target organ damage.

Role of oxalate crystals in granuloma formation

Calcium particles in the biopsies noted are believed to be endogenously derived (9) and appear to be part of the granuloma. Their exact significance is unclear in the entire process, but they could act as a nidus. Calcium oxalate has been implicated in the sequestration of iron and the acute reactant ferritin. *In vitro* experiments have shown that in alveolar macrophages, oxalate crystals form chemical coordinate bonding with iron, which leads to increases in production of ferritin and contribute to giant cell formation. Interleukin 8 and Interleukin 6 have been shown to be stimulated by calcium oxalate in cultured respiratory epithelial cells. These cytokines play a role in granuloma formation. In a rat model system, granuloma formation was found to be associated with an accumulation of pulmonary iron and ferritin, suggesting a role for persistent oxidative stress. Such insults may contribute to granuloma formation in the lungs (10).

Interaction between environmental, genetic and immunological factors

The involvement of environmental agents in the development of granulomatous disease has been documented in the literature (1–5, 11–14). Sarcoid-like pulmonary disease has been reported in New York City fire department rescue

workers, which is suggestive of a strong environmental influence in the disease process (12–14).

The genetic basis has been investigated in diverse study populations. In a Japanese study, the *HLA-DRB1* allele has been implicated as a genetic factor (15). Other studies evaluated Toll-like receptors (TLRs), which are a major part of innate immunity. TLR genes are therefore considered candidates for susceptibility to sarcoidosis. In a gene linkage analysis of 83 families and 180 affected siblings, *TLR4* was the most promising marker for sarcoidosis but failed to confirm the *TLR4* polymorphisms *Asp299Gly* and *Thr399Ile* as susceptibility markers, suggesting that other variants close to this locus may be conferring susceptibility (16). In an earlier study of gene polymorphisms, TLR 4 polymorphisms were associated with the chronic phase, but not the acute phase, of sarcoidosis (17). A meta-analysis of six known polymorphisms of TNF-α and -β showed that the TNF-β gene *A252G* polymorphism may be a risk factor for the development of sarcoidosis (18).

Whether the mechanism of augmented T-cell proliferation in sarcoidosis is a cause or effect of the disease pathogenesis is unclear (19). Th1 immunity is thought to play a large role in sarcoidosis; however, recent data point to IL-23 and Th17 cells (20). IL-35 and IL-27, which have immunosuppressive properties, have been implicated, but future studies may be needed to elucidate their actual role (20). IL-18 was upregulated in sarcoid physiology but not in normal controls. IL-18 induction activated transcription factors AP1 and NF-κ B and upregulated IL-2 gene transcription/IL-2 protein production. The addition of IL-18 to normal epithelial lining fluid (ELF) also induced IL-2 mRNA accumulation, whereas correspondent depletion of IL-18 from sarcoid ELF using neutralizing antibodies abolished all of the effects. These data suggest a role for IL-18 in the pathogenesis of pulmonary sarcoidosis via activation of AP1 and NF-κ B, leading to enhanced IL-2 gene and protein expression and T-cell activation (21).

Interleukin-33 (IL-33) is an inflammatory cytokine upregulated in sarcoidosis but not significantly increased in other granulomatous conditions. IL-33 could therefore possibly be used as an adjunct biomarker in diagnosis (22). The imbalance noted in sarcoidosis between Treg and Th17 cells may open avenues for developing therapeutic protocols. This cellular imbalance appears to resolve with steroid treatment (23). Analysis of circulating memory CD4 (+) T-cell populations in sarcoidosis patients showed increased numbers of IL-17A/IFN-λ and IL-17A/IL-4 double-producing cells. Bronchial mucosal biopsies from sarcoid patients demonstrated increased IL-17A positive T-cells in the granulomatous lesions. Increased levels noted in circulation suggest a role for this pathway to be exploited in therapy using neutralizing antibodies to IL-17A (24).

Use of molecular markers to distinguish granulomatous diseases mimicking sarcoidosis

The uncertainty in diagnosing sarcoidosis may be addressed by developing a panel of biomarkers (20–24). Such an approach will help differentiate sarcoidosis from other granulomatous diseases. The advantage of identifying molecular markers is that they can also be used as therapeutic targets and for monitoring progress and as a guide to therapy. Using molecular and immunochemical staining techniques, biomarkers may be identified in granulomas, which helps distinguish sarcoidosis from its 'look-alike'. Using polarizing light and electron microscopy, particulate matter may be identified to add further definition to the etiology. Multiple levels of testing may be required to arrive at the right diagnosis. A suggested algorithm to assess granulomatous diseases is shown in Figure 1.

Prognosis and treatment of sarcoidosis versus particulate-matter granulomatous diseases

The prognosis of sarcoidosis or sarcoid-like granulomatous diseases depends on the etiology inciting the inflammatory response. Case reports of sarcoid-like disease induced by aluminum dust showed no immunological characteristics of sarcoidosis, though biopsy of the lung showed sarcoid-like epithelioid granulomas and helper T-cells in alveoli, which resolved a year after exposure was discontinued (25). In addition to steroids, a multitude of drugs including methotrexate, leflunomide, infliximab, rituximab and anakinra have been used to treat various forms of sarcoidosis and sarcoid-like diseases with no clear cut delineation of these therapies for the two diseases (26–31). This problem can be solved only if distinct etiologies can be pinned for both diseases, and differences established between sarcoid and sarcoid-like granulomatous diseases. The development of diagnostics and therapeutics also need to be tailored for different organ systems that are affected. Serum Amyloid A is another

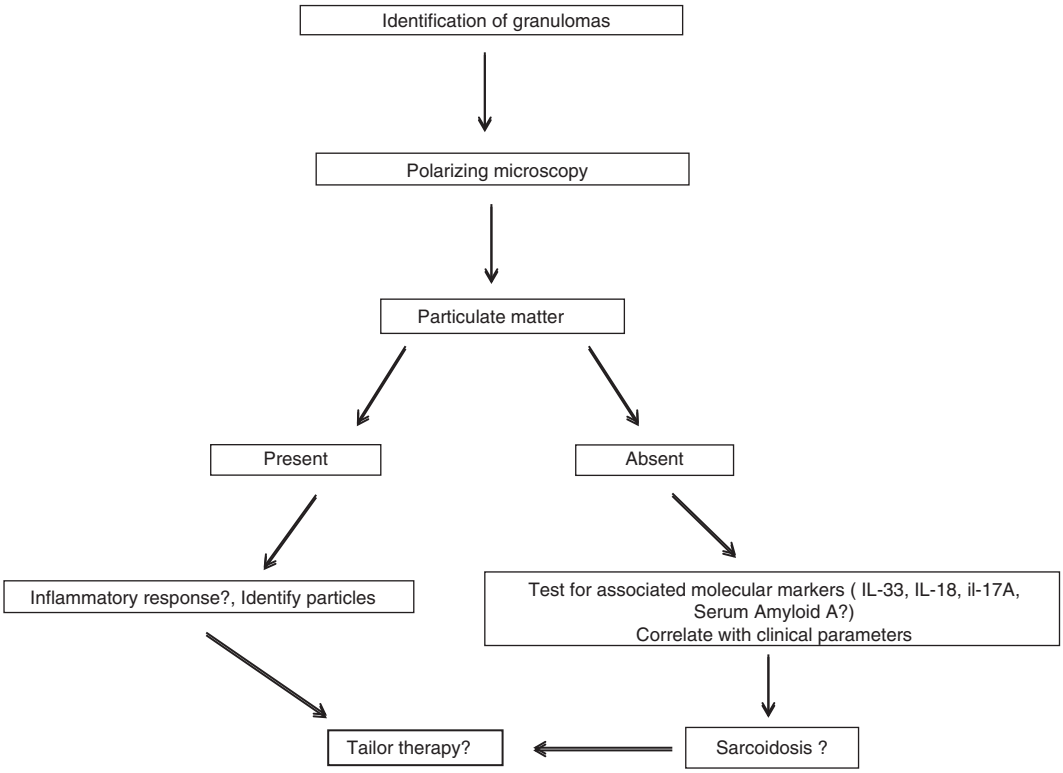

Figure 1: Suggested algorithm for assessing granulomatous diseases.

highly expressed molecule in sarcoid granulomas as compared to other granulomatous diseases (32). A panel of biomarkers in addition to advanced imaging modalities may be required to diagnose the different granulomatous diseases and pave the way to prognosis and specific treatments (33).

It is very likely that direct exposure to environmental agents and systemic distribution of these agents can elicit an exaggerated immune response leading to multiorgan granuloma formation mimicking sarcoidosis. A genetically susceptible host may be necessary to complete the granulomatous response to the particulate matter.

Conclusions

It is evident from the existing literature that differentiating 'true' sarcoidosis from sarcoid- like manifestations remains difficult (34). In this overview we have discussed the uncertainty in the diagnosis of sarcoidosis versus other granulomatous diseases masquerading as sarcoidosis. The diagnostic dilemma was highlighted by a brief case review. Development of newer techniques in molecular biology and identification of a panel of biomarkers in future with appreciable specificity and sensitivity would help in the process. Future studies to determine receiver operating curves (ROC) using multiple biomarker combinations would help develop robust testing. Further studies are also warranted for defining the immunological basis of sarcoidosis. Recent studies also implicate Th17 cells.

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