The Etiologic Role of Infectious Antigens in Sarcoidosis Pathogenesis

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KEYWORDS

• Sarcoidosis • Antimicrobials • Infectious antigens

KEY POINTS

- There is a growing body of literature supporting the role of infectious antigens, in particular mycobacteria and propionibacteria, in sarcoidosis pathogenesis.
- Immunologic studies reveal that mycobacterial virulence factors are the targets of the immune response in sarcoidosis diagnostic bronchoalveolar lavage (BAL).
- Recently, case reports and clinical trials have emerged reporting the efficacy of antimicrobial therapy on cutaneous and pulmonary sarcoidosis. Although the studies are not conclusive, they demonstrate efficacy on endpoints associated with sarcoidosis morbidity and mortality, such as forced vital capacity (FVC).

SARCOIDOSIS EPIDEMIOLOGY SUGGESTS EXPOSURE TO MICROBIAL BIOAEROSOLS

Sarcoidosis is a granulomatous disease of unknown etiology, most commonly involving the lung, skin, lymph node, and eyes.[1] Granulomatous inflammation can be initiated by infectious agents, such as fungi or *Mycobacterium tuberculosis* (MTB), or by noninfectious agents, such as beryllium (chronic beryllium disease). Analysis of sarcoidosis epidemiology suggests that infectious agents have a role in sarcoidosis pathogenesis. Investigators in A Case Control Etiologic Study of Sarcoidosis observed positive associations between sarcoidosis risk and certain occupations, such as agricultural employment, exposure to insecticides, and moldy environments.[2] Another study noted that the hospitalization admissions for African Americans with sarcoidosis in South Carolina increased with proximity to the Atlantic Ocean.[3] A unifying factor in environmental and geographic reports is the possibility of exposure to microbial bioaerosols. Natural waters; water distribution systems; biofilm in pipes; peat and potting soil; water droplets; equipment, such as bronchoscopes and catheters; and moldy buildings are natural habitats for environmental opportunistic mycobacteria.[4] Aerosolization of environmental opportunistic mycobacteria has been associated with the development of other granulomatous diseases of mycobacterial origin, such as hypersensitivity pneumonitis.[5]

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MOLECULAR AND IMMUNOLOGIC INVESTIGATIONS REVEAL MICROBIAL PROTEINS AND DNA

The inability to identify microorganisms by histologic staining or to culture microorganisms from pathologic tissues continues to be one of the strongest arguments against a potential role for infectious agents in sarcoidosis pathogenesis. As molecular analysis continues to grow in sensitivity and specificity, current culture and staining methods are known to identify less than 2% of current microbial communities present within the human biological specimens.[6,7] Advanced molecular techniques, such as deep sequencing technologies, also have demonstrated successful identification of novel microorganisms in pathologic tissues not easily identified by traditional methods.[8,9] Molecular analysis of pathologic tissue for microbial nucleic acids and proteins serves as an alternative means of identifying a putative infectious agent. Polymerase chain reaction (PCR) was used to identify the etiologic agents of Whipple disease (*Tropheryma whippelii*)[10] as well as the novel coronavirus as the agent of severe acute respiratory syndrome.[11]

A growing scientific interest involves defining the microbial community within distinct diseases, that is, microbiome analysis. Microbiome analysis was performed on the upper and lower airway of subjects with interstitial lung diseases, including idiopathic interstitial pneumonia (IIP), non-IIP, and sarcoidosis as well as *Pneumocystis jiroveci* pneumonia and healthy controls. The microbiota in lower airways of a majority of patients (30; 90%) primarily consisted of *Prevotellaceae*, *Streptococcaceae*, and *Acidaminococcaceae*; α and β diversity measurements revealed no significant differences in airway microbiota composition between the 5 different groups of patients. It was concluded that IIP, non-IIP, and sarcoidosis are not associated with disordered airway microbiota and a pathogenic role of commensals in the disease process is therefore unlikely.[12] A more targeted molecular approach for microbial pathogens in sarcoidosis granulomas most strongly supports that propionibacteria and/or mycobacteria have a role in sarcoidosis pathogenesis. Japanese researchers report molecular evidence of *Propionibacterium acnes* DNA in sarcoidosis specimens, although the DNA could also be isolated from control specimens.[13] The distinction lies in the quantitative differences in *P acnes* DNA between sarcoidosis and controls. The number of genomes of *P acnes* in BAL cells was correlated with the serum angiotensin-converting enzyme level and the percentage of macrophages in BAL fluid from patients with sarcoidosis. No significant difference was detected between *P granulosum* and controls.[14] A murine model of sarcoidosis pathogenesis was successfully developed using heat-killed propionibacteria by intratracheal challenge. This model demonstrated the contribution of Toll-like receptor (TLR)-1, TLR-2, and TLR-9 to the development of the polarized, T_H1 immune response.[15] Another study further confirmed the role of TLR-2 in *P acnes*–specific sarcoidosis immune responses by demonstrating that *P acnes*–induced granulomatous pulmonary inflammation was markedly attenuated in TLR-2(−/−) mice compared with wild-type C57BL/6 animals.[16] A recent meta-analysis involving 9 case-control studies of *P acnes* associated with sarcoidosis revealed a significantly elevated sarcoidosis risk (odds ratio 19.58; 95% CI, 13.06–29.36).[17]

Investigations from independent laboratories worldwide have also reported molecular evidence supporting a significant association between mycobacteria and sarcoidosis. One study reported evidence of mycobacterial 16S ribosomal RNA (rRNA) or RNA polymerase B in 60% of the sarcoidosis granulomas and in none of the controls ($P<.00002$, chi-square).[18] Sequence analysis of the 16S rRNA and *rpoB* amplicons revealed the presence of a novel *Mycobacterium*, genetically most similar to MTB complex (99% positional identity). Using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, Song and colleagues[19] found MTB katG peptides in 75% of sarcoidosis specimens compared with 14% of control specimens ($P = .0006$), in situ hybridization localized MTB katG, and 16S rRNA DNA to the inside of sarcoidosis granulomas. Analysis of Polish sarcoidosis lymph nodes revealed MTB complex heat shock protein (HSP) 70, HSP 65, and HSP 16.[20] Molecular analysis of American sarcoidosis granulomas also revealed the presence of nucleic acids of the mycobacterial virulence factor, superoxide dismutase A (sodA), in 70% of the sarcoidosis specimens compared with 12% of controls ($P = .001$). Sequence analysis of the amplicons demonstrated close positional identity with MTB complex, yet genetically distinct.[21] DNA of mycobacterial HSPs has been detected in cutaneous lesions of Chinese sarcoidosis patients but absent from control specimens. Sequence analysis was consistent with MTB, *M chelonae*, and *M gordonae*.[22] Another study reported the ability of real-time PCR analysis to quantitatively differentiate sarcoidosis from tuberculosis using receiver operating characteristic curves.[23] Real-time PCR analysis from these independent laboratories demonstrates that if viable mycobacteria are present within the sarcoidosis