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Sarcoidosis: Can a murine model help define a role for silica?

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ABSTRACT

Both genetic and environmental factors are thought to play a role in the etiology of sarcoidosis. An association of the condition with exposure to environmental microbes has been recognized for many years, and has become stronger in the last 10–15 years with the advent of newer investigative techniques. A body of literature now is accumulating suggesting that silica may be yet another trigger in genetically predisposed persons.

Impressive support for an etiologic role of mycobacteria derives from earlier studies by several investigators in Japan and in Europe and more recently from the US in Baltimore and Nashville. Other investigators have produced evidence that propionibacteria and fungi can also act as environmental triggers in sarcoidosis patients.

We propose that, in an animal model that has been previously sensitized to microbial antigens, the introduction of silica through the gastrointestinal route, or intravenously, may have a granuloma-worsening effect, if the strain of animals is already predisposed to develop granulomatous disease. Here the silica may add a "second hit" to the "first hit" given by the exposure to microbial antigens.

Introduction/background

Sarcoidosis is a chronic granulomatous disease of unknown etiology characterized by the formation of non-caseating granulomas with predominant involvement of the lungs and regional thoracic lymph nodes, sometimes accompanied by involvement of other organ systems. Confirmation of the diagnosis generally requires demonstration of the characteristic granulomatous lesion by biopsy, in the absence of specific known causes of granulomatous inflammation. When treatment is indicated, corticosteroids remain the treatment of choice, although alternative immune-modulating agents are being increasingly used for patients requiring more prolonged therapy [1–3].

Genetic predispositions clearly play a significant role in disease pathogenesis; the strongest associations with sarcoidosis are found within the HLA region on the short arm of chromosome six. In the ACCESS study done in the US [4], patients reported a five times greater frequency than controls of a parent or sibling with a history of sarcoidosis. In some patient subgroups, strong associations have been reported between HLA-DRB1 and acute onset and resolution of sarcoidosis. African Americans are known to have more severe and chronic disease and the genetic associations are different compared to other populations, e.g., stronger associations with HLA-DQB1 than with HLA-DRB1 [5].

Multiple environmental factors have been considered to play major roles in etiology of sarcoidosis. John Chapman from the University of Texas Southwestern Medical School in the late 1950s and early 1960s was the first to describe significantly elevated levels of antimycobacterial antibodies in the sera of sarcoidosis patients [6]. In a subsequent study, he and colleagues found that these elevated antimycobacterial titers were associated with IgM and IgA antibodies in subjects with recent onset of sarcoidosis. When followed up at six to nine months later, these patients were found to have lower or unmeasurable titers of these antibodies, suggesting that recent exposure to nontuberculous mycobacteria may have been, at least in part, responsible for the onset of their initial presentation [7].

In the late 1990s and early 2000s, more studies implicating a bacterial etiology in sarcoidosis appeared [8,9]. The demonstration of significantly greater quantities of nucleic acid residues of propionibacteria, as well as various mycobacteria supported their involvement in disease pathogenesis. In 2002, Drake et al. reported finding *Mycobacterium* species 16S ribosomal RNA and ribosomal polymerase, beta subunit, in tissue specimens from cervical or mediastinal lymph nodes and lung biopsies in 60% of 25 sarcoidosis patients, but none in tissues from 25 control subjects ($p = 0.00002$, chi square) [10–13]. That group later found in both blood and urine lavage specimens greater Th-1 type immunologic responses to several mycobacterial antigens in patients with sarcoidosis than in controls.

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In 2005 Song et al. in Baltimore found *Mycobacterium tuberculosis* catalase-peroxidase protein in lung tissue from approximately 50% of patients with sarcoidosis [14]. Later, Chen and coworkers, collaborating with Swedish investigators documented T-cell responsiveness of similar degree to mycobacterial antigens in sarcoidosis patients studied in Baltimore and Stockholm, respectively [15]. Brownell et al. recently reviewed the literature on the association of mycobacteria with sarcoidosisl [16]. Several European papers have shown evidence of mold and fungal environmental triggers of pulmonary sarcoidosis in recent years [17,18].

There is also evidence to show that silica introduced into the body via the respiratory tract or the gastrointestinal tract can trigger development of sarcoidosis in individuals who may be genetically predisposed to develop the disease. Remarkable support for an oral portal of entry came from a 2009 Lancet article [19] suggesting that silica, present as an excipient in many oral antihypertensive medications, had acted as an exacerbating trigger for the disease in one subject.

Swaisgood et al. [20] have developed a "murine lung granuloma model" of sarcoidosis in which mice sensitized to mycobacterial superoxide dismutase amplicons (SODA) in incomplete Freund's adjuvant are challenged intravenously 14 days later with naked beads, covalently bound either to SODA peptides or to schistosome egg antigens (SEA). Non-caseating granulomas are then seen in the lungs of these mice sensitized to SODA, closely resembling those characteristically found in patients with sarcoidosis. A similar animal model may be useful in revealing whether silica introduced into the body through the gastrointestinal route, or intravenously, will have a granuloma-worsening effect in animals already predisposed to the development of granulomatous disease.

The hypothesis/theory

We propose that the Swaisgood model [20] may be useful in helping answer the question of whether silica introduced into the body by the gastrointestinal or intravenous route can directly worsen the granulomatous lesions of an experimental model resembling human sarcoidosis, possibly adding a "second hit" to a "first hit" given by the exposure to microbial antigens. We also would suggest inclusion of a suitable intravenous preparation containing a soluble silica solution as a separate experimental group, since it is likely that there is translocation from the GI tract to the lungs via the circulatory system in a situation such as occurs in persons ingesting silica compounds as in the subject referred to in the Lancet article.

The potential role of silica in the pathogenesis of sarcoidosis

Since the lungs are the predominant target organ affected in patients with sarcoidosis, it has been generally accepted (and reasonable to presume) that the major portal of entry of any putative etiologic agent of the disease is the respiratory tract. An increased incidence has been reported in several situations where exposure to silica dust in the respiratory tract may have played a role: in US Navy sailors on aircraft carriers, in New York City firefighters, and in a number of persons exposed to excessive amounts of dust after the 9/11 disaster in whom "sarcoid-like" granulomatous pulmonary disease has been reported [21–23]. Workers in Iceland reported an increased risk of sarcoidosis following exposure of subjects to diatomaceous earth and cristobalite (crystalline silica) [24].

The gastrointestinal tract, however, may also be a portal of entry. Comstock et al. [25] had found that there was an increased amount of sarcoidosis among persons who were clay-eaters in Georgia, than in controls who did not report eating clay. The recent 2009 Lancet article suggested that silica could act as an exacerbating trigger. The subject had a history of hypertension, but no previous personal or family history of sarcoidosis. He developed the disease when he was started on an oral antihypertensive medication which contained anhydrous colloidal silica as an excipient. His disease remitted when his blood pressure medication (a beta blocker, containing silica as an excipient) was stopped. Sarcoidosis recurred later, when he was given an alpha blocker for his hypertension (which again contained anhydrous colloidal silica), and symptoms normalized when the drug was withdrawn. Later he was treated for his hypertension with another non-silica-containing medication and remained symptom free at the time of publication of the article in 2009.

Discussion

In previous animal experiments, silica exposure has been shown to cause the development of granulomas similar to those in human sarcoidosis patients. Continuous low dose silica exposure of Lewis rats caused granulomas without much lung inflammation, in contrast to acute exposure to massive doses, which caused severe alveolitis, fibrosis and alveolar lipoproteinosis along with granuloma-like lesions suggesting a dose–response relationship [26,27]. However, it is likely that not all humans exposed to silica will develop sarcoidosis, only those who are genetically predisposed to do so will. In order to study the effects of silica exposure through the GI tract, one would need to utilize rodent species having immune defects predisposing them to develop granulomas like those typically seen in sarcoidosis.

The role of the inflammasome in the pathogenesis of silica-triggered sarcoidosis

The innate immune system provides a critical protective function during the initial phase of microbial invasion as well as a homeostatic safeguard for the detection of a variety of endogenous products, e.g., uric acid, and exogenous products, e.g., alum, asbestos, silica which can also initiate inflammatory responses through the production of a wide variety of pro-inflammatory cytokines [28,29]. In the detection of these external and internal pathogenic signals, the innate immune system utilizes a variety danger sensing molecules that are generically referred to as pathogen recognition receptors (PRRs). In the case of bacterial microbes, there are 10 major types of microbe-detecting systems in the human called toll-like receptors (TLRs) and TLR2 is used for the detection of the tubercle bacillus, and likely also for nontuberculous mycobacteria, and possibly other microbial triggers of sarcoidosis. The major pathway utilized by the innate immune system to detect inanimate substances, such as silica, is another specialized cytoplasmic multimeric protein structure called the inflammasome. The basic structure of the inflammasome consists of three major components, (1) a **N**OD-**l**ike **r**eceptor (**NLR**); (2) an adapter protein; and, (3) a caspase (i.e. a **c**ysteine-**asp**artate prote**ase**) capable of cleaving a pro-inflammatory form of a cytokine to its active form, e.g., a pro-IL-1 to active IL-1.

Current evidence concerning the formation of the inflammasome suggests at least a 2-phase process. The first is related to the activation of the cell through stimulation of various PRR sensors (e.g., TLRs, NLRs) leading to the synthesis of proIL-1β. The second event is the activation of a NLR by the presence of various secretion systems and by potassium efflux and generation of reactive oxygen species. This disruption of the cellular membrane works as a signature for the presence of pathogenic bacteria in the cell and allows the completion of any of two events that trigger the final signal to initiate the activation of the inflammasome: (1)

the secretion of ATP that is linked to changes in the electrolyte composition of the cytoplasm due to the formation of bacterial induced pores, i.e. the efflux of potassium outside of the cell that contributes to the activation of the NALP 3 inflammasome, or (2) the formation of reactive oxygen species (ROS) that are attributed to the stimulation of NADPH oxidase pathway, that is related to frustrated phagocytosis of large particles, e.g., silica, alum, uric acid that are unable to be taken up by the macrophages, i.e. uric acid crystals. These molecular events are finding clinical application based on a better understanding of the role of the immune system in health, e.g., use of alum adjuvants in vaccines, as well as in disease, e.g., gout and sarcoidosis. The proposed "two hit hypothesis" described above is consistent with this 2-phase process involving a "first hit" generated by a microbial TLR-mediated encounter followed by a "second hit" engendered by silica driven inflammasome activation.

Conclusion

The causes of sarcoidosis have long remained elusive. The studies proposed in the present report suggesting use of a murine model that explores a "two hit" hypothesis may offer new inroads into a better understanding of the pathogenesis of this clinically perplexing disease.

Conflict of interest

None declared.

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