

# Sarcoidosis—recent progress in etiology and pathogenesis

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**D**ESPITE its recognition more than ninety years ago, and the development of an extensive literature representing investigative studies, sarcoidosis remains an enigma. With the increased screening of patients by means of roentgenograms of the chest, and the decreasing incidence of tuberculosis, new interest has arisen in this widespread granulomatous disease—sarcoidosis.

The clinical manifestations and current therapy of sarcoidosis are well known and are beyond the scope of this paper. Many excellent reviews<sup>1-6</sup> are available which summarize these aspects in detail. What perhaps is not so well known are the many small bits of information that support some of the recent concepts about the etiology and possible pathogenesis of this masquerader of many diseases.

The development of our present knowledge about sarcoidosis has been described as occurring in three stages.<sup>7</sup> Originally recognized in the second half of the nineteenth century as a skin disease, it was not until Boeck<sup>8</sup> in 1899 noted involvement in the lungs and named the process “sarkoid”, because he believed it was in some way related to benign sarcomas, that a new stage began, stressing the internal manifestations of the disease. In 1917, Schaumann’s<sup>9</sup> important work was published, noting the generalized systemic involvement of the disease, of which skin lesions were only a small part. In the next several decades, experience was gained through numerous clinical observations, but there was little advancement in the understanding of the basic pathophysiology. Interest flourished, especially in the Scandinavian countries, in what was thought to be an atypical form of tuberculosis.

The third stage occurred after World War II. In Europe, rampant tuberculosis stimulated mass chest roentgenographic screening of the population. From these surveys a mild form of sarcoidosis, manifested by bilateral hilar adenopathy, was recognized in asymptomatic patients, and a greatly increased incidence was uncovered. Investigators, being unable to find a specific etiologic agent, undertook vast epidemiologic surveys to find some

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common denominator among sarcoid patients. Most physicians no longer thought that tuberculosis was the etiologic agent, but were divided into two groups: those who considered sarcoidosis to be a type of histologic pattern produced by a wide variety of inciting agents such as beryllium, tubercle bacilli, fungal infection, and other stimuli; and those who thought that a single, as yet unknown, extrinsic agent or intrinsic defect was responsible for the syndrome or disease.

Today, the development of our knowledge concerning sarcoidosis is in a fourth stage, stimulated by recent advances in immunology and bacteriology. This report presents what has recently been learned about sarcoidosis, and discusses some of the current and fascinating theories about its etiology and pathogenesis.

#### IMMUNOLOGIC AGENTS

Interest in the immunology of sarcoidosis began in 1917 with Schaumann's<sup>10</sup> observation that most patients with sarcoidosis had negative reactions to tuberculin skin tests at a time when most adults were tuberculin-positive. This was the first evidence of an immune defect in patients who had sarcoidosis. Wide variations in the percentages of nonreactors have been noted in numerous series.<sup>11</sup> Many of the variations probably represent differences in technic, for several authors<sup>12, 13</sup> noted that the percentage of positive reactions could be significantly enhanced through the use of depot tuberculin or by the addition of cortisone to the antigen, implying that the person's sensitivity was merely depressed and not absent. In 1953, Nitter<sup>14</sup> documented the loss of tuberculin sensitivity in patients with active sarcoidosis, who had been positive reactors in the past, and observed the return of their hypersensitivity as the disease went into remission. In 1965, though, Israel and Sones<sup>15</sup> noted that most of their patients with sarcoidosis failed to regain tuberculin hypersensitivity, and furthermore remained unreactive even after bacille Calmette Guérin (BCG) vaccination. Subsequently they<sup>16</sup> showed that in patients who have recovered, and in those with active disease alike, hypersensitivity to tuberculin did not develop, even after vaccination with a strain of BCG that converted 95 percent of control subjects. They<sup>16</sup> observed that three of these patients had not converted with previous BCG vaccination before the onset of clinically evident sarcoidosis. From this observation they concluded that a more basic manifestation of sarcoidosis than even the positive Kveim test was this loss of the ability to acquire and to maintain tuberculin sensitivity, and that this defect may be necessary for the occurrence of the disease. However, Sutherland, Mitchell, and Hart<sup>17</sup> in a large survey found no greater incidence of sarcoidosis among young adults who were tuberculin-negative than among those who were tuberculin-positive.

The inability of sarcoidosis patients to acquire delayed hypersensitivity has not been limited to tuberculin, but includes histoplasmin, trichophyton, oidiomycin, mumps virus, and pertussis agglutinin, yet most serum antibody titers are normal, even to some of these same antigens.<sup>18, 19</sup> This non-specific defect would appear to be strong evidence against those theories that linked sarcoidosis with tuberculosis on the basis of a specific "anergy."<sup>11, 19</sup> Despite this immune deficit, no increased susceptibility to infection has been noted,<sup>20</sup> with the possible exception to tuberculosis, and this is most likely a manifestation of the pathologic changes produced by the disease.

The nature of this immunologic abnormality is now fairly well known. In 1952<sup>21</sup> it was shown, by means of Lawrence's passive transfer technic in which leukocytes from tuberculin-sensitive patients are injected into the skin of tuberculin-negative sarcoidosis recipients, that a normal tuberculin reaction could be elicited, thus demonstrating that no cutaneous abnormality existed and implicating leukocytes as the site of the defect.

In 1964, Hirschhorn and associates<sup>22</sup> showed that lymphocytes cultured from the peripheral blood of patients with sarcoidosis reacted abnormally when exposed to phytohemagglutinin, a crude chemical extract of the kidney bean, which induces blast transformation and mitosis in normal, cultured lymphocytes, similar to that induction seen when they are exposed to an antigen to which they have already been sensitized.<sup>23</sup> In comparison with cells from normal control subjects, these sarcoid cells were extremely slow to respond, and reacted less actively. When sarcoid lymphocytes were exposed to Kveim antigen, a higher than normal rate of blast formation occurred, while cells from control subjects or tuberculin-positive patients did not react abnormally. This impaired immunologic response is also known to occur in other disorders, such as Hodgkin's disease, which affects the reticuloendothelial system and exhibits a depression in delayed hypersensitivity.<sup>22, 24</sup>

Of great speculative interest is the observation of a transient loss of delayed hypersensitivity during some viral infections,<sup>25</sup> correlating with the time of interferon production by leukocytes.<sup>26</sup> It has also recently been reported that human leukocytes, stimulated by phytohemagglutinin, produce an interferon-like virus inhibitor and are no longer capable of stimulation by previously sensitized antigen.<sup>27</sup> Thus, it would seem that more likely explanations for the loss of delayed hypersensitivity in patients with sarcoidosis are either that the immunologically active cells are preoccupied with some other task,<sup>28</sup> possibly the production of an interferon-like material or some other antigen, or perhaps they are from a genetically incompetent clone, as postulated by Burnet.<sup>29</sup>

Buckley, Nagaya, and Sieker,<sup>28</sup> having studied both cellular and sero-

logic aspects of immunologic responses in patients with sarcoidosis, found that the impaired lymphocyte stimulation by phytohemagglutinin correlated with the clinical activity of the disease; that is, cells from patients in remission acted like those from control subjects, while those patients with the most active disease had lymphocytes with the weakest responses. This work correlates with the clinical observations of Sutherland, Mitchell, and Hart,<sup>17</sup> who noted that the depression of hypersensitivity to tuberculin occurs at the onset of the acute phase of the illness. Buckley, Nagaya, and Sieker<sup>28</sup> also observed a slight increase in serum complement activity, and a disproportionate increase in IgA, a small increase in IgG, and an insignificant increase in IgM in the sera of patients with active sarcoidosis, and that these increases in immunoglobulins followed the normal pattern in response to known stimulation of antibody production,<sup>30</sup> but at a much slower rate. The levels of serum complement activity were in the same range as those found in association with tuberculosis and other chronic infections in which an intracellular organism is the infecting agent. It was believed that these studies supported the new concept of Mankiewicz and Van Walbeek<sup>31</sup> that an intracellular organism may be the etiologic agent of sarcoidosis. Their concept is discussed later.

Clinicians have observed some phenomena associated with sarcoidosis, which, despite the impairment of delayed hypersensitivity, suggest that there are factors of hypersensitivity in the disease. One of these features, especially in young Scandinavian women, is erythema nodosum, occurring in a frequency as high as 50 percent in some series,<sup>32</sup> but seemingly influenced by geographic or ethnic factors, being quite uncommon in the United States.<sup>33</sup>

The Kveim test is the best known example of hypersensitivity in patients with sarcoidosis. In this test, sarcoid lymph nodal or splenic tissue free of all contamination is ground in a mortar with saline to make a 10 percent suspension, is sterilized, and then from 0.10 to 0.20 ml is injected intradermally in the patient as in the Mantoux test. A positive test often yields an indolent papule at the injection site, but the test site is always biopsied after from four to six weeks, since in some patients biopsy specimens are positive though there is no obvious papule. A positive biopsy yields a typical histologic pattern "the naked tubercle," which is characteristic for the disease and is indistinguishable from the systemic lesions of sarcoidosis. It should be noted that the Kveim test is usually only positive in the active phase of sarcoidosis.<sup>34</sup>

The specificity of the Kveim test is now recognized, and with experienced clinicians only 3 percent or less false-positive reactions occur. However, the effectiveness of different preparations varies considerably. In evaluating 38 different tissue suspensions, Siltzbach<sup>35</sup> found that only 18, or less

than half, were satisfactory. It now appears that much criticism of the test occurred because either preparations that were not standardized were used, or interpretations of the biopsies were inaccurate. At Mt. Sinai Hospital, in New York City, 1,013 patients were tested, with the occurrence of 85 percent positive reactions in patients with biopsy proved active sarcoidosis, and with less than 1 percent false-positive reactions.<sup>35</sup> When these same Kveim preparations were tested in 1,228 patients in 21 countries by 40 different investigators, 60 percent of patients with biopsy proved active sarcoidosis yielded positive reactions, with only two false-positive reactions noted.<sup>33</sup> These results support the concept that sarcoidosis is a worldwide disease with a common etiology and pathogenesis.

Despite its widespread use for more than 20 years, the exact nature of the Kveim reaction is still not known. The suspensions are usually stable for long periods, resistant to acid and lipid extractions and to filtration with bacterial filters, but quite sensitive to mild alkali.<sup>33</sup> Rogers and Haserick<sup>36</sup> believed that the Kveim antigen was likely to be involved in the etiology of sarcoidosis, that it produced when injected a localized form of the disease in sensitized subjects; i.e., patients with sarcoidosis who had antibodies against the etiologic agent. Further evidence, that the Kveim suspension may contain an antigen, comes from the report of the successful passive transfer of Kveim reactivity to unreactive normal subjects by the use of leukocytes.<sup>37</sup> This transfer is characteristic of the delayed type of antigen-antibody reaction. However, information concerning the exact nature of the Kveim reaction will probably not be available until the pathogenesis of sarcoidosis is better understood, but the diagnostic significance of the test seems now to have been accepted, and it provides another means of approach to a wider understanding of the disease.

Serologic evidence for hypersensitivity in patients with sarcoidosis was presented by Sands and associates<sup>38</sup> in 1955 when they showed that sarcoid patients, along with those with collagen disorders, had higher titers of agglutinins against injected mismatched blood than those of normal controls. Pepys and associates<sup>39</sup> noticed that one third of the series of patients with sarcoidosis exhibited precipitating antibodies against hay and molds similar to those of patients with farmer's lung, yet had had no exposure to these antigens.

#### BACTERIOLOGIC ASPECTS

One of the most exciting theories has developed recently from the work of Mankiewicz and associates.<sup>31, 40, 41</sup> If their hypothesis proves to be correct, an entirely new type of infectious process will have been delineated, which may have widespread implications for many, as yet puzzling, diseases. In 1962, Mankiewicz and Van Walbeek<sup>31</sup> reported the paradox of a

reduction or absence in mycobacteriophage-neutralizing antibodies in the sera of patients with sarcoidosis, and the presence of phages lytic for mycobacteria in all their feces.

This work has been partially confirmed by Kallings and Löfgren,<sup>42</sup> who found an absence of phage-neutralizing antibodies to the *Escherichia coli* phage, T<sub>2</sub>, in 22 percent of sarcoid patients, but the presence in all of control subjects. An obvious implication of the presence of parasitic mycobacteriophages in sarcoid patients is that mycobacteria must be present to allow the phage to thrive. Patients with tuberculosis had a lower incidence of phages in their feces, but their sera contained high concentrations of antibodies that when added to the phage cultures prevented lytic action, by the phages, on the mycobacteria. Patients with other types of chronic lung disease had only a rare phage isolated from their feces, but patients with positive isolates also had neutralizing antibody titers against the phage.

These observations obtain etiologic significance from Mankiewicz and Van Walbeek's<sup>31</sup> findings that mycobacteria in the presence of high concentrations of virulent mycobacteriophages in vitro are either destroyed or undergo changes that render them bacteriologically or serologically unrecognizable as mycobacteria. The changes are thought to be secondary to the mycobacteria lysogenic mutation by the phages. In vitro colonies of virulent human mycobacteria, when exposed both to phages and to high concentrations of neutralizing antibodies produce occasional colonies that closely resemble unclassified, atypical, mycobacteria, yet are immune to mycobacteriophages, thus probably representing lysogenic forms. These lysogenic forms represent sensitive mycobacteria that, instead of being destroyed by the infecting phage, merely carry the phage genetic material, called prophage, with the potential of releasing new generations of phages when appropriate stimulation occurs. Since these prophages may produce no characteristic changes in their host *Bacterium*, their presence is detected by their host's immunity to phage infection through a mechanism as yet not known.

In 1964, Mankiewicz<sup>40</sup> reported isolating seven of these lysogenic variants of mycobacteria from the biopsied tissues of 12 patients with sarcoidosis. Furthermore, mycobacteriophages were cultured from the same tissues.

In 1964, Mankiewicz and Béland<sup>41</sup> reported that "sarcoid-like" lesions were produced in guinea pigs by the simultaneous inoculation of large doses of mycobacteriophages with small numbers of virulent human mycobacteria. All of the animals so treated showed high titers of neutralizing antibodies to the infecting phages, and when pretreated with pharmacologic doses of hydrocortisone in an attempt to simulate the anergy known to be produced in patients with sarcoidosis, only low titers were demon-

strable. However, the only differences between the animals that received hydrocortisone and those that did not, were a slower progression of the disease with a lower death rate and the absence of hyperplastic lymphoid follicles in the treated guinea pigs. In previous attempts to produce sarcoid-like lesions in guinea pigs, with a 10 times greater inoculum of mycobacteria, plus the same phage, a rapidly fatal form of tuberculosis occurred with unchanged virulent mycobacteria isolated in perfusion. The authors<sup>41</sup> speculated that the smaller dose of tubercle bacilli hindered the evolution of the disease and allowed the effects of the phage to become manifest. From the sarcoid-like lesions that were produced, atypical strains of mycobacteria were isolated, some of which produced lytic phage. No tubercle bacilli were isolated from these lesions. When the lysogenic atypical variants were injected back into normal guinea pigs, only an inflammatory type of reaction developed. Mankiewicz and associates' <sup>40, 41</sup> work has gained considerable indirect support from many sources, but direct confirmation is lacking.

Beginning with Michael and associates' <sup>43</sup> study of sarcoidosis among military personnel after World War II, and Cummings and associates' <sup>44</sup> even larger series, it became evident that the greatest concentration of sarcoidosis in the United States occurred in the southeastern part of the country. The search for some unique etiologic factor operating in this area seemed at first successful. Pine pollen enjoyed a brief period of notoriety,<sup>45</sup> but after exhaustive and often provocative studies, it has been conditionally abandoned by its chief protagonist.<sup>46</sup> The prevalent soil type of the tidal basins in the south also was implicated,<sup>47</sup> but, like pine pollen, its proponents are unable to explain how typical sarcoidosis can occur in regions in which neither is present, unless sarcoidosis is a syndrome with multiple etiologies.<sup>44, 46</sup>

Then in 1958, Edwards and Palmer<sup>48</sup> showed, in a large survey of naval recruits from all over the United States, a striking predilection for individuals from the southeast to be positive to skin tests for atypical mycobacteria. When Israel and associates<sup>49</sup> tested patients with active sarcoidosis with a similar antigen, less than half as many reacted as did control subjects. (This might be explained by the loss of delayed hypersensitivity seen in the active disease.) Chapman,<sup>50, 51</sup> and Chapman and Speight<sup>52</sup> observed that there might be a relationship between the findings of Edwards and Palmer<sup>48</sup> and the prevalence of sarcoidosis in the Southeast. They studied 280 patients with sarcoidosis, from 11 states and seven foreign countries, for the presence of serum antibodies to atypical mycobacteria. These sera were tested against antigens from 24 strains of atypical mycobacteria, normal tubercle bacilli, several saprophytic mycobacteria, and six common



systemic fungi, by the gel-diffusion technic. The control subjects were healthy medical students, patients with chronic lung disease, asthma, berylliosis, tuberculosis, or uveitis.

The sera of sarcoidosis patients yielded the highest number of significant reactions to atypical mycobacteria. Only patients with uveitis or proved mycobacterial disease produced more than one half the reactions of those of the sarcoid patients. Reactions to human tubercle bacilli were two times as common among the uveitis, berylliosis, and tuberculosis patients as among sarcoid patients. No geographic differences were found. Of note were the long-term data from 16 sarcoid patients whose progress was followed from active disease to remission, while their sera changed from weakly positive or negative to quite reactive, as radiographic evidence of clearing was demonstrated, just the opposite of what is seen with the Kveim test. Tests of the more active sarcoid sera against lepramin and Kveim antigen were negative, as might be expected, since only cellular antibodies are postulated in these tests.

Chapman and associates<sup>53</sup> have obtained further evidence linking atypical mycobacteria to sarcoidosis by studying the response to skin testing in contacts of known sarcoidosis patients with antigens of atypical mycobacteria. They found that sarcoid contacts react to atypical antigens with more than twice the frequency of that of the control subjects, and in similar proportions as contacts to patients with proved infection with the atypical *Mycobacterium*, *M. kansasii*. The degree of tuberculin hypersensitivity in both groups was essentially the same as that of the control subjects.

A most intriguing epidemiologic study reported the attempt to find some reason for the rural predominance of sarcoidosis which had been noted in most surveys. Evidence had accumulated that mycobacteria were frequently isolated from cattle and other farm animals. Chapman<sup>54</sup> reported that 50 percent of 50-ml samples of raw milk were positive for a wide variety of unclassified mycobacteria, and that these organisms were not all destroyed by pasteurization. He points to the peculiar worldwide distribution of sarcoidosis as being not incompatible with the concept that farm products may have some role in the epidemiology of the disease.

In summary then, the etiology of sarcoidosis is still not proved, but recent work suggests that much of the confusion and contradiction in the published reports may now be explained; that in the past, sarcoidosis was considered by many to be any noncaseating granulomatous process, but as investigators acquired the ability to understand and analyze these cases, a certain number have been found to have known etiologies such as: zirconium granules, talc and other crystals, tuberculosis, fungal infections, berylliosis, tuberculoid leprosy, syphilis, and finally some of the lymphoma groups of diseases.<sup>3, 4, 13, 55</sup> As more known causes have been elucidated and



removed from the sarcoid spectrum, an increasingly uniform and characteristic systemic process has remained.

### PATHOGENESIS

We can only suggest the pathogenesis of sarcoidosis. If serial sections of the Kveim test are used as a model,<sup>36</sup> the *naked tubercle* that is characteristic of sarcoidosis begins with a degeneration of perivascular collagen accompanied by the migration into the region by histiocytes, followed by small lymphocytes and other mononuclear cells. Then the formation of giant cells occurs. After about two weeks, epithelioid cells begin to appear and the inflammatory response subsides, leaving "naked" the clusters of epithelioid cells. Eventually, hyalinization occurs with fibrosis. Some recent reports<sup>13, 56-58</sup> suggest that although corticosteroids are of value in controlling the acute complications of sarcoidosis, such as hypercalcemia, erythema nodosum, and ocular disease, the steroids only suppress the tissue reaction and thus do little to alter the basic process.

Refvem<sup>55</sup> has shown that histiocytes become transformed into epithelioid cells through the focal accumulation of phospholipids. He believed that these are produced by antigen-antibody reactions involving small lymphocytes, and are induced by a wide variety of dissimilar stimuli. These small lymphocytes have been intensely studied, and appear to possess immunologic memory, the ability to produce antibodies, and are the principal cells involved in delayed hypersensitivity.<sup>59</sup> Thus there are at least two factors in the pathogenesis of sarcoidosis: an initial infection, followed by a widespread immunologic reaction predominantly by cellular elements. Since in the early stages the disease frequently affects the hilar nodes, it seems likely that a respiratory route of infection is involved.

If, as has been postulated,<sup>27, 33</sup> a predisposing immunologic alteration prevents phage-neutralizing antibodies from developing in some persons when they become infected, and allows unchecked interaction between phage and host mycobacteria within their systems, a symbiotic relationship might then develop between the phage and its lysogenic host. In an intracellular location, a chronic, smoldering process would stimulate the reticuloendothelial system, resulting in subsequent reactions between cellular antibodies and antigen, provoking the widespread granuloma formation and impairment of delayed hypersensitivity so characteristic of the disease.

Much is yet to be proved, and many questions are still unanswered, such as: What is the nature of this altered immunity that predisposes to disease? Is it genetically determined, or the result of some undiscovered more basic process? Why do some persons have asymptomatic limited benign disease, and others have widespread granulomas, progressive fibrosis, and even die from involvement of vital organs? If as much is learned within the next

decade as has been in the last, we may then be able to unmask this challenging masquerader.

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