S A R C O I D O S I S

Jonathan C. Weissler, M.D.

Medical Grand Rounds
Parkland Memorial Hospital
University of Texas
Southwestern Medical School
August 19, 1993

Sarcoidosis is a multiorgan granulomatous disease of unknown etiology. The initial description of the disease by Jonathan Hutchinson in 1877 focused on skin involvement and a report in 1910 by Bering observed eye involvement (1). However with the advent of chest roentgenograms in 1915 two Swedish physicians Jorgen Scheumann, a dermatologist and Sven Lofgren, a chest physician, defined the characteristic involvement of mediastinal and hilar lymph nodes with noncaseating granulomata. Although many organs may be involved in sarcoid, the lung is most commonly affected and accounts for the majority of morbidity and mortality from the disease. This review will focus primarily on current concepts concerning the immunopathogenesis and management of pulmonary sarcoid.

Epidemiology

Sarcoidosis has been reported from virtually every corner of the globe, though some populations demonstrate a markedly increased incidence. In Sweden, screening roentgenograms suggested an incidence of 64/100,000 though autopsy studies indicated that the incidence might actually be ten fold higher (2). In Japan, the incidence was less than 1/100,000 and cases were clustered in northern and central Japan (3). In the United States the prevalence of disease is approximately 5/100,000 in whites and 40/100,000 in blacks (4). A similar six-fold increase in the black population has also been reported in South Africa. Interestingly the disease is uncommon in Chinese and American Indians.

Most series report a 2:1 female:male predominance in the United States while some series from Europe have demonstrated an even distribution. Although the majority of cases are diagnosed between age 20-40, 10% of cases make their initial presentation after age 60 and the disease is well described in pediatric populations. Some studies comparing the incidence in monozygotic versus dizygotic twins have suggested a familial form of the disease though this appears to be uncommon (5,6).

Based on most epidemiologic studies it is apparent that the majority of patients with sarcoidosis are either asymptomatic or have such minor symptoms that medical attention is not sought. Thus it has been estimated that 80% of all cases of sarcoid go undetected.

Clinical Presentation

Patients who develop symptoms related to sarcoidosis may present with generalized complaints, symptoms attributable to extrapulmonary involvement, or findings localized to the respiratory tract. In several large series (7-9) (Table 1) 40-50% of patients complain of constitutional symptoms including malaise, weight loss, fevers, sweats and myalgias. Approximately 15-30% of patients display signs of peripheral lymphadenopathy, splenomegaly or hepatic enlargement.

Table 1

Clinical Symptoms at Presentation In 448 Patients With Sarcoidosis

	Number	%
Asymptomatic	31	7
Respiratory	226	50
Skin	137	30
Constitutional	210	47

Skin lesions may be found in at least 15% of sarcoid patients (10). These lesions may be either specific or nonspecific for sarcoid. Lupus pernio, the most classic skin lesion in sarcoid is a violaceous indurated lesion which preferentially involves the ear, lips, and nose. The nasal lesion is usually associated with granulomatous involvement of the nasal mucosa. This lesion is most common in women with extensive pulmonary and extra-pulmonary involvement.

Other common skin lesions include: a) plaques usually located on the limbs, face and back in a symmetrical distribution; b) maculopapular eruptions on the face and upper back which occur more frequently in black patients; and c) subcutaneous nodules (Darier-Roussy lesions) representing dermal and subcutaneous granulomata. Erythema nodusum is quite common in European patients but occurs in less than 10% of American patients. This lesion is commonly associated with fever, malaise and polyarthralgia. Of note this syndrome of a marked inflammatory response in patients with sarcoidosis is generally viewed as presaging a benign clinical course.

A variety of other organs may be involved with sarcoid including the eyes, central nervous system, heart and joints. Considerable morbidity may result from these processes, though symptoms related to involvement of these organs are rarely the cause for presentation.

Eye involvement occurs in 5-10% of patients (11). A granulomatous uveitis may present acutely with redness of the eyes and blurred vision. Chronic uveitis may present with pain or may be subclinical. Opthalmologic evaluation should thus be performed on all sarcoid patients. Retinal involvement has been described but is rare. Anterior uveitis can often be treated with topical steroid drops but posterior involvement requires systemic therapy.

Central nervous system involvement may present with signs of a basilar meningitis, psychiatric dysfunction, or a space

occupying lesion (12-14). CNS involvement occurs in <10% of patients. The basilar meningitis may be associated with cranial nerve palsies, or hypothalamic/pituitary dysfunction. Peripheral neuropathy may also complicate sarcoid. High dose corticosteroid therapy is the treatment of choice for neurosarcoid.

Cardiac sarcoid is an important and largely undiagnosed complication. Although clinically evident cardiac involvement is rare, autopsy series disclose a 25-30% incidence of cardiac involvement (15-19). Although the literature often stresses the infiltrative nature of involvement, overt restrictive or constrictive disease is rare. Unfortunately ventricular fibrillation may be the initial presentation of cardiac sarcoid and this diagnosis should be entertained in young African Americans with unexplained ventricular ectopy.

Involvement of the respiratory tract produces symptoms in 30-50% of individuals with sarcoidosis. Characteristic complaints include dyspnea on exertion, chest pain, and a nonproductive cough. More importantly 40-70% of patients with sarcoidosis display reductions in lung volumes or diffusion capacity for carbon monoxide (DLCO). Significant resting hypoxemia is uncommon in sarcoid, though arterial desaturation may occur with exercise (20-22).

Granulomatous involvement of the airways may be found in both the upper and lower respiratory tract (23). Although overt sinusitis is present is only 5% of sarcoid patients, endobronchial involvement is common. In approximately 20% of sarcoid patients significant reductions in expiratory flow rates indicating airways obstruction may be noted on spirometry. Some authors contend that all sarcoid patients display evidence of obstruction if sensitive techniques are utilized and many patients with sarcoid demonstrate abnormally hyper-reactive responses to methacholine.

Chest Roentgenograms

Radiologic studies have contributed considerable insight into the course of sarcoid (24,25). The most commonly utilized staging system has been developed by Siltzbach (Table 2). Stage 0, a normal radiograph occurs in less than 10% of patients at presentation. Stage I, indicating bilateral hilar adenopathy without parenchymal involvement, occurs in 35-45% of patients. Mediastinal adenopathy without hilar involvement or unilateral hilar involvement can occur but is relatively uncommon.

Table 2

Frequency of Chest Roentgenographic Findings in 764 Patients With Sarcoidosis

Stage 0	8%
Stage I	37%
Stage II	34%
Stage III	21%

Stage II sarcoid demonstrates both hilar adenopathy and parenchymal lung disease. Stage II disease is present in approximately 35% of patients at presentation. Stage III disease, defined as parenchymal disease only, occurs in 20% of presenting patients. At its extreme, Stage III disease may show cystic changes consistent with pulmonary fibrosis.

High resolution CT (HRCT) scanning may provide more definitive description of alveolar architecture. However in most studies HRCT and plain roentgenograms provide similar assessments of disease severity and the role of HRCT in sarcoid thus appears limited (26-28). Longitudinal studies have demonstrated that Stage I disease may progress to Stage II disease, and that as Stage II disease progresses hilar adenopathy may regress until Stage III sarcoid is present. Although there is a wide variation between series it is generally agreed that radiographic stage at presentation is an important prognostic sign (Table 3). Spontaneous radiographic resolution occurs in 50-80% of Stage I disease, 50-60% of Stage II disease and 30% of Stage III disease. Radiographic resolution occurs within 12-24 months of diagnosis in 85% of patients ultimately demonstrating radiographic clearing.

Table 3

Frequency of Radiographic Resolution in Sarcoidosis

Stage I	50-80%
Stage II	50-60%
Stage III	20-30%

It is important to note, however, that abnormalities of the chest radiograph correlate poorly with either pulmonary function testing or clinical symptoms (29,30) (Table 4). Radiographic abnormalities may thus be of limited use in following a patient with sarcoid once the diagnosis is established. Nevertheless, studies which have evaluated the clinical course of sarcoid suggest that the initial stage at presentation correlates with

ultimate outcome. In a study from a primary care setting (Table 5), Reich (31) reported a favorable outcome in over 80% of Stage I or II patients, but only 56% of Stage III patients.

Table 4

Correlation of Radiographic Stage With Pulmonary Function Testing

Stage	Range of FVC (% pred)	Range of DLCO (% pred)
I	62-140	44-120
II	53-120	24-100
III	27-97	17-108

Table 5

Prognosis of Patients With Sarcoidosis In a Primary Care Setting (52 month F/U)

	Same or Improved
Stage I	88%
Stage II	83%
Stage III	56%

Am. J. Med. 78:61, 1985

Diagnosis of Sarcoid

The currently accepted diagnostic standard for sarcoidosis involves the demonstration of non-caseating granulomas in one or more organs in the setting of radiographic or clinical findings consistent with sarcoid. Because granulomas may be found in a variety of diseases including infection, malignancy, hypersensitivity lung diseases and local reactions to inert foreign substances it should be recognized that sarcoid in many instances is a diagnosis of exclusion.

However, the finding of bilateral hilar adenopathy (BHA) is uncommon in diseases other than sarcoid. In a series (Table 6) from the pre-HIV era sarcoid (32) accounted for 74% of consecutive cases of BHA while lymphoma contributed 20%. More importantly all asymptomatic patients with BHA in this series had sarcoid. Whether similar results would be expected in the post-HIV era is uncertain.

Table 6

**Causes of Bilateral Hilar Adenopathy (BHA)
In 100 Consecutive Patients**

Diagnosis	Patients Sampled	Patients with BHA	%Total
Sarcoid	99	74	74
Lymphoma	212	20	9.4
Lung Carcinoma	500	4	0.8
Extrathoracic Malignancy	1201	2	0.2

For the patient with symptoms and clinical presentation suggestive of sarcoidosis most authors agree with the need for a tissue diagnosis. Although a variety of sites (33-44) have previously been utilized for biopsy with varying rates of success (Table 7) the currently accepted approach to sarcoid patients is fiberoptic bronchoscopy with transbronchial biopsy. Diagnostic yield ranges between 85%-95% regardless of the radiographic stage of disease.

Table 7

Yield of Biopsies For Diagnosing Sarcoidosis

Site	%Positive
Skin	88
Salivary Glands	58
Conjunctiva	55
Liver	82
Lymph nodes	86
Mediastinum	94
Transbronchial	85

Furthermore, though the lung can be involved with granuloma from a variety of processes relatively few are likely to produce lesions accessible by transbronchial biopsy (Table 8). Thus in the appropriate clinical setting and in the absence of positive tissue cultures, the findings of non-caseating granuloma on transbronchial biopsy is highly indicative of sarcoidosis.

Table 8

Diseases Where Transbronchial Biopsy Commonly Yields Granuloma

Sarcoidosis

Tuberculosis

Fungal Infection

Berylliosis

Immunopathogenesis of Sarcoid

Alterations of immune function have long been described in the context of sarcoidosis (45, 46). Most common amongst these abnormalities has been the findings of polyclonal hyperglobulinemia in roughly 2/3 of patients and the presence of cutaneous anergy in 50-60%. Interestingly, although some authors have reported a somewhat increased incidence of lymphoma (47-49) in sarcoid patients, there is otherwise no clinical data to suggest that sarcoid patients are immunocompromised (50). Cutaneous anergy likely reflects the action of both suppressor cells in the skin and a relative depletion of CD4 lymphocytes in the peripheral circulation (see below). Indeed in studies utilizing highly purified blood T cells the proliferation of lymphocytes to antigen was similar in sarcoid patients and healthy controls.

The central finding in sarcoidosis suggesting an altered immune response is the widespread distribution of granulomata (51-53). The lungs are involved out of proportion to other organs in most series with granulomata found primarily in the alveolar septa, around lymphatics and occasionally in perivascular regions. The granuloma consists of a follicle of macrophages and multi-nucleated giant cells which are rich in secretory granules. Around this central lesion exists a predominance of CD4 lymphocytes. As the lesion progresses, the total number of lymphocytes and CD4 cells may decrease and CD8 lymphocytes may predominate (54,55). B cells and plasma cells are also present indicating the synthesis of immunoglobulin around the granuloma.

An identical granuloma in skin can be induced by the introduction of a crude extract from spleen or lymph node preparations taken from sarcoid patients (56-58). This test, called the Kveim-Siltzbach test, induces non-caseating granuloma formation 4-6 weeks post intradermal inoculation in 75% of sarcoid patients. The popularity of this test in diagnosing sarcoid is limited, however, due to a lack of standardization and availability.

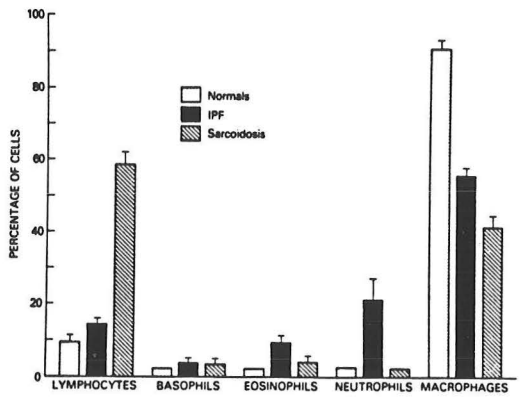
In many granulomata an increase in fibroblasts is noted. However the development of diffuse fibrosis is unusual and most granulomata ultimately resolve with little permanent fibrosis. This resolution occurs despite the production of numerous pro-fibrogenic cytokines (59-62) suggesting that release of these mediators alone is insufficient for the development of permanent fibrosis. Because the respiratory tract is the predominant site of disease and the radiographic changes in sarcoid are consistent with an immune response to an inhaled antigen, attention has focused on the local pulmonary immune response in sarcoid.

The wide spread use of bronchoscopy, which began in the mid 1970's, quickly lead to further investigation of the pathogenesis of sarcoidosis. These studies have contributed significant understanding to the regulation of immune processes in the lung.

The Role of T Lymphocytes

Seminal observations made in the late 1970's and early 1980's established a compartmentalization of activated CD4 lymphocytes in the lungs of patients with sarcoidosis (54, 63-66). In normal individuals lymphocytes comprise 10% or less of the total mononuclear cell population in the alveolus as determined by bronchoalveolar lavage (BAL). In patients with sarcoidosis an early study (67) demonstrated that 60% of cells obtained by BAL were lymphocytes (Figure 1).

Figure 1



Cellular composition of BAL of normal subjects and patients with idiopathic pulmonary fibrosis or sarcoidosis

The finding of increased lymphocytes in BAL correlated with the number of lymphocytes found infiltrating the pulmonary interstitium. The term "active alveolitis" was thus defined (68)

as a BAL lymphocyte count >28%, since this number appeared to differentiate between an active histological picture of inflammation and a more quiescent lesion. Interestingly the number of BAL lymphocytes did not correlate with the extent of granuloma formation (69,70) and indeed the finding of an alveolitis appeared to predate the development of granuloma (Table 9).

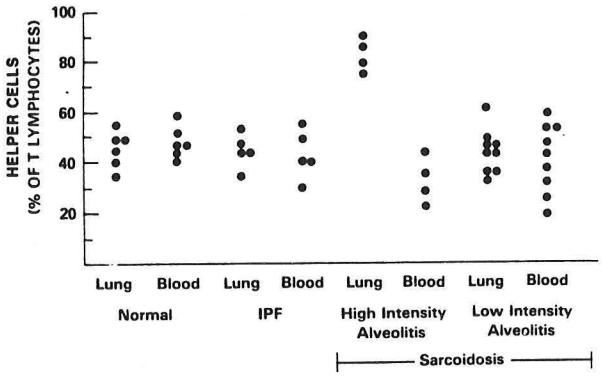
Table 9

Relationship Between Lymphocytic Alveolitis and Extent of Parenchymal Granulomas

	Granulomas			
Alveolitis	0	1+	2+	3+
0	28%	30%	45%	50%
1+	11%	40%	35%	50%
2+	61%	30%	19%	0%

Monoclonal antibody evaluation of lymphocyte markers quickly established the characteristic patterns of sarcoid lung involvement (64,71). While the mean number of CD4+ lymphocytes in peripheral blood was reduced, Hunninghake and colleagues (63) demonstrated a marked increase in CD4+ cells in BAL (Figure 2). CD8+ cells in contrast were enriched in blood but reduced in the alveolus. Similar patterns were observed at other sites of disease activity including skin, lymph node, and spleen (Table 10).

Figure 2



Proportions of CD4 cells in lung and blood of normal subjects and patients with interstitial lung disease

Table 10

Local CD4/CD8 Ratios In Sarcoidosis

Tissue	Sarcoid	Control
Lung	9	2
Lymph node	24	5
Liver	18	2
Skin	20	-

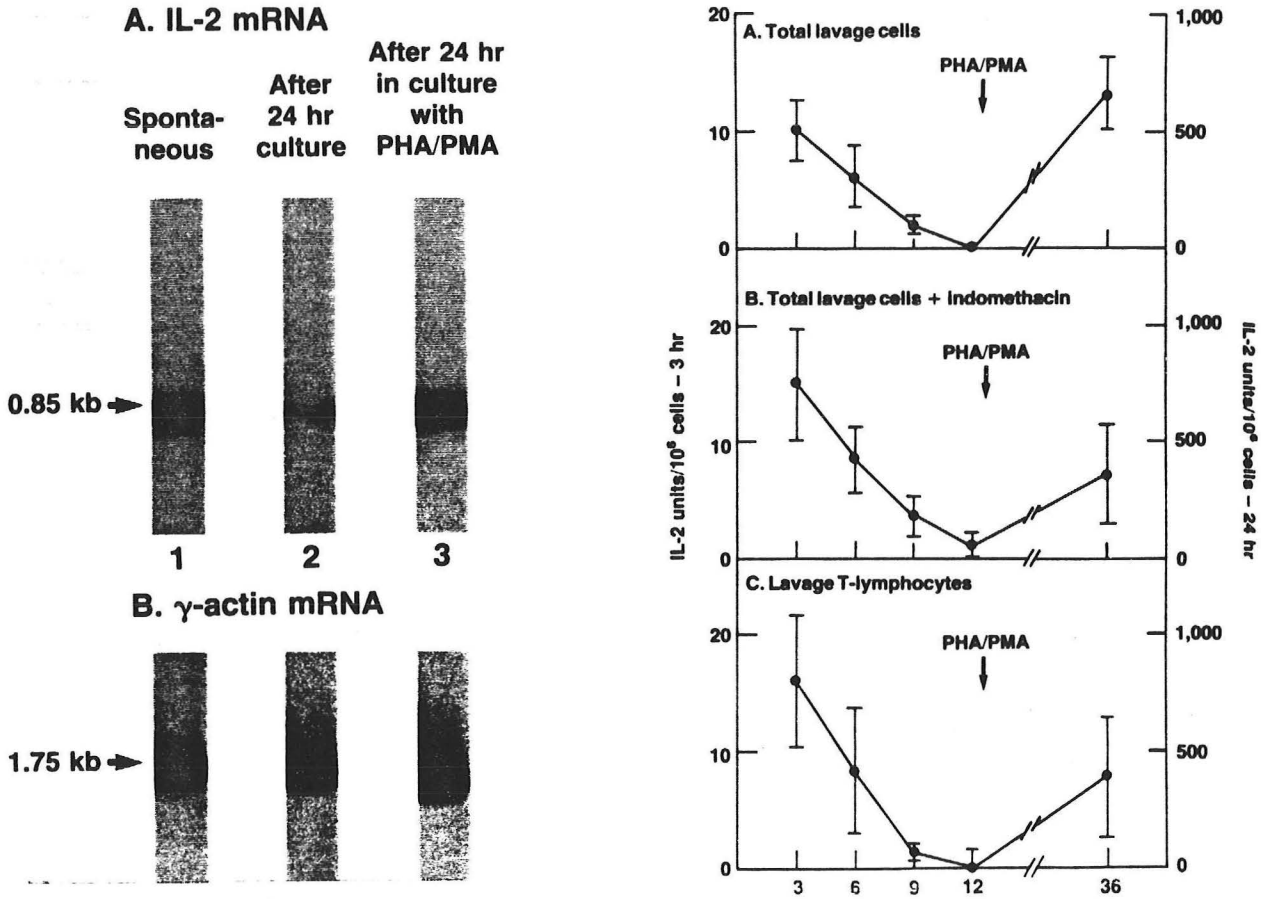
Further analysis of BAL CD4+ cells revealed that the majority of these cells expressed CD29, a marker thought to be consistent with a helper-inducer function while they were CD45 RA- (the subset functioning as a suppressor-inducer fraction). However subsequent advances in the study of T cell maturation are more consistent with the CD4+/CD29+/CD45RA- cell being a previously activated memory cell which has infiltrated the area of disease activity (72,73). Indeed these cells in BAL have been demonstrated to express the CD45 RO marker consistent with memory T cell function (74,75).

Evidence of activation of these CD4+ lung cells has been several fold. First, lung T cells but not blood lymphocytes from sarcoid patients demonstrate increased expression of several activation markers including Class II major histo-compatability (MHC) antigen, CD71 (the transferrin receptor), and the VLA-1 late activation antigen (76-78). More importantly, isolated lung T cells have been demonstrated to spontaneously release IL-2, a cytokine important for ongoing lymphocyte proliferation and express mRNA for IL-2 (79-82). These cells also spontaneously produce chemotactic factors for monocytes and macrophages, release the potentially macrophage-activating cytokine gamma interferon, and induce blood B cells to produce immunoglobulin (63). Finally, lung T cells spontaneously incorporated tritiated thymidine indicating that they were actively proliferating (83,84). The central hypothesis for the immunopathogenesis of sarcoid in the early 1980's was thus one of a disease perpetuated by an intrinsic alteration (63) in lung T cell function. These cells were capable of releasing cytokines which recruited and activated other immune cells with resultant granuloma formation. Indeed several studies evaluated the possibility that T cells had been permanently transformed by a retrovirus (85,86). However the concept that sarcoid reflects an intrinsic abnormality of T cells has come to be modified by several subsequent observations.

First, lung lymphocytes from sarcoid patients quickly lost their "activated" phenotype, stopped producing IL-2, and ceased proliferation within 24 hours of in vitro culture (Figure 3) (79). These observations suggested that ongoing T cell

activation was stimulated by a local factor in the pulmonary milieu and did not reflect an intrinsic property of sarcoid lung T cells. Secondly, studies analyzing the T cell antigen receptor have demonstrated that lung T cells have decreased expression of the alpha/beta T cell receptor compared to blood T cells when they are freshly isolated but increased mRNA transcripts for the beta chain (87,88). These studies are most consistent with a recent activation of lung T cells through the T cell receptor following the presentation of antigen in the pulmonary milieu. Finally several studies have reported a preference for T cell receptor phenotypes in the lungs of sarcoid patients which would be most consistent with stimulation by a specific antigen (see below). Attention has therefore focused on the local stimulation of T cells by antigen in the pulmonary milieu, particularly by lung macrophages.

Figure 3



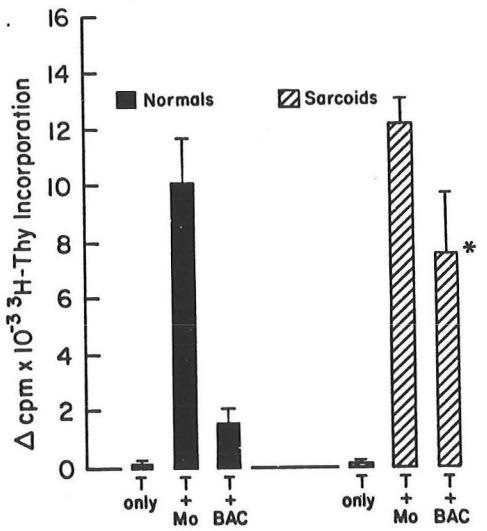
Time course of IL-2 mRNA and IL-2 production by BAL T cells obstained from sarcoid patients. Spontaneous production fell to baseline within 12-24 hours. Cells were capable of response to mitogen (PHA/PMA) indicating continued viability.

The Role of Lung Macrophages

The alveolus is normally a poor environment for the activation and proliferation of T cells in response to antigen. Numerous studies have demonstrated that alveolar macrophages (AM), which comprise >90% of normal BAL mononuclear cells, are poor stimulators of primary T cell proliferation in response to antigen compared to peripheral blood monocytes (89,90). In addition normal AM appear capable of suppressing T cells and inhibiting T cell activation through the T cell receptor, in part by ablating the generation of second messengers necessary for T cell proliferation (91,92).

In contrast, AM from patients with sarcoidosis are efficient stimulators of T cell proliferation in response to soluble antigen (90,93) (Figure 4). Indeed sarcoid AM are as efficient as peripheral blood monocytes and markedly superior to normal AM. The enhanced stimulatory capacity of sarcoid AM appears to be explained in part by the presence of recently recruited peripheral blood mononuclear phagocytes (94,95). Analysis of sarcoid AM phenotye with monoclonal antibodies demonstrated increased expression of markers (CD11b, CD13, CD14) found on monocytes but less commonly on normal AM.

Figure 4



Effect of monocytes (Mo) and bronchoalveolar cells (BAC) on antigen induced T cell proliferation

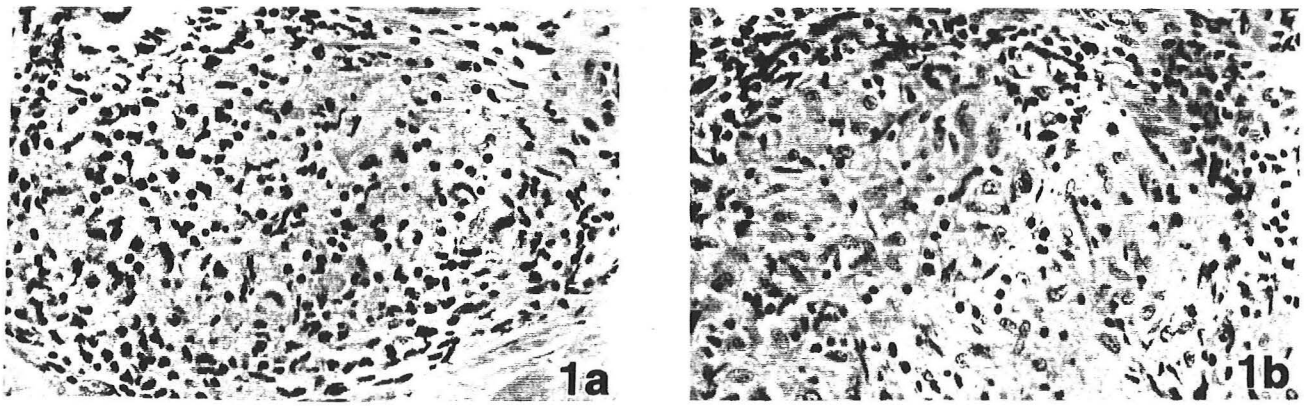
Considerable data suggests that sarcoid AM are in an immunologically activated state. Phenotypic studies demonstrated increased density of Class II MHC molecules, transferin receptors

(CD71) and the alpha chain of the IL-2 receptor (CD25) on sarcoid AM. More importantly sarcoid AM release pro-inflammatory cytokines (96-98) such as tumor necrosis factor-alpha (TNF), IL-6, IL-1 beta and can release factors important for fibroblast growth (99-101) including fibronectin and alveolar macrophage derived growth factor. The production of 1,25 vitamin D and angiotensin converting enzyme by AM has also been demonstrated to reflect the activation of AM by lymphokines (102-104).

However, the role of AM in the sarcoid lung appears complex. Because normal AM appear highly suppressive of lymphocyte responses, some changes in "activation" may actually reflect a loss of suppressive properties. Arachidonic acid metabolism is altered in sarcoid AM in a manner likely to reduce suppression of lymphocyte responses (105). Similarly, a recent study demonstrates that sarcoid AM are permissive of early events in T cell activation stimulated through the T cell receptor, in sharp contrast to normal AM (106).

Perhaps the most striking evidence of the role of AM in stimulating the T cell alveolitis and subsequent granuloma formation has been reported by Holter (107). In this study, intradermal injection of nonviable autologous AM from sarcoid patients induced the formation of granuloma 4-6 weeks later in 41% of patients. The histology of these lesions was identical to that induced by injection of the Kveim-Siltzbach reagent (Figure 5). In sum, our current knowledge of AM function in sarcoidosis strongly suggests that the development of a T cell alveolitis and parenchymal granuloma formation reflects the local presentation of antigen by macrophages to T lymphocytes. The release of pro-inflammatory, chemotactic, and fibrogenic mediators reflect ongoing macrophage-T cell interaction. The identity of the antigen being presented has thus become of central interest in the study of sarcoidosis.

Figure 5



Intradermal granulomas produced by autologous sarcoid AM (1a) and the Kveim-Siltzbach antigen (1b).

Approaches to Identifying the Antigen in Sarcoid

Inert, noninfectious particles are capable of inducing granuloma formation. Additionally, chronic inhalation of beryllium salts leads to a clinical and radiographic syndrome with many similarities to sarcoidosis (108). However while many inert substances can induce granuloma formation (a foreign body granuloma), the histology of these granuloma tends to differ from sarcoid or berylliosis as few activated lymphocytes surround the periphery (109,110). To date no evidence has been furnished in histologic series to suggest that sarcoid is caused by poorly digested, inert foreign particles.

It has long been hypothesized that sarcoidosis is a systemic response to an infectious agent. Epidemiologic studies (111,112) conducted on the Isle of Man demonstrated that 40% of sarcoid cases had contact with a known case prior to diagnosis as compared to 2% of a control group. These studies also demonstrated space-time clustering in the diagnosis of "outbreaks" of sarcoid. These studies may however have been biased by a high reported incidence (14.7/100,000) on a relatively small island (30 x 10 miles). Evidence that sarcoid is a communicable infectious process has not been firmly established in the literature.

Several infectious agents have been suggested as the potential cause for sarcoidosis. Histologic analysis of sarcoid lesions was said to disclose weakly acid fast cocco-bacilli in a small number of patients (113). Non-diptheria corynebacteria may cause pneumonitis with granulomatous involvement of regional lymph nodes and skin lesions but a definitive link of these bacilli to sarcoid has not been established (114). Evaluation of a retroviral cause was reported (115) by assaying for reverse transcriptase (RT) production amongst cultured peripheral blood mononuclear cells from sarcoid patients. In this report 1/26 patients had detectable RT levels and this was associated with syncytia formation. While granuloma from sites of disease were not evaluated this study suggests that a retrovirus is unlikely to cause sarcoid.

Table 11

Detection of Mycobacterial DNA In Sarcoid Lesions By Polymerase Chain Reaction (PCR)

Patient Location	Probe	Sarcoid	TB Control	Normal Control
United Kingdom	IS6110,groEL	10/20	8/13	2/22
United Kingdom	" "	7/16	2/4	1/16
France	" "	2/14	ND	0/11

A continuing controversy has revolved around the role of mycobacteria in the pathogenesis of sarcoid. Recently a number of studies have utilized the polymerase chain reaction (PCR) to detect signs of mycobacterial infection in sarcoid granuloma. Two studies from the United Kingdom probed for two distinct gene sequences found in mycobacteria. The IS6110 insert is relatively specific for M. tuberculosis though it can also be found in M. bovis. The mycobacterial groEL gene, which codes for the 65KD heat shock protein produced by mycobacteria, served as a probe for all mycobacteria. In one study (116) (Table 11) 10/20 sarcoid patients had some evidence of mycobacterial involvement as compared to 8/13 patients with active and 16/49 patients with inactive TB or 2/22 controls. In another study (117) 7/16 sarcoid patients had mycobacterial DNA detected compared to 1/16 controls. Of note, no information was provided as to whether these individuals had been immunized with BCG, which might be detected by the sequences utilized. Another study from the United Kingdom utilized mycobacterial ribosomal RNA probes (118) to demonstrate a five fold increase in hybridization amongst sarcoid spleens compared to controls. The authors of these reports concluded that significant evidence existed to link mycobacteria to sarcoid.

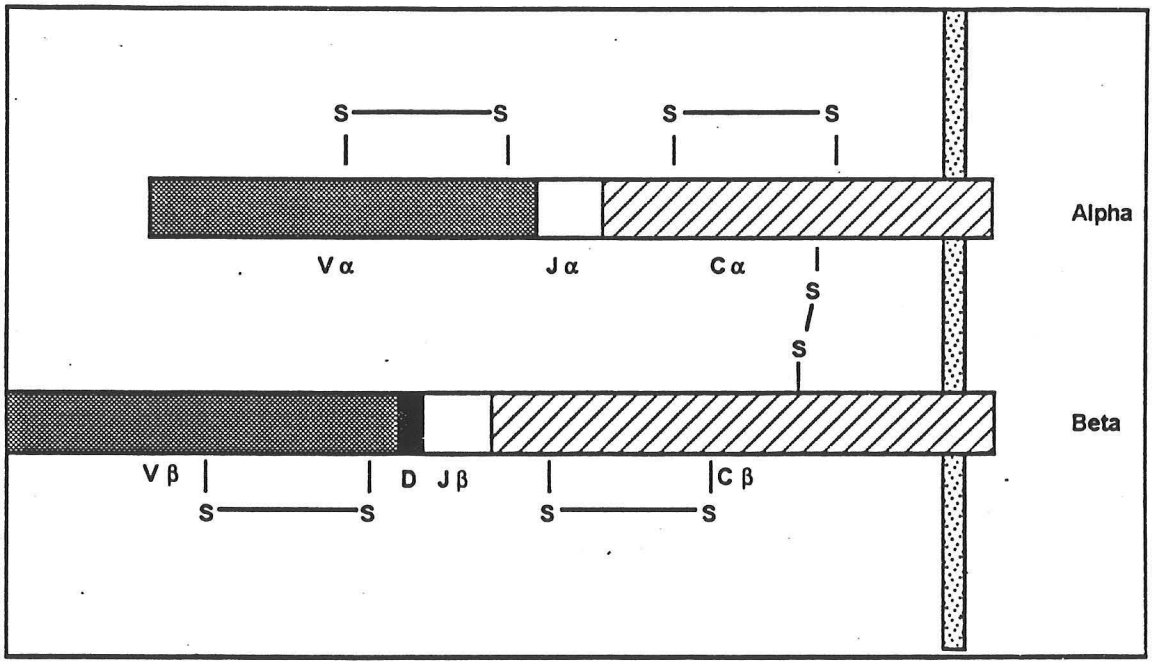
These studies have been countered by an exhaustive PCR evaluation performed by a group of French investigators (119). Using probes similar to the U.K. groups only 2/14 sarcoid patients had convincing evidence of mycobacteria in granuloma. Two other (120,121) groups from Europe utilized PCR techniques somewhat different from the above. A German group reported that 14/14 sarcoid patients had a negative PCR for the 16S DNA sequence of mycobacteria, while a group from Glasgow utilized different primers to evaluate the specificity of amplified groEL DNA. In this study only 1/14 sarcoid lymph nodes produced a mycobacterial DNA product. The authors of all these studies contended that mycobacteria are not associated with most cases of sarcoid and suggested that the positive results of other investigators were the result of contamination.

In attempting to reconcile these studies several technical questions emerge which might bias either the sensitivity or the specificity of the results. However a major issue relates to the value of PCR when probing for a common infectious agent, such as M. TB, which is capable of producing asymptomatic lesions in a large number of patients. Without a firm epidemiologic link PCR may prove too sensitive to establish a causal relationship. The second broader issue, however, is whether sarcoid is caused by the same antigen in all populations. Indeed one explanation for the disparate PCR results might be that the etiologic agent for sarcoid in the United Kingdom is different from that in France. One approach to solving this possibility has been the analysis of the T cell receptor phenotype at sites of disease in sarcoid patients.

The proliferation of CD4 T cells to antigen depends upon the interaction of the T cell receptor (TCR) with a macrophage or

other accessary cell bearing the antigen in context with Class II MHC molecules. The fine specificity of the TCR relies on the presence of both variable and constant regions of the T cell receptor which are coded for by separate genes (Figure 6). CD4 cells express both an alpha and a beta chain of the T cell receptor (122). Each chain is comprised of a variable (V), junctional (J) and constant (C) region, while the beta chain has an additional diversity (D) region not found in the alpha chain. It is estimated that there are approximately 10^{15} possible combinations for T cell receptors in any individual. Cross reactivity between a given antigen and multiple T cell receptors exists and it is thus estimated that $1/10^5$ resting T cells is capable of responding to a given peptide antigen.

Figure 6



Structure of the T cell antigen receptor (Courtesy of Dr. David Karp).

If sarcoidosis were caused by a common antigen one might hypothesize that an over-representation of a few T cell receptor phenotypes might be observed amongst affected individuals. Several investigators (123-126) have described an increased expression of certain V beta phenotypes in lymphocytes from sarcoid lung including V beta 8 (Table 12) and V beta 14. Biases in variable regions from alpha chains (V alpha 2.3) and beta chain constant regions (C beta) have also been reported. However, it should be noted that variable region biases have occurred in a <u>minority</u> of sarcoid patients.

Table 12

T Cell Receptor Biases In Sarcoid Lung

Center	Phenotype	% with overexpression
Bethesda	V beta 8	38%
Stockholm	V alpha 2.3	27%
Bethesda	V gamma 9	~15-20%
Denver	V delta 1	27%

Several confounding variables in the interpretation of these studies exist including the possibility that only a few T cell phenotypes in any given individual are capable of being recruited to the lung. Indeed a recent study of expression of the gamma and delta chains of the T cell receptor on the small subset of T cells which are both CD4 and CD8 negative, suggests that the heterogeneity of these cells may be highly restricted in most normal individuals (127). Of note, several studies have demonstrated a restriction of gamma/delta phenotypes in lung lymphocytes of some patients with sarcoidosis. Whether a similar restriction would occur in a given individual with any form of lymphocytic alveolitis remains an unanswered question.

More importantly it is clear from animal models that the initial response to antigen may be restricted to a limited number of T cell receptor phenotypes but becomes more heterogeneous as the disease progresses (128). Because sarcoid patients present at varying stages of disease, it is possible that over-expression of one TCR type may be missed or mis-interpreted.

Thus analysis of T cell receptor phenotypes in sarcoid has suggested a common antigen may be the etiology in some individuals, but this technology appears limited for exploring the etiology of sarcoid. Fortunately, techniques have been developed which may facilitate identification of peptide antigens being presented by macrophages. These techniques involve the precipitation of Class II molecules, the interruption of peptide bonds and subsequent analysis by mass spectrometry and Edman sequencing to provide peptides being presented by macrophages (129). This would seem to be a promising approach which should overcome the limitations of TCR phenotype and generate candidate peptides as etiologic agents. It should be remembered however that non-peptide antigens, such as superantigens, characteristically produce V Beta biases and could be the etiologic agent in some cases of sarcoidosis.

Summary

Most data would suggest that sarcoidosis results from continued presentation of antigen by tissue macrophages to T lymphocytes at the site of disease. The modulation of the inflammatory response occurs via a variety of cytokines. In a majority of individuals the inflammatory process resolves without lasting functional sequelae. This may reflect clearance of the inciting antigen, development of cellular or humoral suppressor mechanisms, or a genetic predisposition which limits the immune response. In some patients however the inflammatory response is progressive with the development of fibrosis. Considerable effort has been devoted to assessing whether evidence of continued immune activation is useful in determining disease severity, predicting which patients are likely to deteriorate, or respond to anti-inflammatory therapy.

Clinical Application of Markers of Inflammation in Sarcoidosis

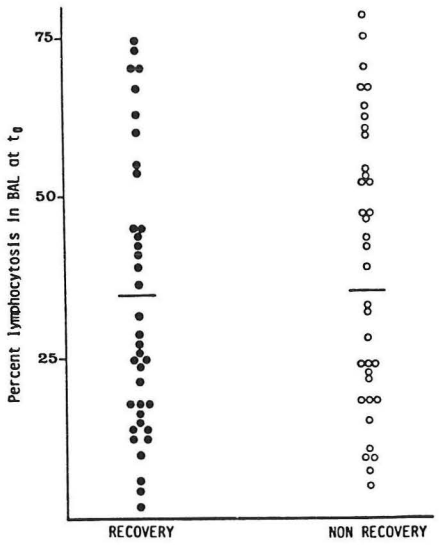
The central issue in the utilization of inflammatory markers for the clinical management of sarcoidosis is whether inflammation itself necessarily connotes a bad prognosis. Several clinical observations, in fact, suggest that active inflammation may portend a satisfactory outcome. First, patients with erythema nodusum who present with clinical parameters of active inflammation such as fever, myalgias and malaise have a uniformly benign course. Secondly, patients with an abrupt onset of symptoms in general have a better prognosis than those with insidious disease. Finally, the high incidence of spontaneous clinical remission in the majority of patients with Stage I or II sarcoid suggests that evaluation of the inflammatory state at a single time point may be misleading.

BAL Studies and Gallium Scanning

A combination of BAL lymphocyte count with another marker of active pulmonary inflammation, Ga^{67} scanning, has been utilized in several studies. Initial reports (68) prospectively followed 19 patients with BAL, gallium scanning and pulmonary function testing every 6 months. 5/14 patients had a high intensity alveolitis as defined by BAL lymphs >28% or uptake of Ga^{67}. This group had a somewhat greater deterioration over a 6 month period. However, spontaneous changes between high intensity and low intensity alveolitis were observed. Several subsequent studies lessened the enthusiasm for BAL and Ga^{67} scanning. In one study (130) 32 patients with sarcoid were followed before and after the institution of steroids. There was no correlation between either BAL lymphocyte counts or Ga^{67} scanning and clinical course during an almost 2 year follow-up. A French study (131) revealed no difference in initial BAL lymphocyte count between those patients subsequently recovered or with continued disease (Figure 7). In another study 98 patients with sarcoid were followed over a 2 year period (132). Approximately 32% of patients with high BAL lymphocytes and 18% of patients with low BAL lymphs were treated

with steroids. No significant change in total lung volumes, vital capacity or DLCO was found at follow-up between patients with an initial high or low BAL lymphocyte count. An initial high lymphocyte count weakly correlated with improvement in FVC but not DLCO or lung volumes during steroid treatment. Several studies have demonstrated that a BAL neutrophilia may be present during the fibrotic phase of sarcoid. Overall, however, BAL lymphocyte counts appear to poorly reflect physiologic impairment or prognosis.

Figure 7



Initial values of total BAL lymphocytes for patients deemed ultimately recovered or non-recovered at 2 years. Mean values were 35% in both groups (p>0.5).

Analysis of CD4/CD8 lymphocyte ratios in BAL may have advantages over the total lymphocyte count alone. Several investigators (133, 134) have demonstrated that a fall in CD4/CD8 ratio in BAL usually corresponds to improved function in studies where sequential lavage has been performed. Evidence suggests that an elevated CD4/CD8 ratio occurs relatively early in the course of sarcoid and often correlates with an abrupt onset. Since these parameters are themselves associated with a good prognosis, the independent value of CD4/CD8 ratios remains uncertain.

More specific markers of immune activation have also been studied. Analysis of release of inflammatory mediators including TNF, IL-1 and PGE_2 by sarcoid AM have not correlated with clinical parameters of disease severity or BAL lymphocyte count (135).

The independent utilization of Ga^{67} scanning has also been evaluated. Ga^{67} is taken up by activated mononuclear phagocytes in the lung and has been utilized as a marker of lung inflammation in a variety of populations. Increased uptake of Ga^{67} has been reported in 60-95% of sarcoid patients and thus would not be expected to predict the clinical course. Using a subjective grading system for uptake gallium scanning correlated with an increased likelihood of response to steroids in some studies (136,137) while in others it did not (130,134,138). Given the expense and lack of definitive data, gallium scanning has fallen into disfavor in the evaluation of sarcoid.

Serum Studies

Serum levels of angiotensin converting enzyme (ACE) are elevated in 30-80% of patients with sarcoid. Elevations appear to correlate overall with clinically active disease though not necessarily with BAL or Ga^{67} assessment of immune activation (139-144). In one study (145), ACE levels were elevated in 77% of patients with clinically progressive disease as opposed to 12% with stable disease. Another study (144) found elevated ACE levels in 62% of patients with progressive disease but only 5% with quiescent disease. Overall, most data suggests that serial changes in ACE levels mirror the routine clinical assessment of disease (Table 13). However a single isolated ACE level does not correlate with either clinical course or likelihood of response to corticosteroids. In one study 3/13 patients with normal ACE levels deteriorated over the next two years. Approximately 50% of symptomatic patients with high ACE levels in this study responded to steroid therapy suggesting limited prognostic value in terms of predicting steroid response. The overall contribution of ACE levels to sarcoid management remains controversial though most authors advise against basing therapeutic decisions on serum ACE levels alone.

Table 13

Comparison of Clinical and ACE Indices of 143 Paired Observations In 61 Patients With Sarcoidosis

	ACE Index		
Clinical Index	**Worse**	**Stable**	**Improved**
Worse	27	6	0
Stable	13	23	3
Improved	3	11	57

Another marker of immune activation in sarcoidosis is measurement of soluble IL-2 receptors (IL-2R) in serum (146,147). IL-2R are released as T cells are activated and thus constitute a method for evaluating ongoing T cell activation. Of note, these studies can not differentiate between activation of CD4 and CD8 cells or other cells expressing IL-2R. In one study (148) a five fold increase in serum IL-2R was noted in patients with sarcoid compared to normal. This elevation did not correlate with ACE levels or BAL lymphocytosis but did decrease with steroid therapy. A larger study (149) of 116 sarcoid patients demonstrated that patients with clinically active disease had higher serum IL-2R than those with inactive disease and that the level dropped with steroid therapy. However serum IL-2R levels did not correlate with BAL lymphocyte number, CD4/CD8 ratio or phenotypic markers of lung lymphocyte activation. Given the observation in this study that 73% of patients with active disease had elevated IL-2R levels it is unlikely that this marker would adequately predict either prognosis or response to therapy.

Directly measuring serum cytokine levels has been attempted as well. Eighty percent of sarcoid patients had elevated serum levels of gamma interferon in one study (150). However, those with the highest levels actually had the best prognosis. In addition, those patients with the best response to steroids demonstrated the smallest drop in gamma interferon levels.

In summary, the high frequency with which BAL, radionucleide, or serum markers of inflammation are abnormal in sarcoidosis appears to limit their utility in identifying the subset of patients likely to deteriorate or respond to steroids. The management of sarcoid patients thus relies on the evaluation of routine clinical parameters.

Management of Pulmonary Sarcoidosis

Given the high rate of asymptomatic sarcoid and of spontaneous remission in patients presenting with Stage I or II sarcoid, it is not surprising that data on the benefit of anti-inflammatory therapy is difficult to interpret. Several studies demonstrate a 70-90% response rate with corticosteroid therapy and 30-50% of patients may relapse after discontinuation of therapy. A prospective trial of low dose Prednisone (15 mg/day for 3 months) versus placebo disclosed (151) a somewhat greater improvement in functional status at 3 months in patients with Stage II or III sarcoid but not Stage I disease. However, longer term follow-up revealed no difference amongst the groups.

A larger prospective randomized trial (152) followed 94 patients randomized to high doses of Prednisone (40mg/d x 3 months, then 20mg/d x 2 years) or 65 patients randomized to placebo. Patients were followed for a mean of 3 years. No significant difference was observed in radiographic progression (Table 14) or pulmonary function testing (Table 15), though there was a tendency for a steroid response.

Table 14

Radiographic Response of Sarcoidosis to Prednisone

	Prednisone	Placebo
Resolution	27%	23%
Partial Improvement	39%	35%
Unchanged	27%	30%
Worse	5%	10%

Table 15

Pulmonary Functions In Sarcoid Patients Following Therapy

	FVC		DLCO	
	Prednisone	Placebo	Prednesone	Placebo
Same	39%	35%	43%	47%
Improved	40%	30%	43%	35%
Worse	20%	35%	13%	18%

The available evidence would suggest that corticosteroids may accelerate symptomatic improvement but appear not to affect the long range prognosis in most patients. These recent recommendations appear in an official publication of the American Thoracic Society (153) for the management of pulmonary sarcoid:

1. Asymptomatic patients with mild to moderate impairment of pulmonary function should be followed clinically and with pulmonary function testing.

2. Asymptomatic patients with severe abnormalities of pulmonary function should receive steroid therapy.

3. Patients with dyspnea, cough, chest pain, or exercise intolerance and abnormal pulmonary functions should be treated.

The optimum regimen for managing pulmonary sarcoid remains to be established. Most current protocols utilize a short course of 15-20mg of Prednisone/day for patients with Stage I disease and symptoms unresponsive to nonsteroidal anti-inflammatory drugs. For patients with profound abnormalities of pulmonary function 40-60mg of Prednisone/day for 8-12 weeks with gradual tapering to 10-20mg every other day over a 6-12 month period has been suggested. Many authors recommend at least one year of therapy prior to discontinuation. Serial study of FVC and DLCO are necessary to judge the efficacy of therapy and vital capacity appears to correlate best with symptoms. Serial changes in the chest roentgenogram should not be utilized to guide therapeutic decisions.

If a patient with pulmonary sarcoidosis does not respond to corticosteroids there are relatively few therapeutic options available. The most successful alternative therapy appears to be methotrexate (154). Low dose methotrexate, 10mg orally once/week, produced subjective improvement in 14/15 patients. However only 36% of patients demonstrated improvement in pulmonary functions. Utility of methotrexate appears greatest in patients with extensive skin involvement. Unfortunately chronic methotrexate therapy has a high incidence of hepatotoxicity and may require serial liver biopsies.

Other agents which have been utilized include cyclosporin A. However in one series (155) this drug failed to induce clinical improvement over a 6 month period. Cytoxan (156) and chloroquine (157) also appear to be of limited value in the therapy of pulmonary sarcoid.

Relatively few patients with sarcoid have undergone lung transplantation. Anecdotal reports at national meetings and in personal communications suggest that involvement of the transplanted organ with sarcoid occurs in some recipients, similar to that observed in cardiac allografts of sarcoid

patients. However sarcoid is not considered a contra-indication for lung transplantation.

Conclusion

Sarcoidosis is a common disease which is asymptomatic in a majority of individuals and appears to have a benign course in many patients. Current data suggests that presentation of a foreign antigen in the pulmonary milieu is responsible for the development of granuloma. The identity of the foreign antigen as well as the mechanisms ultimately determining resolution or progression to fibrosis remain unknown. A commendable effort to utilize local markers of inflammation to predict outcome has taken place over the past decade. Unfortunately these markers appear to have little role in the management of sarcoid. Given the usually benign course of the disease it is apparent that only individuals with impressive symptoms or significant impairment of pulmonary function should receive corticosteroid therapy. For other patients the best advice comes from literature:

> "All of human wisdom is summed up in two words -
> wait and hope".
>
> Alexandre Dumas
> The Count of Monte Cristo

REFERENCES

1. Young RC, Rachal RE, Cowan CL: Sarcoidosis-The beginning: Historical highlights of personalities and their accomplishments during the early years. J. Natl. Med. Assoc. 76:887-896, 1984.

2. Teirstein A, Lesser M: Worldwide distribution and epidemiology of sarcoidosis in Fanburg BL (ed): Sarcoidosis and other granulomatous diseases of the lung. NY, Marcel Dekker, Inc. pp. 101-134, 1983.

3. Yamaguchi M, Hosoda Y, Sasaki A, et al: Epidemiological study on sarcoidosis in Japan. Sarcoidosis 6:138-146, 1989.

4. James DG, Neville E, Siltzbach LE, et al: A world view of sarcoidosis. Ann. NY Acad. Sci. 278:321-332, 1976.

5. British Tuberculosis and Thoracic Association 1973: Familial association in sarcoidosis. Tubercle 54:87-97, 1973.

6. James DG: Genetics and familial sarcoidosis, in Fanberg BL (ed): Sarcoidosis and other granulomatous diseases of the lung. NY, Marcell Dekker, Inc. p. 15, 1985.

7. Siltzbach LE, James DG, Neville E, Turiaf J, Battesti JP, Sharma OP, Hosoda Y, Mikami R, Odaka M: Course and prognosis of sarcoidosis around the world. Am. J. Med. 57:847-852, 1974.

8. Mayock RL, Bertrand P, Morrison CE, Scott JH: Manifestations of sarcoidosis. Analysis of 145 patients, with a review of nine series selected from the literature. Am. J. Med. 35:67-89, 1963.

9. Kataria YP, Shaw RA, Campbell PB: Sarcoidosis: an overview II. Clinical Notes on Respiratory Diseases 20:3, 1982.

10. Sharma OP: Cutaneous sarcoidosis: Clinical features and management. Chest 61:320-330, 1972.

11. Karma A: Ophthalmic changes in sarcoidosis. Acta Ophthalmol. 57:1-94, 1979.

12. Delaney P: Neurologic manifestations in sarcoidosis. Review of literature with a report of 23 cases. Ann. Int. Med. 87:336-345, 1987.

13. Sharma OP, Anders A: Neurosarcoidosis: A report of ten patients illustrating some usual and unusual manifestations. Sarcoidosis 2:96-100, 1988.

14. Graham E, James DG: Neurosarcoidosis. Sarcoidosis 5:125-131, 1988.

15. Fleming HA: Sarcoid heart disease. Sarcoidosis 2:20-21, 1985.

16. Flora G, Sharma OP: Myocardial sarcoidosis: A review. Sarcoidosis 2:20-24, 1985.

17. Pehrsson SK, Tornling G: Sarcoidosis associated with complete heart block. Sarcoidosis 2:135-141, 1985.

18. Stewart RE, Graham DM, Godfrey GW, et al: Rapidly progressive heart failure resulting from cardiac sarcoidosis. Am. Heart J. 115:1324-1326, 1988.

19. Silverman KJ, Hutchins GM, Buckley BH: Cardiac sarcoid: A clinicopathologic study of 84 unselected patients with systemic sarcoidosis. Circulation 58:1204-1215, 1978.

20. Sharma OP, Colp C, Williams, Jr., MH: Pulmonary function studies in patients with bilateral sarcoidosis of hilar lymph nodes. Arch. Int. Med. 117:436-439, 1966.

21. Athos L, Mohlar JG, Sharma OP: Exercise testing in physiologic assessment of sarcoidosis. Ann. NY Acad. Sci. 465:491-501, 1986.

22. Sharma OP, Johnson R: Airway obstruction in sarcoidosis: A study of 123 nonsmoking American black patients with sarcoidosis. Chest 94:343-346, 1988.

23. Polet MA, Lefebyre J, Minet JC, et al: The larynx and sarcoidosis. Acta Otorhino. Belg. 38:192-197, 1984.

24. Siltzbach LE: Sarcoidosis: Clinical features and management. Med. Clin. North Am. 51:483-502, 1967.

25. DeRemee RA: The roentgenographic staging of sarcoidosis: historic and contemporary perspectives. Chest 83:128-32, 1983.

26. Uller NL, Mawson JB, Mathieson JR, Abboud R, Ostrow DN, Champion P: Sarcoidosis: Correlation of extent of disease at CT with clinical, functional and radiographic findings. Radiology 171:613-618, 1989.

27. Brauner MW, Grenier P, Mompoint D, Lenoir S, de Cremoux H: Pulmonary Sarcoidosis: Evaluation with high-resolution CT. Radiology, 172:467-471, 1989.

28. Bergin CJ, Bell DY, Coblentz CL, Chiles C, Gamsu G, MacIntyre NR, Coleman RE, Putman CE: Sarcoidosis: Correlation of pulmonary parenchymal pattern at CT with results of pulmonary function tests. Radiology 171:619-624, 1989.

29. DeRemee RA, Andersen HA: Sarcoidosis: a correlation of dyspnea with roentgenographic stage and pulmonary function changes. Mayo Clin. Proc. 49:742-745, 1974.

30. Sharma OP, Colp C, Williams, Jr MH: Course of pulmonary sarcoidosis with and without corticosteroid therapy as determined by pulmonary function studies. Am. J. Med. 41:541-550, 1966.

31. Reich JM, Johnson RE: Course and prognosis of sarcoidosis in a nonreferral setting: analysis of 86 patients observed for 10 years. Am. J. Med. 78:61-67, 1985.

32. Winterbauer RH, Belic N, Moores KD: A clinical interpretation of bilateral hilar adenopathy. Ann. Int. Med. 78:65-71, 1973.

33. Hughes GRV, Gross NJ: Diagnosis of sarcoidosis by labial gland biopsy. Br. Med. J. 3:215, 1972.

34. Nessan VJ, Jacoway JR: Biopsy of minor salivary glands in the diagnosis of sarcoidosis. N. Eng. J. Med. 301:922, 1979.

35. Khan F, Wessely Z, Chazin SR, Seriff NS: Conjunctival biopsy in sarcoidosis: a simple, safe and specific diagnostic procedure. Ann. Ophthalmol. 9:671, 1977.

36. Mikhail JR, Mitchell DN, Drury RAB, Sutherland I: A comparison of the value of mediastinal lymph node biopsy and the Kveim test in sarcoidosis. Am. Rev. Respir. Dis. 104:544, 1971.

37. Israel HL, Goldstein RA: Hepatic granulomatosis and sarcoidosis. Ann. Int. Med. 79:669, 1973.

38. Koontz CH, Joyner LR, Nelson RA: Transbronchial lung biopsy via the fiberoptic bronchoscope in sarcoidosis. Ann. Int. Med. 85:64, 1976.

39. Gilman MJ, Wang KP: Transbronchial lung biopsy in sarcoidosis. An approach to determine the optimal number of biopsies. Am. Rev. Respir. Dis. 122:721-724, 1980.

40. Koerner SK, Sakowitz AJ, Appelman RI, Becker NH, Schoenbaum SW: Transbronchial lung biopsy for the diagnosis of sarcoidosis. N. Eng. J. Med. 293:268, 1975.

41. Anees KM, Corona F, Masson RG, Whitcomb ME: Transbronchial lung biopsy for sarcoidosis. N. Eng. J. Med. 295:225, 1976.

42. Poe RH, Israel RH, Utell MJ, Hall WJ: Probability of a positive transbronchial lung biopsy result in sarcoidosis. Arch. Int. Med. 139:761, 1979.

43. Roethe RA, Fuller PB, Byrd RB, Hafermann DR: Transbronchoscopic lung biopsy in sarcoidosis. Optimal number and sites for diagnosis. Chest 77:400, 1980.

44. Wall CP, Gaensler EA, Carrington CB, Hayes JA: Comparison of transbronchial and open biopsies in chronic infiltrative lung diseases. Am. Rev. Respir. Dis. 123:280, 1981.

45. Hunninghake GW, Crystal RG: Mechanisms of hypergammaglobulinemia in pulmonary sarcoidosis: site of increased antibody production and role of T-lymphocytes. J. Clin. Invest.. 67:86-92, 1981.

46. Daniele RP, Dauber JH, Rossman MD: Immunologic abnormalities in sarcoidosis. Ann. Int. Med. 92:406-416, 1980.

47. Bogaers Y, Van Der Straeten M, Tasson J, et al: Sarcoidosis or malignancy. A diagnostic dilemma. Eur. J. Respir. Dis. 64:541-550, 1983.

48. Brincker H: Coexistence of sarcoidosis and malignant disease: Causality or coincidence. Sarcoidosis 6:31-43, 1989.

49. Sharma OP, Meyer PR, Akil B, et al: Sarcoidosis and lymphoma: An unusual association. Sarcoidosis 4:58-63, 1987.

50. Winterbauer RH, Kraemer KG: The infectious complications of sarcoidosis. Arch. Int. Med. 136:1356, 1976.

51. Mitchell DN, Scadding JG: Sarcoidosis. Am. Rev. Respir. Dis. 110:774-802, 1974.

52. Jones-Williams W, Erazmus DA, Valerie-James EM, Davies T: The fine structure of sarcoid and tuberculous granulomas. Postgrad Med. J. 46:496-500, 1970.

53. Mitchell DN, Scadding JG, Heard BE, et al: Sarcoidosis: histopathological definition and clinical diagnosis. J. Clin. Pathol. 30:395-408, 1977.

54. Hunninghake GW, Crystal RG: Pulmonary sarcoidosis: a disorder mediated by excess helper T-lymphocyte activity at sites of disease activity. N. Eng. J. Med. 305:429-34, 1981.

55. Semenzato G, Zezzutto A, Pizzolo G, et al: Immunohistological study in sarcoidosis: evaluation at different sites of disease activity. Clin. Immun. Immunopathol. 30:29-40, 1984.

56. Siltzbach LE: The Kveim test in sarcoidosis: A study of 750 patients. JAMA 178:476-482, 1961.

57. Douglas AC, Wallace A, Clark J, Stephens JH, Smith IE, Allan NC: The Edinburgh spleen: Source of a validated Kveim-Siltzbach test material. Ann. NY Acad. Sci. 670-680, 1976.

58. Israel HL, Goldstein RA: Relation of Kveim-antigen reaction to lymphadenopathy: Study of sarcoidosis and other diseases. N. Eng. J. Med. 284:345-349, 1971.

59. Okabe T, Fujisawa M, Watanabe J, Takaku F: Production of colony-stimulating factor by sarcoid granulomas in vitro. Jpn. J. Med. 26:36-40, 1987.

60. Rennard SI, Hunninghake GW, Bitterman PB, Crystal RG: Production of fibronectin by the human alveolar macrophage: mechanism for the recruitment of fibroblasts to sites of tissue injury in interstitial lung diseases. Proc. Natl. Acad. Sci. USA 78:7147-7151, 1981.

61. de Rochemonteix-Galve B, Dayer JM, Junod AF: Fibroblast-alveolar cell interactions in sarcoidosis and idiopathic pulmonary fibrosis: evidence for stimulatory and inhibitory cytokine production by alveolar cells. Eur. Respir. J. 3:653-664, 1990.

62. Bitterman PB, Adelberg S, Crystal RG: Mechanisms of pulmonary fibrosis. Spontaneous release of the alveolar-macrophage-derived factor in the interstitial lung disorders. J. Clin. Invest. 72:1801-1813, 1983.

63. Crystal RG, Roberts WC, Hunninghake GW, et al: Pulmonary sarcoidosis: a disease characterized and perpetuated by activated lung T-lymphocytes. Ann. Int. Med. 94:73-94, 1981.

64. Semenzato G, Agostini C, Zambello R, et al: Activated T cells with immunoregulatory functions at different sites of involvement in sarcoidosis: phenotypic and functional evaluations. Ann. NY Acad. Sci. 465:56-73, 1986.

65. Semenzato G, Pezzutto A, Chilosi M, Pizzolo G: Redistribution of T lymphocytes in the lymph nodes of patients with sarcoidosis. N. Eng. J. Med. 306:48-49, 1982.

66. van Maarsseveen AC, Mullink H, Alons CL, Stam J: Distribution of T-lymphocyte subsets in different portions of sarcoid granulomas: immunohistologic analysis with monoclonal antibodies. Hum. Pathol. 17:493-500, 1986.

67. Hunninghake GW, Fulmer JD, Young, Jr. RC, Gadek JE, Crystal RG: Localization of the immune response in sarcoidosis. Am. Rev. Respir. Dis. 120:49, 1979.

68. Keogh BA, Hunninghake GW, Line BR, Crystal RG: The alveolitis of pulmonary sarcoidosis: evaluation of natural history and alveolitis-dependent changes in lung function. Am. Rev. Respir. Dis. 128:256-265, 1983.

69. Rosen Y, Athanassiades TJ, Moon S, Lyons HA: Nongranulomatous interstitial pneumonitis in Sarcoidosis: Relationship to the development of epithelioid granulomas. Chest 74:122-125, 1978.

70. Bernaudin JF, LaCronique J, Soler P, Lange F, Kawanami O, Basset F: Alveolitis and granulomas: sequential onset and evolution in pulmonary sarcoidosis. Bull. Eur. Physio. Respir. 17:27-64, 1981.

71. Rossi GA, Sacco O, Cosulich E, et al: Helper T-lymphocytes in pulmonary sarcoidosis. Functional analysis of a lung T-cell sub-population in patients with active disease. Am. Rev. Respir. Dis. 133:1086-1090, 1986.

72. Gerli R, Darwish S, Broccucci L, et al: Helper inducer T cells in the lungs of sarcoidosis patients. Analysis of their pathogenic and clinical significance. Chest 95:811-816, 1989.

73. Sanders ME, Makgoba MW, Shaw S: Human naive and memory T cells: reinterpretation of helper inducer and suppressor inducer subsets. Immun. Today 9:195-198, 1988.

74. Gerli R, Darwish S: Memory phenotype of alveolar T cells in sarcoidosis. 98:250-251, 1990.

75. Saltini C, Kirby M, Trapnell BC, et al: Biased accumulation of T-lymphocytes with "memory"-type CD45 leukocyte common antigen gene expression on the epithelial surface of the human lung. J. Exp. Med. 171:1123-1140, 1990.

76. Costabel U, Bross KJ, Ruhle KH, Lohr GW, Matthys H: Ia-like antigens on T-cells and their subpopulations in pulmonary sarcoidosis and in hypersensitivity pneumonitis. Am. Rev. Respir. Dis. 131:337-342, 1985.

76b. Saltini C, Helmer ME, Crystal RG: T-lymphocytes compartmentalized on the epithelial surface of the lower respiratory tract express the very late activation antigen complex VLA-1. Clin Immun. Immunopathol. 46:221-233, 1988.

77. Gerli R, Darwish S, Broccucci L, et al: Analysis of CD4-positive T cell subpopulation in sarcoidosis. Clin. Exp. Immun. 73:226-229, 1988.

78. Costabel U, Bross KJ, Ruhle KH, et al: Ia-like antigens on T-cells and their subpopulations in pulmonary sarcoidosis and in hypersensitivity pneumonitis: analysis of bronchoalveolar and blood lymphocytes. Am. Rev. Respir. Dis. 131:337-342, 1985.

79. Muller-Quernheim J, Saltini C, Sondermeyer P, Crystal RG: Compartmentalized activation of the interleukin-2 gene by lung T-lymphocytes in active pulmonary sarcoidosis. J. Immun. 137:3475-3483, 1986.

80. Hunninghake GW, Bedell GN, Zavala DC, Monick M, Brady M: Role of interleukin-2 release by lung T cells in active pulmonary sarcoidosis. Am. Rev. Respir. Dis. 128:634-638, 1983.

81. Saltini C, Spurzem JR, Lee JJ, Pinkston P, Crystal RG: Spontaneous release of interleukin-2 by lung T lymphocytes in active pulmonary sarcoidosis is primarily from the Leu3+DR+ T cell subset. J. Clin. Invest. 77:1962-1970, 1986.

82. Pinkston P, Saltini C, Muller-Quernheim J, Crystal RG: Corticosteroid therapy suppresses interleukin-2 release and spontaneous proliferation of lung T lymphocytes of patients with active pulmonary sarcoidosis. J. Immun. 139:755-760, 1987.

83. Hunninghake GW, Bedell GN, Zavala DC, et al: Role of interleukin-2 release by lung T-cells in active pulmonary sarcoidosis. Am. Rev. Respir. Dis. 128:634-638, 1983.

84. Pinkston P, Bitterman PB, Crystal RG: Spontaneous release of interleukin-2 by lung T lymphocytes in active pulmonary sarcoidosis. N. Eng. J. Med. 308:793-800, 1983.

85. Blayney DW, Rohatigi PK, Hines W, et al: Sarcoidosis and the human T cell leukemia-lymphoma virus. Ann. Int. Med. 99:409, 1983.

86. Pinkston P, Sarin P, Saxinger WC, et al: Evaluation of the human T cell leukemia lymphoma virus as a possible etiology of sarcoidosis. Am. Rev. Respir. Dis. 129:A21, 1984.

87. Yamaguchi E, Okazaki N, Itoh A, et al: Modulation of accessory molecules on lung T cells. Chest 97:1393-1400, 1990.

88. Du Bois, RM, Kirby M, Balbi B, Saltini C, Crystal RG: T-lymphocytes that accumulate in the lung in sarcoidosis have evidence of recent stimulation of the T-cell antigen receptor. Am. Rev. Respir. Dis. 145:1205-1211, 1992.

89. Toews GB, Vial WC, Dunn MM, et al: The accessory cell function of human alveolar macrophages in specific T cell proliferation. J. Immun. 132:181-189, 1984.

90. Venet A, Hance AJ, Saltini C, et al: Enhanced alveolar macrophage-mediated antigen-induced T-lymphocyte proliferation in sarcoidosis. J. Clin. Invest. 75:293-301, 1985.

91. Rich EA, Cooper C, Toossi Z, et al: Requirement for cell-to-cell contact for the immunosuppressive activity of human alveolar macrophages. Am. J. Respir. Cell Mol. Biol. 4:287-294, 1991.

92. Yarbrough, Jr., WC, Wilkes DS, Weissler JC: Human alveolar macrophages inhibit receptor-mediated increases in intracellular calcium concentration in lymphocytes. Am. J. Respir. Cell Mol. Biol. 5:411-415, 1991.

93. Lem VM, Lipscomb MF, Weissler, JC, et al: Bronchoalveolar cells from sarcoid patients demonstrate enhanced antigen presentation. J. Immun. 135:1766-1771, 1985.

94. Agostini C, Trentin L, Zambello R, Luca M, Masciarelli M, Cipriani A, Marcer G, Semenzato G: Pulmonary alveolar macrophages in patients with sarcoidosis and hypersensitivity pneumonitis: Characterization by monoclonal antibodies. J. Clin. Immun. 7:64-70, 1987.

95. Hance AJ, Douches S, Winchester RJ, Ferrans J, Crystal RG: Characterization of mononuclear phagocyte subpopulations in the human lung by using monoclonal antibodies: Changes in alveolar macrophage phenotype associated with pulmonary sarcoidosis. J. Immun. 134:284-292, 1985.

96. Hunninghake GW: Release of interleukin-1 by alveolar macrophages of patients with active pulmonary sarcoidosis. Ann. Rev. Respir. Dis. 129:569-572, 1984.

97. Hasday JD, Bachwich PR, Lynch JP, Sitrin RS: Tissue thromboplastin and urokinase activities in bronchoalveolar lavage fluid of patients with pulmonary sarcoidosis. Exp. Lung Res. 14:261-278, 1988.

98. Bachwich PR, Lynch JP, Larrick J, Spengler M, Kunkel SL: Tumor necrosis factor production by human sarcoid alveolar macrophages. Am. J. Pathol. 125:421-425, 1986.

99. Meyer KC, Kamininski MJ, Calhoun WJ, Auerbach R: Studies of bronchoalveolar lavage cells and fluids in pulmonary sarcoidosis: Enhanced capacity of bronchoalveolar lavage cells from patients with pulmonary sarcoidosis to induce angiogenesis in vivo. Am. Rev. Respir. Dis. 140:1446-1449, 1989.

100. Moseley PL, Hemken C, Monick M, Nugent K, Hunninghake GW: Interferon and growth factor activity for human lung fibroblasts. Chest 89:657-662, 1986.

101. Baughman RP, Strohofer SA, Buchsbaum J, Lower EE: Release of tumor necrosis factor by alveolar macrophages of patients with sarcoidosis. J. Lab. Clin. Med. 115:36-42, 1990.

102. Thomas PD, Hunninghake GW: Current concepts of the pathogenesis of sarcoidosis. Am. Rev. Respir. Dis. 135:747-760, 1987.

103. DeLuca HF: Evidence that increased circulating 1-alpha, 25-dihydroxyvitamin D is the possible cause for abnormal calcium metabolism in sarcoidosis. J. Clin. Invest. 64:218-225, 1979.

104. Mason RS, Frankel T, Chan Y-L, Lissner D, Posen S: Vitamin D conversion by sarcoid lymph node homogenate. Ann. Int. Med. 100:59-61, 1984.

105. Bachwich PR, Lynch JP, Kunkel SL: Arachidonic acid metabolism is altered in sarcoid alveolar macrophages. Clin. Immun. Immunopathol. 42:27-37, 1987.

106. Weissler, JC, Mendelson C, Moya F, Yarbrough, Jr, WC: Effect of interstitial lung disease macrophages on T cell signal transduction. Am. Rev. Respir. Dis., 1993 (In press).

107. Holter JF, Park HK, Sjoerdsma KW, Kataria YP: Nonviable autologous bronchoalveolar lavage cell preparations induce intradermal epithelioid cell granulomas in sarcoidosis patients. Am. Rev. Respir. Dis. 145:864-871, 1992.

108. Saltini C, Winestock K, Kirby M, Pinkston P, Crystal RG: Maintenance of alveolitis in patients with chronic beryllium disease by beryllium-specific helper T cells. N. Eng. J. Med. 320:1103-1109, 1989.

109. Adams DO: The granulomatous inflammatory process. A review. Am. J. Pathol. 84:164-191, 1976.

110. Boros DL: Granulomatous inflammation. Prog. Allergy 24:183-267, 1978.

111. Parkes SA, Baker SBC, Bourdillon RE, Murray CRH, Rakshit M: Epidemiology of sarcoidosis in the Isle of Man: A case controlled study. Thorax 42:420-426, 1987.

112. Hills SE, Parkes, SA, Baker SBC: Epidemiology of sarcoidosis in the Isle of Man: Evidence for space-time clustering. Thorax 42:427-430, 1987.

113. Cantwell AR: Histologic observations of variably acid-fast pleomorphic bacteria in systemic sarcoidosis: a report of 3 cases. Growth 46:113-121, 1982.

114. Lipsky BA, Goldberger AC, Tomkins LS, Plorde JJ: Infections caused by nondiphtheria corynebacteria. Rev. Inf. Dis. 4:1220-1235, 1982.

115. Lyons DJ, Sinclair A, Smith HG, Mitchell DN, Dalgleish AG: Search for a retroviral cause of sarcoidosis: no evidence from peripheral blood studies. Eur. Respir. J., 4:445-449, 1991.

116. Saboor SA, Johnson NM, McFadden J: Detection of mycobacterial DNA in sarcoidosis and tuberculosis with polymerase chain reaction. Lancet 339:1012-1015, 1992.

117. Fidler HM, Rook GA, Johnsom NM, McFadden J: Mycobacterium tuberculosis DNA in tissue affected by sarcoidosis. BMJ 306:546-549, 1993.

118. Mitchell IC, Turk, JL, Mitchell DN: Detection of mycobacterial rRNA in sarcoidosis with liquid-phase hybridisation. Lancet 339:1015-1017, 1992.

119. Bocart D, Lecossioer D, De Lassence A, Valeyre D, Battesti J, Hance AJ: A search for mycobacterial DNA in granulomatous tissues from patients with sarcoidosis using the polymerase chain reaction. Am. Rev. Respir. Dis. 145:1142-1148, 1992.

120. von Segesser LK, Schaffner A, Stocker R, Lachat M, Speich R, Baumann PC, Turina M: Mycobacterial nucleic acids in sarcoid lesions. Lancet 339:1536-1537, 1992.

121. Thakker B, Black M, Foulis AK: Mycobacterial nucleic acids in sarcoid lesions. Lancet 339:1537, 1992.

122. Marrack P, Kappler JW: The T cell receptor. Chem. Immun. 47:69-81, 1990.

123. Moller DR, Konishi K, Kirby M, et al: Bias toward use of a specific T cell receptor beta-chain variable region in a subgroup of individuals with sarcoidosis. J. Clin. Invest. 82:1183-1191, 1988.

124. Tamura N, Moller DR, Balbi B, Crystal R: Preferential usage of the T-cell antigen receptor ß-chain constant region Cß1 element by lung T-lymphocytes of patients with pulmonary sarcoidosis. Am. Rev. Respir. Dis. 143:635-639, 1991.

125. Grunewald J, Jason CH, Eklund A, et al: Restricted V alpha 2,3 gene usage by CD4+ T-lymphocytes in bronchoalveolar lavage (BAL) fluid from sarcoidosis patients correlates with HLA-DR3. Proceedings of the XII World Conference on Sarcoidosis and Other Granulomatous Diseases, Kyoto, Sept., 1991.

126. Tamura N, Holroyd KJ, Banks T, et al: Diversity in junctional sequences associated with the common human V gamma 9 and V delta 2 gene segments in normal blood and lung compared with the limited diversity in a granulomatous disease. J. Exp. Med. 172:169-181, 1990.

127. Beldjord K, Beldjord C, Macintyre E, Even P, Sigaux F: Peripheral selection of V delta 1+ cells with restricted T cell receptor delta gene junctional repertoire in the peripheral blood of healthy donors. J. Exp. Med. 178:121-127, 1993.

128. Offner H, Buenafe AC, Vainiene M, Celnik B, Weinberg AD, Gold DP, Hashim G, Vandenbark AA: Where, when, and how to detect biased expression of disease-relevant Vß genes in rats with experimental autoimmune encephalomyelitis. J. Immun. 151:506-517, 1993.

129. Chicz RM, Urban RG, Gorga JC, Vignaili DAA, Lane WS, Strominger JL: Specificity and promiscuity among naturally processed peptides bound to HLA-DR alleles. J. Exp. Med. 178:27-47, 1993.

130. Turner-Warwick M, McAllister W, Lawrence R, et al: Corticosteroid treatment in sarcoidosis: Do serial lavage lymphocyte counts, serum angiotensin converting enzyme measurements and gallium-67 scans help management? Thorax 41:903-913, 1986.

131. Israel-Biet D, Venet A, Chreitien J: Persistent high alveolar lymphocytosis as predictive criterion of chronic pulmonary sarcoidosis. Ann. NY Acad. Sci. 465:395-406, 1986.

132. Laviolette M, La Forge J, Tennia S, et al: Prognostic value of bronchoalveolar lavage lymphocyte count in recently diagnosed pulmonary sarcoidosis. Chest 100:380-384, 1991.

133. Ceuppens JL, Ludovicus M, Lacquet GM, Maurits D, Andre VDE, Stevens E: Alveolar T-cell subsets in pulmonary sarcoidosis: Correlation with disease activity and effect of steroid treatment. Am. Rev. Respir. Dis. 129:563-569, 1984.

134. Costabel U, Bross KJ, Guzman J, Nilles A, Ruhle KH, Matthys H: Predictive value of bronchoalveolar T cell subsets for the course of pulmonary sarcoidosis. Ann. NY. Acad. Sci. 465:418-426, 1986.

135. Steffen M, Petersen J, Oldigs M, Karmeier A, Magnussen H, Thiele HG, Raedler A: Increased secretion of tumor necrosis factor-alpha, interleukin-1-beta, and interleukin-6 by alveolar macrophages from patients with sarcoidosis. J. Allergy Clin. Immun. 91:939-49, 1993.

136. Baughman RP, Fernandez M, Bosken CH, Manitl J, Hurtubise P: Comparison of gallium-67 scanning, bronchoalveolar lavage, and serum angiotensin-converting enzyme levels in pulmonary sarcoidosis: Predicting response to therapy. Am. Rev. Respir. Dis. 129:676-681, 1984.

137. Line BR, Hunninghake GW, Heogh BA, Jones AE, Johnston GS, Crystal RG: Gallium-67 scanning to stage the alveolitis of sarcoidosis: Correlation with clinical studies, pulmonary function studies, and bronchoalveolar lavage. Am. Rev. Respir. Dis. 123:440-446, 1981.

138. Hollinger WM, Staton GW, Fajman WA, Gilman MJ, Pine JR, Check IJ: Prediction of therapeutic response in steroid-treated pulmonary sarcoidosis: Evaluation of clinical parameters, bronchoalveolar lavage, gallium-67 scanning, and serum angiotensin-converting enzyme levels. Am. Rev. Respir. Dis. 132:65-69, 1985.

139. DeRemee RA, Rohrbach MS: Normal serum angiotensin converting enzyme activity in patients with newly diagnosed sarcoidosis. Chest 85:45-48, 1984.

140. DeRemee RA, Rohrbach MS: Serum angiotensin-converting enzyme activity in evaluating the clinical course of sarcoidosis. Ann. Int. Med. 92:361-365, 1980.

141. Rohatgi PK, Ryan JW, Lindeman P: Value of serum measurement of serum angiotensin converting enzyme in the management of sarcoidosis. Am. J. Med. 70:44-50, 1981.

142. Lieberman J: Elevation of serum angiotensin-converting enzyme (ACE) level in sarcoidosis. Am. J. Med. 59:365-372, 1975.

143. Rossman MD, Dauber JM, Cardillo ME, Daniele RP: Pulmonary sarcoidosis: Correlation of serum angiotensin-converting enzyme with blood and bronchoalveolar lymphocytes. Am. Rev. Respir. Dis. 125:366-369, 1982.

144. Turton CW, Grundy E, Firth G, Mitchell D, Rigden BG, Turner-Warwick M: Value of measuring serum angiotensin 1 converting enzyme and serum lysozyme in the management of sarcoidosis. Thorax 34:57-62, 1979.

145. Klech H, Kohn H, Kummer F, Mostbeck A: assessment of activity in sarcoidosis: Sensitivity and specificity of 67 Gallium scintigraphy, serum ACE levels, chest roentgenography, and blood lymphocyte subpopulations. Chest 82:732-738, 1982.

146. Semenzato G, Cipriani A, Trentin L, et al: High serum levels of soluble interleukin-2 receptors in sarcoidosis. Sarcoidosis 4:25-27, 1987.

147. Lawrence EC, Berger MB, Brousseau KP, et al: Elevated serum levels of soluble interleukin-2 receptors in active pulmonary sarcoidosis: relative specificity and association with hypercalcemia. Sarcoidosis 4:87-93, 1987.

148. Keicho N, Kitamura K, Takaku F, Yotsumoto H: Serum concentration of soluble interleukin-2 receptor as a sensitive parameter of disease activity in sarcoidosis. Chest 98:1125, 1990.

149. Muller-Quernheim J, Pfeifer S, Strausz J, et al: Correlation of clinical and immunological parameters of the inflammatory activity of pulmonary sarcoidosis. Am. Rev. Respir. Dis. 144:1322-1329, 1991.

150. Prior C, Haslam PL: Increased levels of serum interferon-gamma in pulmonary sarcoidosis and relationship with response to corticosteroid therapy. Am. Rev. Respir. Dis. 143:53-60, 1991.

151. James DG, Carstairs LS, Trowell J, Sharma OP: Treatment of sarcoidosis: Report of a controlled therapeutic trial. Lancet 2:526-528, 1967.

152. Azki MH, Lyons HA, Leilop L, Huang CT: Corticosteroid therapy in sarcoidosis: A five-year, controlled follow-up study. NY State J. Med. 87:496-499, 1987.

153. Sharma OP: Pulmonary sarcoidosis and corticosteroids. Am. Rev. Respir. Dis. 147:1598-1600, 1993.

154. Lower EE, Baughman RP: The use of low dose methotrexate in refractory sarcoidosis. Am. J. Med. Sci. 299:153-157, 1990.

155. Martinet Y, Pinkston P, Saltinic, et al: Evaluation of the in vitro and in vivo effects of cyclosporine on the lung T-lymphocyte alveolitis of active pulmonary sarcoidosis. Am. Rev. Respir. Dis. 138:1242-1248, 1988.

156. Demeter SL: Myocardial sarcoidosis unresponsive to steroids. Treatment with cyclophosphamide. Chest 94:202-203, 1988.

157. British Tuberculosis Association: Chloroquine in the treatment of sarcoidosis. Tubercle 48:257-272, 1967.