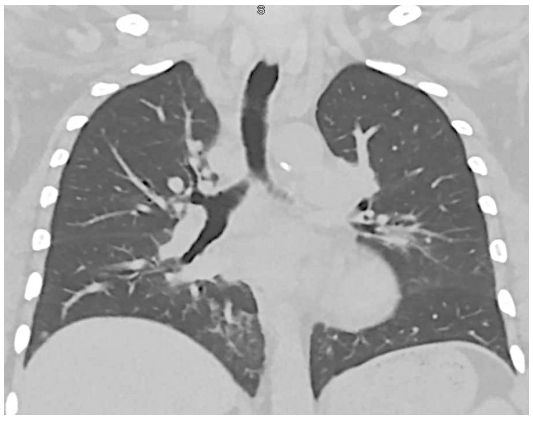
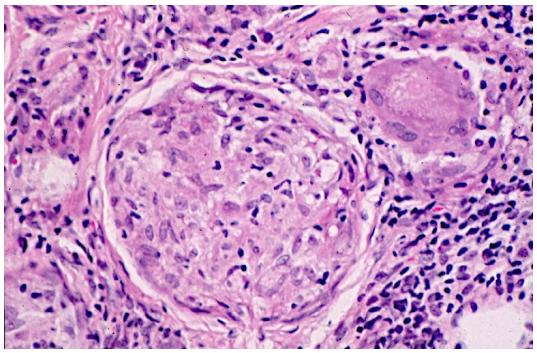
# TOWARDS A UNIFYING CLINICOPATHOLOGICAL UNDERSTANDING OF SARCOIDOSIS





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**INTERNAL MEDICINE GRAND ROUNDS**

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*Dr. Hsia does not have any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Hsia will not be discussing off-label uses in this presentation.*

## Personal Statement

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As a pulmonologist and a pulmonary physiologist, my research interests center on understanding the mechanisms of lung repair and compensatory alveolar-capillary growth, and relating the molecular-cellular responses to their structure-function consequences. In addition, I have maintained a longstanding clinical interest in sarcoidosis. I attend at the Sarcoidosis Clinic at Parkland Hospital and UT Southwestern, manage a Patient Registry, and participate in local and national efforts to facilitate clinical research and patient education with the goal of advancing knowledge and improving management of this multi-system disease.

## Purpose:

Review and update current knowledge of the pathogenesis and pathophysiology of sarcoidosis and its impact on management approaches.

## Objectives:

At the end of this lecture, the learner should be able to:

1. Describe the common clinical manifestations of sarcoidosis
2. Describe the basic cellular processes in granuloma formation
3. Identify potential causative factors of granulomatous inflammation

Sarcoidosis is typically defined as “a multisystem disorder of unknown etiology characterized pathologically by the presence of non-caseating granulomatous inflammation in the involved organs”.

Clinico-pathological understanding of this disease is marked by slow evolution over some 140 years. Progress has been hampered by the heterogeneous nature in nearly every aspect of this condition, including the confusing nomenclature and definition, myriad putative causal agents, distinct demographic-geographic-socioeconomic disparity, diverse clinical manifestations, unpredictable natural course and variable response to therapy.

The milestones in the development of medical knowledge of this condition are summarized below:

*Early clinical diagnosis based on cutaneous findings*

1878 – Jonathan Hutchinson, a famous English surgeon, dermatologist, ophthalmologist, venereologist and pathologist who authored more than 1,200 publications, described a coal worker with purplish skin plaques on both shins, gout and renal failure. He named it “*livid papillary psoriasis*”. This is considered the first recognizable case of sarcoidosis.

1889 – Ernest Besnier, a French dermatologist and histologist who invented the word “biopsy”, recorded a male patient with chronic ulcerations in the ears, violaceous swelling on the nose, erosions in the nostrils, and digital synovitis. Besnier considered the condition an infiltrating form of tuberculosis, and named it “***lupus pernio***”, a misnomer that stuck to this day.

1898 – Jonathan Hutchinson reported more cases, including a 65 year old woman with bilateral symmetrical dusky-red nonulcerating skin plaques that progressed slowly. He considered the term “*lupus vulgaris multiplex nonulcerans*” but in a pioneering spirit decided to recognize the patient by naming it “***Mortimer’s malady***”. Mrs. Mortimer died 6 years later of probable renal failure. Autopsy showed benign enlargement of her lymph nodes.

*Recognition of granuloma pathology and multi-organ involvement*

1899 – Caesar Boeck, a Norwegian dermatologist, removed skin nodules from a patient with widespread lesions, and recorded the classical microscopic observations (**Figure 1**):

“*sharply circumscribed foci of new growth…of epithelioid connective-tissue cells...The nuclei were sometimes multiple…(some) giant cells of the sarcomatous type…”.* He noted that most foci developed in perivascular lymph spaces and the lymph nodes.

Boeck published his findings in an article titled "***Multiple benign sarcoid of the skin***". Later he changed his mind and called the condition “*benign miliary lupoid*”. Others began referring to “*Boeck’s sarcoidosis*” or “*Hutchinson-Boeck’s disease*”.

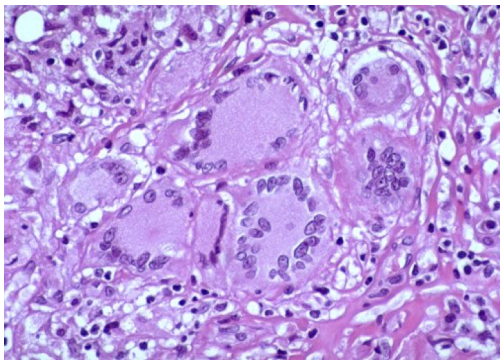


**Figure 1.** Granuloma containing Langhans type giant cells, which were initially described in tuberculosis, but they may be found in any form of granuloma.

1909 – Christian Frederik Heerfordt, a Danish ophthalmologist, described his eponymous syndrome associated with fever, enlarged parotid gland, uveitis, and cranial nerve palsy (usually 7$^{th}$ nerve). He called it “*uveo-parotid tuberculosis*”, later known as “***uveo-parotid fever***”. ***Heerfordt’s syndrome*** is rare, usually chronic and may be associated with cerebrospinal fluid pleocytosis.

1914 – Jörgen Nielsen Schaumann, a Swedish dermatologist, proposed that granulomas contained agents that cause the disease. He described "***Schaumann bodies***"– cytoplasmic inclusions within giant cells that may contain calcium, phosphorus and various proteins. These are related to the "***asteroid bodies***" composed of cytoskeletal filaments, microtubules, centrioles and/or myelinoid membranes. These inclusion bodies can be found in sites of sarcoidosis, tuberculosis, leprosy, fungal and parasite infections, and lipoid and foreign body reaction. He used the term "***benign lymphogranulomatosis***" to distinguish it from Hodgkin's malignant lymphoma. Others began referring to "*Besnier-Boeck-Schaumann disease*" and "*Schaumann-Hutchinson-Boeck disease*".

*Examples of inclusion bodies*

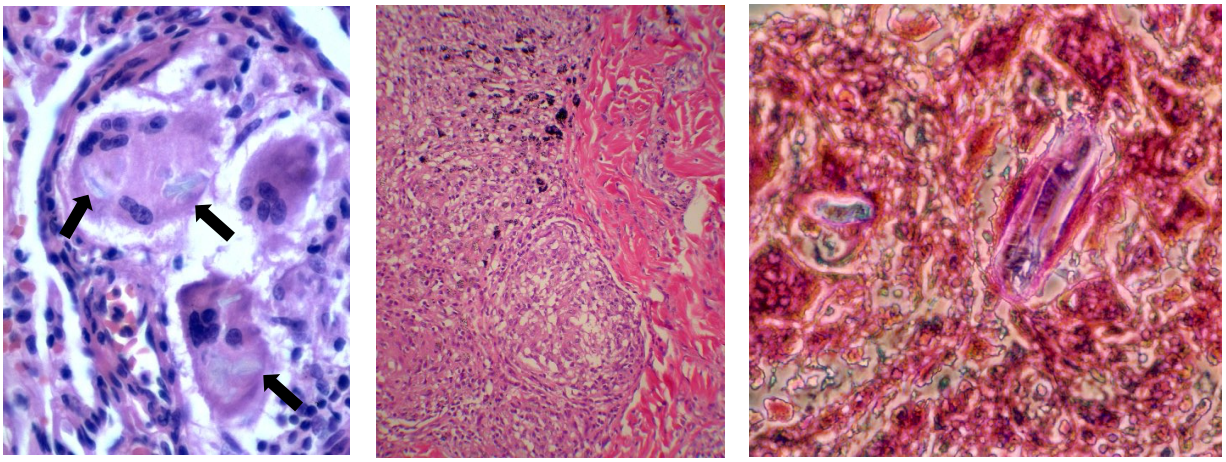


**Figure 2.** Granulomas with giant cells that contain foreign materials: calcium oxalate crystals (left, arrows), tattoo pigments (middle), and magnesium trisilicate (talc) crystals (right).

1940 – Sven Havlar Löfgren, a Swedish internist, described a "*bilateral hilar lymphoma syndrome*" – hilar adenopathy, erythema nodosum, fever, arthralgia – in Caucasian patients.

***Löfgren's syndrome*** is often acute onset with relatively good prognosis. Only 10-20% of patients require therapy beyond 2 years. More frequently seen in Caucasian patients. Those with HLA type DRB1*03 tend to have self-limited disease whereas 50% of those without HLA type DRB1*03 tend not to resolve in 2 years.

*Diagnostic approaches*

1941 – Morten Ansgar Kveim, a Norwegian dermatologist, observed that a suspension of homogenized lymph node tissue from a patient with confirmed sarcoidosis when injected intradermally into another patient with sarcoidosis gave rise in 4-6 weeks to local papules that contained non-caseating granulomas. Control injection with tuberculin produced no reaction. He concluded that active sarcoid tissue contained unknown granuloma-inducing agent(s).

Louis Siltzbach, a New York physician, modified and popularized the ***Kveim-Siltzbach test*** using spleen tissue. The test is positive in 78% of sarcoid patients (range 54%-92%) with ~1% false positive rate. The induced granulomas contain clonal T-lymphocytes. Despite concerns with its reagent source, preparation, standardization, validation, and safety, the Kveim-Siltzbach test was used worldwide as a diagnostic aid to differentiate sarcoidosis from tuberculosis until the advent of bronchoscopy and transbronchial lung biopsy.

1945 – Sheila Sherlock, a British hepatologist, reported ***aspiration liver biopsy*** as a useful tool for diagnosing sarcoidosis. In 1959, her physician husband, David Geriant James, organized the first international medical conference on sarcoidosis, and opened the first dedicated sarcoidosis clinic in London.

1960 – Lynne Reid, a Thoracic Pathologist at Harvard, and Gerard Lorriman reported the use of ***open lung biopsy*** with excision of a hilar node to diagnose sarcoidosis, with special stains to exclude infectious agents and distinguish hyaline material from caseation, and birefringence to detect crystals and polarizable particles.

1961 – Nils Svanborg, a Swedish pulmonary physiologist, published the first monograph on ***pulmonary function abnormalities*** in sarcoidosis. Normal lung function is a common finding despite significant radiologic abnormalities. Typical impairment consists of reduced lung diffusing capacity and lung volumes. Obstructive defects and airway hyperreactivity may be present if granulomas involve airways.

Later measurements of pulmonary function from rest to exercise in sarcoidosis patients show that at a comparable severity of anatomical lung destruction, alveolar-capillary recruitment for lung diffusing capacity, and consequently arterial $O_2$ saturation, are better preserved in sarcoidosis than in idiopathic pulmonary fibrosis (IPF) [1]. The difference reflects the distinct pathophysiology in sarcoidosis, i.e., discrete granuloma deposition with sparing of adjacent parenchyma, vs. diffuse alveolar septal inflammation in IPF.

1975 – Spencer Koerner et al [2] reported the use of fiberoptic bronchoscopy with ***transbronchial lung biopsy*** for the diagnosis of sarcoidosis, with a positive yield in excess of 90%. Subsequent studies reported overall sensitivity of ~60% even when combined with endobronchial biopsy.

A recent randomized international multicenter trial [3] found a significantly higher diagnostic yield for detecting granulomas and granulomatous inflammation by **endobronchial ultrasonography (EBUS)-guided transbronchial needle aspiration** of intrathoracic lymph nodes (80%) compared to that by bronchoscopy + endobronchial or transbronchial biopsy (53%), and by bronchoalveolar lavage – CD4/CD8 ratio (54%) or flow cytometry (24%).

*Radiological Staging*

1961 – Guy Scadding, a British physician, popularized a simple phenotyping system based on chest x-ray staging of pulmonary involvement to provide an estimate of the likelihood of resolution in 5 years. [4]

Stage I: bilateral hilar adenopathy, normal lung parenchyma.
Stage II: bilateral hilar adenopathy, abnormal lung parenchyma.
Stage III: No hilar adenopathy, abnormal lung parenchyma.
Stage IV: fibrotic parenchymal changes.

*Limitations*: Radiological stages do not correlate well with symptoms, pulmonary function, inflammation in other organs, or clinical need for treatment. Staging using chest x-ray does not always correspond to that using chest CT.

*Phenotypic cluster analysis*:

Recently, the *Genotype-Phenotype Relationship in Sarcoidosis study* [5] – a multinational European collaboration (31 centers, 2,163 Caucasian patients) – identified 5 organ-based hierarchical clusters:

- Pulmonary and intrathoracic lymph node involvement
- Abdominal involvement
- Musculoskeletal-cutaneous involvement
- Oculo-cardiac-central nervous system-cutaneous (OCCC) involvement
- Other extrapulmonary involvement

Cluster analysis remains imprecise because occult asymptomatic organ involvement is common, often detected only at autopsy. The number of organs identified depends on the intensity of screening tests and/or biopsy procedures. Clustering may differ in other demographic groups.

Examples of clusters:

European Caucasian females – More frequent eye and skin involvement and fatigue.

U.S. patients – More frequent liver, spleen, skin involvement with constitutional symptoms

African Americans – More frequent liver, spleen and bone marrow involvement

Chinese patients – Older at diagnosis. Less severe lung involvement. Lower frequency of extra thoracic involvement. Higher incidence of hypercalcemia or hypercalciuria.

Japanese patients – More frequent skin, eye and cardiac involvement compared to Caucasian and African American patients.

Löfgren's syndrome – More frequent musculoskeletal and cutaneous manifestations.

Screening for occult organ involvement should be individualized as they incur high cost for laboratory tests, risks associated with radiation exposure or invasive procedures, and may not change treatment decision or outcome. For example, ophthalmological examination is recommended when there are ocular symptoms or positive clinical findings, and in patients with known neurosarcoidosis. Cardiac MRI is recommended in patients with cardiac symptoms or dysfunction, rhythm disturbances, or EKG abnormalities.

*Epidemiology*

Incidence, prevalence and mortality of sarcoidosis are increasing worldwide, in both urban and rural settings. Whether this is due to a true increase in occurrence rate, a generally aging population, greater recognition of the diagnosis especially in developing countries, increased use of diagnostic tools for tissue confirmation, longer survival, or some degree of all of the above, is unclear.

Patient characteristics vary with location: Usually presents in middle age. The mean age of the patient population has been rising in the last 20 years. The disease is more common in females than males; it is both more common and more severe in African Americans than Caucasians. Certain occupations – in construction, heavy metal industry, agriculture, and firefighting – are associated with higher rates of sarcoidosis.

*Genetic Predisposition*

- Familial clustering – 15% of cases. Higher concordance in monozygotic twins
- Disparity in clinical manifestations among racial/ethnic groups
- Genome-wide association studies show polymorphism of genes coding for T cell activation and function (NOTCH4, ANXA11), antigen presentation locus 6p21.3 (dendritic and T cell regulation), and IL-23 receptor associated with sarcoidosis.

- HLA-DRB1*03, DQB1*0201 –associated with Löfgren's syndrome, good prognosis
- HLA-DRB1*15, DQB1*0601 – associated with chronic inflammation

*Poor prognostic signs:*

Severe physiological impairment
Pulmonary fibrosis
Pulmonary hypertension
Extra-pulmonary organ failure

*Etiological Antigens*

Organic
- Infectious organisms (mycobacteria, fungal, viral)
- Non-infectious agents – nucleic acids, proteins (animal or plant origin), tumor antigens

Inorganic
- Chemicals, metals, tattoo pigments, mixed dust, cement, air pollution nanoparticles

Since the 1970's it has been recognized that sarcoidosis is associated with impaired proliferative response of blood monocytes to antigens in vitro, peripheral T-lymphopenia, and accumulation of activated T-lymphocytes within the affected organs. [6]

*Granuloma Formation*

Granuloma represents a host defense mechanism against persistent irritants or chronic infection and occurs as a consequence of the host's failure to eliminate the offending antigens. When the offending antigen is large and indigestible, multi-nucleated giant cells are formed via fusion of monocyte–macrophage lineage cells. [7]

Most commonly, submicroscopic antigens enter the body via the lungs, skin, and mucosa (conjunctiva, nasopharyngeal cavity); these are the most frequently involved organs in sarcoidosis. The antigens are picked by interstitial dendritic cells (DCs), releasing the DCs from normal macrophage inhibition (**Figure 3**) and allowing the DCs to mature and migrate to regional lymph nodes. The DCs interact with naïve CD4+ T helper cells (Th0) and secrete IL-12 and IL-18 to induce T cell proliferation and differentiation into type-1 (Th1) or type-17 (Th17) T helper cell phenotypes (**Figure 4**).

*Polarization of T helper cells* [8]

Th1 type cells
- Polarization is induced by IL-12 and IL-18
- Produce INF-$\gamma$, IL-2 and TNF-$\beta$
- Activate macrophages and phagocytosis
- Evolved for immune response against infections by intracellular bacteria and viruses
- Activated in organ-specific autoimmune responses in genetically susceptible individuals

Th17 type cells
- Polarization is induced by IL-6 and TGF-$\beta$ and maintained by IL-23
- Produce IL-17

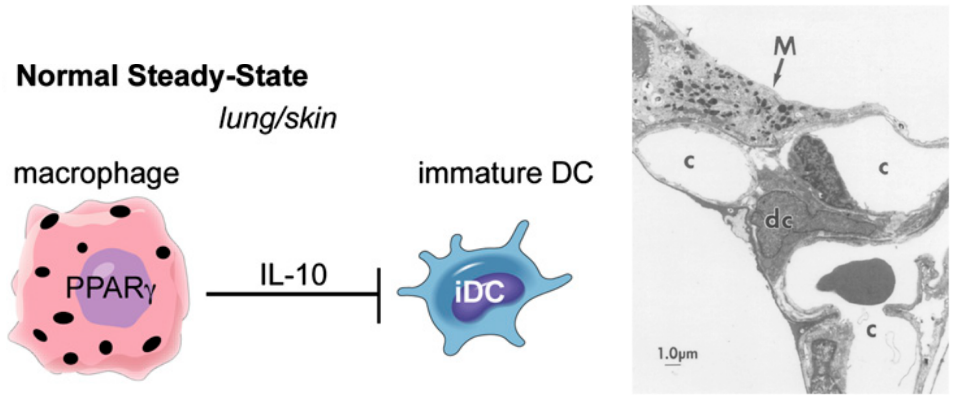



**Figure 3. Normal quiescent steady-state.** *Left:* Macrophages constitutively express peroxisome proliferator–activated receptor (PPAR)γ, a transcription factor that induces macrophage production of IL-10 to inhibit myeloid dendritic cells (DCs, also called antigen-presenting cells). *Right:* Electron micrograph of rat alveolar septum showing an interstitial dendritic cell (dc) with an irregularly shaped indented nucleus in close contact with an alveolar macrophage (M) containing many electron-dense vacuoles. c: capillary. Adapted from Zaba et al [9].

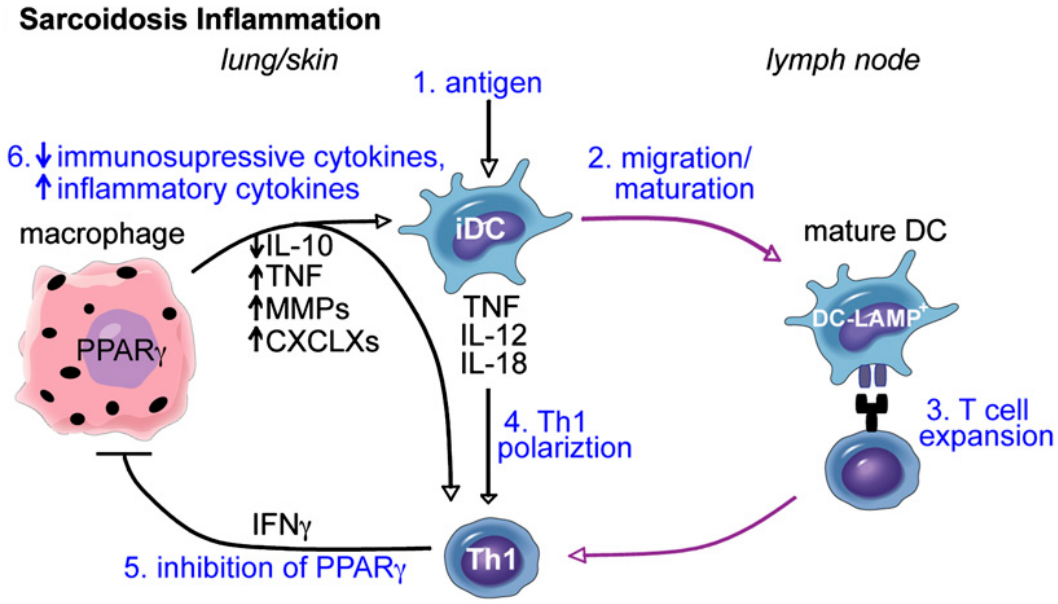



**Figure 4. Self-amplifying cycle of inflammation in sarcoidosis.** The incoming antigen (**1**) activates interstitial DCs that mature and migrate to the lymph node (**2**), where they induce T cell expansion and differentiation into Th1 cells (**3**). Polarized Th1 cells secrete inflammatory mediators TNF, IL-12, and IL-18 (**4**). Th1 T cells also express IFN-γ, which directly inhibits macrophage PPARγ (**5**), resulting in decreased expression of immunosuppressive cytokine IL-10 and increased expression of inflammatory cytokines TNF, MMPs, and CXCLX chemokines (**6**). Decreased IL-10 and increased TNF expression releases DCs from macrophage inhibition, completing the self-amplifying inflammatory loop. *Purple lines* indicate cell migration to and from the lymph node; *black lines* indicate cytokines released from one cell acting upon another; *arrowheads* indicate cell activation; and *flat lines* indicate cell inhibition. Adapted from Zaba et al [9].

*Pathogenic Mediators produced by granulomatous immune cells*

Various *chemokines* – attract myeloid cells and T cells to sites of aggregation.

*Interferon-gamma (IFN-$\gamma$)* – induces monocytes to secrete a fusion molecule – dendritic cell-specific transmembrane protein (*DC-STAMP*), that promotes giant cell formation. [7] IFN-$\gamma$ also activates alveolar macrophages to secrete tumor necrosis factor (TNF) and other mediators of fibrosis, DC maturation, T-cell survival and proliferation, and leukocyte chemotaxis.

Activated neutrophils secrete matrix *metalloproteinases (MMPs),* causing tissue breakdown and cell necrosis. The more intense neutrophilic response in mycobacterial and fungal infections may explain the propensity towards caseation within infectious granulomas.

*TGF-$\beta$* – promotes fibrosis.

*B-cell activating factor* – Signals to B lymphocytes and plasma cells surrounding granulomas can lead to auto-antibody production. A positive antinuclear antibody occurs in up to 30% of sarcoidosis cases. Polyclonal or monoclonal gammopathy also frequently develop.

*Granuloma Maintenance*

The antigen(s) safely sequestered inside granuloma cells may cause no further reaction, or subclinical local inflammation. Some antigen(s) are slowly detoxified or cleared from the body, leading to spontaneous resolution of inflammation. Other antigens require ongoing efforts to fortify their encasement. Chronic efforts by immune cells to build a secure "border wall" may lead to calcification and fibrosis. Depending on antigenic stimuli and the extent and chronicity of granulomas, enlargement of the lymph nodes may or may not be reversible.

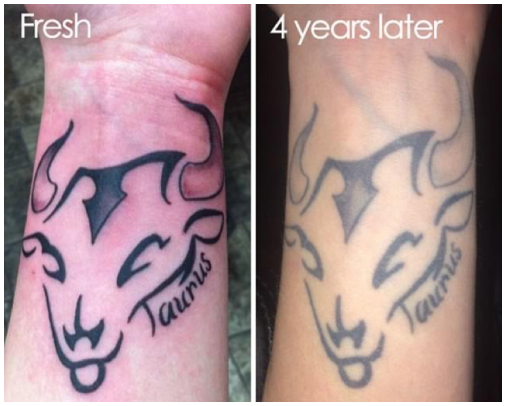



**Figure 5.** The fading tattoo illustrates the slow rate of antigen clearance.

Maintenance of granuloma integrity is critical to successful antigen sequestration, and requires ongoing balance between mediators that promote and degrade barrier functions and between pro- vs. anti-inflammatory activities. Physiological stress, infection, metabolic derangement, and other processes that weaken immune defenses or increase neutrophil secretion of MMPs, could breach of granuloma integrity, leading to antigen escape into surrounding tissue or systemic circulation, and causing renewed inflammation culminating in clinical exacerbation (e.g., delayed systemic reaction to an old tattoo). Thus, persistence of a small amount of antigen can go a long way in causing repetitive cycles of inflammation and injury. This scenario is analogous to that in reactivation tuberculosis.

**Patient 1:**

58 year old Caucasian female presented with dyspnea and chest discomfort. Chest CT shows enlarged paratracheal, hilar and mediastinal lymph nodes, multiple small lung nodules, a large mass in the left lower lobe, background emphysematous changes. Pulmonary function tests show mild restriction with normal $DL_{CO}$. She underwent a left lower lobectomy. The large lung mass was a *carcinoid tumor* ($T_{1a}N_0M_0$)

Biopsy of intrathoracic lymph nodes and smaller lung nodules showed extensive noncaseating granulomas. Negative special stains. TB spot test and fungal serology were negative.

Serum ANA and SMA were positive. Serum protein electrophoresis showed monoclonal gammopathy - IgG-l and IgM-k. Skeletal survey showed diffuse osteopenia without discrete lesions. Patient was given the diagnosis of sarcoidosis, received no specific treatment, and remained stable after 3-years with shrinking lymph nodes.

This patient mounted a robust regional granulomatous response to sequester the tumor antigens with evidence of both T cell and B cell activation. Removal of the tumor eliminates the source of antigenic stimulation, leading to subsequent gradual improvement.

**Patient 2:**

69 year old African American female, a native of Dallas, first presented in 1989 (age 41) with lupus pernio and fulminant multi-system granulomatous inflammation also involving the lung (stage 2 with unusual exudative pleural effusion), cardiomyopathy, central nervous system complications (stroke, panhypopituitarism), manubrial bone cyst, and debilitating constitutional symptoms (night sweats, generalized myalgia, arthralgia). No environmental, occupational, or life style triggering factor could be clearly identified. Over the years, she developed atrial fibrillation and congestive heart failure requiring an AICD, and chronic respiratory failure requiring home oxygen. Chronic therapy using prednisone was complicated by obstructive sleep apnea and osteopenia. Additional treatments included methotrexate and hydroxychloroquine. In the last 2 years, she has responded well to infliximab infusions with resolution of skin lesions and stabilization of organ impairment. However, treatment has been complicated by esophageal candidiasis and infliximab had to be withheld periodically.

The initial fulminant multi-system presentation suggests hematogenous dissemination of a large antigen load that overwhelmed endogenous immune defenses. The progressive inflammatory activity despite suppressive therapy suggests *persistent antigen exposure,* either exogenously introduced or endogenously "recycled" due to impaired ability in maintaining granuloma integrity leading to periodic granuloma breakdown, antigen release and renewed Th1/macrophage stimulation, i.e., reactivation granulomatous inflammation. A third possibility is that of true auto-immunity with runaway immune cell activation in the absence of antigenic stimulation.

*Systemic Treatment*

*Indications/Goals:*

- Symptomatic relief
- Stabilization/reversal of functional impairment
- Prevention of exacerbation

*Mild manifestations:*

NSAID – Mild constitutional symptoms and in mild Löfgren's Syndrome
Hydroxychloroquine – Constitutional symptoms, cutaneous lesions, hypercalciuria
Doxycycline – Cutaneous lesions
Inhaled glucocorticosteroids

*Moderate to severe manifestations:*

*First line:* Systemic glucocorticosteroids

*Second line:* Immunosuppressants – Methotrexate, Azathioprine, Mycophenolate

*Third line:* Anti-TNF agents – Etanercept, Adalimumab, Infliximab

*Paradoxical granuloma formation during anti-TNF therapy*

There have been more than 50 case reports of paradoxical development of sarcoidosis in patients treated with anti-TNF agents for other inflammatory conditions such as psoriasis, rheumatoid arthritis, Crohn's disease, and ankylosing spondylitis. Discontinuation of therapy leads to improvement or resolution of granulomatous inflammation.

One possible explanation for anti-TNF-induced granulomatous disease is a "class-dependent" effect rather than an immuno-allergic reaction. That is, the phenomenon is caused by *cytokine imbalance due to prolonged TNF suppression*, which can trigger a range of autoimmune responses including a "lupus-like" syndrome and leukocytoclastic vasculitis in addition to granulomas. [10,11].

Another possible explanation is supported by the heightened risks of anti-TNF associated reactivation of latent tuberculosis and hepatitis B infection. [12,13] In these infections, the mycobacterial or viral organism or DNA persist in the infected tissue but are safely sequestered. Anti-TNF therapy weaken the integrity of the barriers, allowing the escape of previously encapsulated antigen and triggering renewed inflammation.

*Role of Granuloma Production of 25-Hydoxyvitamin D 1-α Hydroxylase*

An important trail of bread crumbs in following the pathophysiology of granulomatous inflammation is the production of 1-α hydroxylase (CYP27B1) by immune active cells. 1-α hydroxylase is a mitochondrial oxidase belonging to the cytochrome P450 class of hemoproteins. 1-α hydroxylase is the product of a single structural gene present in ancient single cell organisms that catalyzes localized conversion of pro-hormone 25-hydroxyvitamin D (25OHD) to biologically active 1,25-dihydroxyvitamin D (1,25$OH_2$D), which binds to ubiquitous vitamin D receptors (VDRs) to serve two distinct categories of functions (**Table 1**). [14]

**Table 1.**

**Evolutionarily distinct functions of 1,25-dihydroxyvitamin D**

| As Cytokine | As Hormone |
|---|---|
| Primitive function | Advanced function |
| Host protection | Skeletal homeostasis |
| Made by macrophages | Made by renal peritubular epithelial cells |
| Locally acting | Acts at a distance |
| Regulated by immune factors; IFNs, IL15 | Regulated by other hormones; PTH, FGF23 |
| ↓ 25D leads to ↑ 1,25D synthetic rate | ↓ 25D leads to ↑ 1,25D synthetic rate |

The primitive phylogenetic function of all hemoproteins including 1-α hydroxylase is *xenobiotic detoxification* – eliminating microbes or toxic substances by promoting their conversion to nontoxic metabolites or physical removal from the cell. Consistent with the primitive 1-α hydroxylase function, the ancient phylogenetic function of 1,25$OH_2$D is as an *inflammatory*

*mediator* that augments innate immunity to promote microbial killing and downregulates adaptive immunity to mitigate antigen-induced inflammation.

In contrast, the *calciotropic hormonal actions* of 1,25$OH_2$D evolved much later. As marine animals evolved onto land, a strong bony skeleton was required that in turn necessitated development of homeostatic mechanisms to regulate bone metabolism and coordinate the massive traffic of phosphate and calcium and their clearance by the kidney. In vertebrates, renal 1-$\alpha$ hydroxylase and mineral regulatory activities came under the regulation of fibroblast growth factor (FGF)-23 and parathyroid hormone (**Figure 6**).

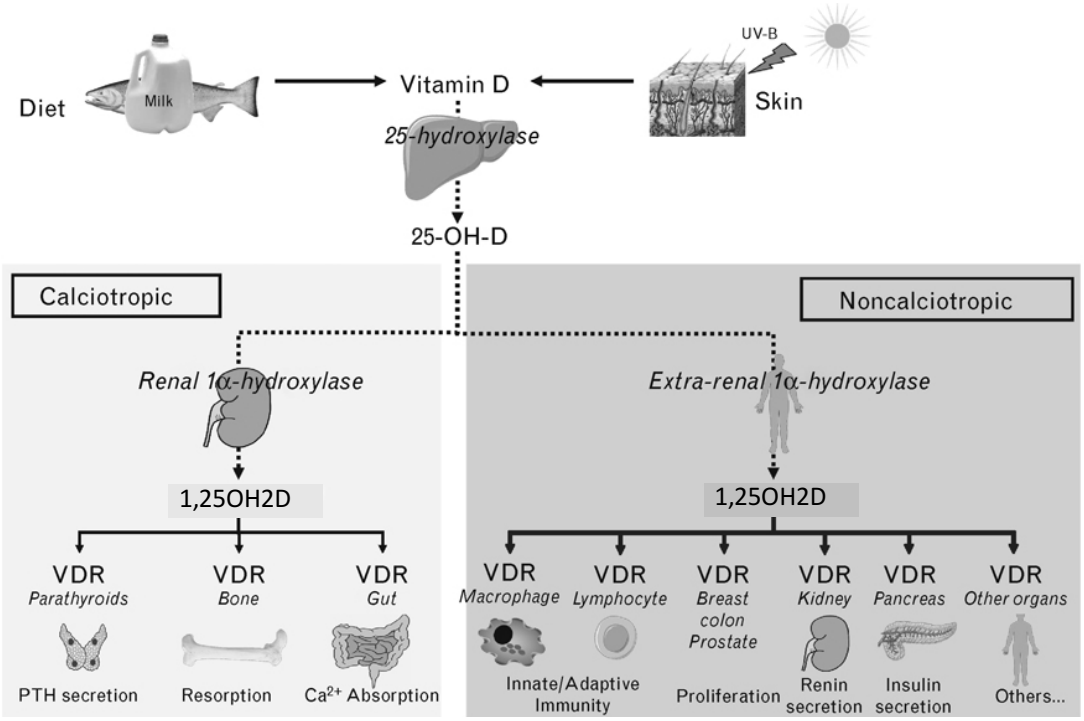



**Figure 6.** Vitamin D precursor is obtained through the diet or photochemically produced in the skin, hydroxylated in the liver to the storage form 25OHD. Further hydroxylation of 25OHD occurs in the kidney or extra-renal sites to the active form 1,25$OH_2$D, which exerts multiple calciotropic and non-calciotropic immune-modulatory actions mediated via the ubiquitous vitamin D receptor (VDR). Adapted from Maalouf [15].

In humans, 1$\alpha$-hydroxylase is expressed primarily in the placenta, kidney, immune cells and skin. Significant extra-renal 1-$\alpha$ hydroxylase functional gene production that is capable of increasing the circulating concentrations of 1,25$OH_2$D and acting in an endocrine mode only occurs in the placenta and in disease-activated tissue macrophages and dendritic cells. The 1,25$OH_2$D produced by activated cells also act locally by binding to VDRs on other activated immune cells, thus contributing to cross-regulation among the network of inflammatory cytokines within the granuloma. [14] Elevated 1,25$OH_2$D production is associated with numerous human granulomatous diseases (**Table 2**).

**Human granulomatous diseases associated with extrarenal overproduction of 1,25-dihydroxyvitamin D**

| Noninfectious | Infectious | Neoplastic |
|---|---|---|
| Sarcoidosis | Tuberculosis | B-cell lymphoma |
| Crohn's disease | Leprosy | Hodgkin's disease |
| Silicone granulomata | Candidiasis | Lymphomatoid granulomata |
| Paraffin granulomata | Histoplasmosis | Dysgerminoma |
| Berylliosis | Coccidiomycosis | Seminoma |
| Wegener's | Cat scratch fever | Mesothelioma |

**Table 2.**

Activity of 1α-hydroxylase and synthesis of $1,25OH_2D$ the cytokine by inflammatory cells is *substrate dependent*, i.e., extracellular concentration of free 25OHD determines how much $1,25OH_2D$ is produced. When serum 25OHD level is low, rate of $1,25OH_2D$ synthesis in immune cells also declines. In contrast, renal production of $1,25OH_2D$ is not substrate-dependent. (**Figure 7**). Serum $1,25OH_2D$ concentration correlates significantly with serum angiotensin converting enzyme (ACE) and serum and urinary [$Ca^{2+}$], and inversely with bone mineral density [16,17].

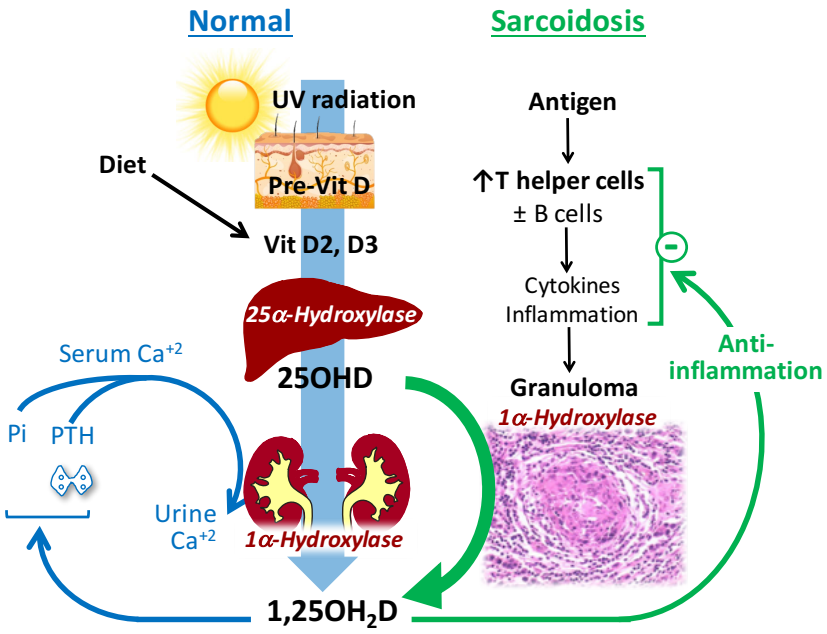



**Figure 7. Normal (renal) and antigen-stimulated granulomatous (extra-renal) vitamin D pathways in sarcoidosis.** Diet and skin-derived vitamin $D_2/D_3$ are hydroxylated in liver to 25OHD, and then via 1α-hydroxylase in kidneys to the active metabolite $1,25OH_2D$. Normal renal pathway (*left*) is primarily regulated by parathyroid hormone (PTH), inorganic phosphate (Pi), and calcium ($Ca^{+2}$) but not by serum 25OHD [18,19]. Active $1,25OH_2D$ exerts both *mineral regulatory* [20] (increasing $Ca^{+2}$/Pi absorption & turnover, *arrows to the left*) and *anti-inflammatory, anti-microbial*, *tissue-protective actions*[21,22 23] (*arrows to the right*). In sarcoidosis (*right*), granuloma immune cells possess receptors for $1,25OH_2D$ and express 1α-hydroxylase to convert 25OHD to $1,25OH_2D$ independent of PTH, Pi, $Ca^{+2}$ or kidney [18] but dependent on substrate [19], i.e., increases with high and decreases with low

[25OHD] [24]. Thus, low serum 25OHD concentration can impair 1,25$OH_2$D-mediated protection against antigen-stimulated inflammation in sarcoidosis.

*Vitamin D and granulomatous infection by M. tuberculosis:*

Vitamin D insufficiency is present in 50-80% of general population [23,25,26], and a risk factor for cardiovascular diseases, diabetes, cancer, asthma and *M. tuberculosis* infection [23,27]. TB granulomas produce 1,25$OH_2$D as an adaptive response to attenuate inflammatory cytokines and promote antimicrobial peptide synthesis for optimal pathogen elimination [28]. Low serum 25OHD concentration impairs microbe-stimulated synthesis of 1,25$OH_2$D and antimicrobial peptides, predisposing to aggressive *M. tuberculosis* infection especially in African American patients [28] while vitamin D repletion reduces inflammation and facilitates microbial clearance [29-31]. Persistence of *M. tuberculosis* antigen (even without active infection) has long been considered a potential trigger of granuloma formation in sarcoidosis.

*Vitamin D status in African American and Caucasian sarcoidosis patients*

To assess the scope of vitamin D derangement in sarcoidosis, we studied an initial 44 patients followed at PHHS Sarcoidosis Clinic Registry. None was taking therapeutic doses of vitamin D. Any over-the-counter vitamin D supplement was discontinued at least 1 week before study entry [26]. All had lung involvement; 68% had multi-organ disease requiring systemic therapy; 48% taking prednisone.

The mostly (93%) African American Dallas cohort was compared with a mostly (95%) Caucasian cohort with biopsy-proven active sarcoidosis followed in university-affiliated clinics in Naples, Italy [26]. All except one patient had [1,25$OH_2$D] within normal range;

Baseline vitamin D and mineral parameters in Dallas African American and Italian Caucasian patients were not different regardless of systemic corticosteroid use; both have high rates of vitamin D insufficiency (74-86%), although African American patients exhibited greater pulmonary dysfunction. Higher urinary $Ca^{+2}$ excretion is associated with renal stones [26]. Lung dysfunction is more severe, and serum 1,25$OH_2$D higher, in African American patients. Vitamin D insufficiency (25OHD<75 nmol/L) is extremely common among both the general African American population in Dallas (97%, Dallas Heart Study) [32] and Caucasian Italians (72%, Pro.V.A. Study) [33]. In both African American and Caucasian sarcoidosis patients, prevalence of low 25OHD stores is also high (86% and 74%, respectively) but not higher than in the location- and race-matched general populations.

*Effect of vitamin D repletion in sarcoidosis*

Sixteen vitamin D-insufficient (25OHD <75 nmol/L or 30 ng/mL) African American patients with normal serum ionized [$Ca^{2+}$] and normal renal function received standard repletion dose of Ergocalciferol ($D_2$, 50,000 IU per week p.o. for 12 weeks). Serum [$Ca^{+2}$] and 24-hr urine $Ca^{+2}$/creatinine (Cr) ratio were measured every 4 weeks [26]. Supplement was well tolerated. Asymptomatic mild elevation in serum $Ca^{2+}$ or urinary $Ca^{+2}$/Cr developed in 3 subjects (**Figure 8a-b**); all reversed without complication upon stopping treatment at the end of study [26]. As serum 25OHD increased (**Figure 8c**), serum PTH was unchanged while selective inflammatory markers ($\gamma$-globulins and a previously elevated ACE) declined. Serum 1,25$OH_2$D level was variable at baseline, but consistently declined post-repletion (**Figure 8d**). Changes in serum [25OHD] and [1,25$OH_2$D] were inversely correlated (**Figure 8e**). There was no significant change in lung function measured at rest pre- to post-treatment.

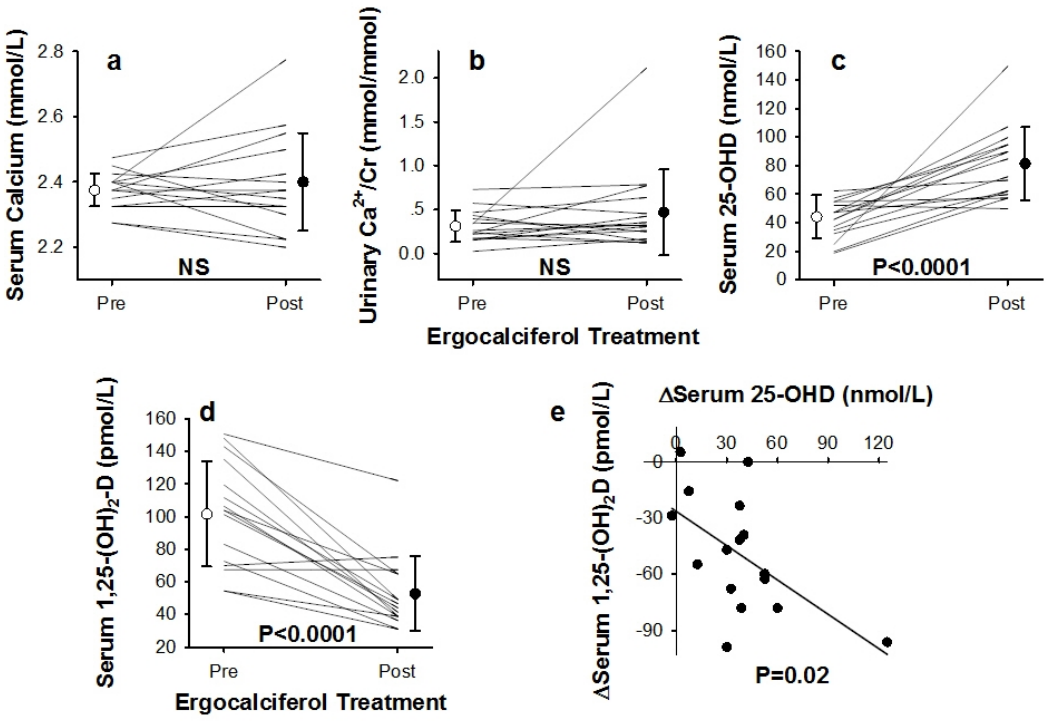
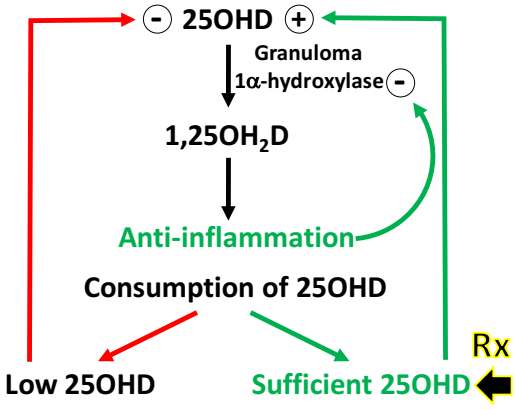



**Figure 8. Vitamin D repletion in patients with reduced serum [25OHD].** (a) Serum total $Ca^{2+}$. (b) 24h urine $Ca^{2+}$/Creatinine. (c) Serum 25OHD. (d) Serum 1,25$OH_2$D. Individual data points and group mean ± SD are shown. P values: pre- vs. post-repletion, paired t-test. (e) Inverse correlation between (post–pre) treatment changes (Δ) in serum 1,25$OH_2$D and 25OHD ($R^2$=0.33, p=0.02).

Thus, in vitamin D-insufficient patients, 12-weeks of repletion is safe and effective in normalizing 25OHD stores. However, calcium balance should be monitored during treatment. Contrary to conventional paradigm that increasing [25OHD] stimulates extra-renal 1,25OH2D synthesis and increases risks of mineral complications, the inverse relationship between serum [25OHD] and [1,25$OH_2$D], and the lowering of γ-globulins and previously elevated ACE levels, suggest that 25OHD repletion reduced extra-renal 1,25$OH_2$D synthesis. Findings are consistent with the hypothesis that repletion enhances intrinsic vitamin D-mediated anti-inflammation that in turn downregulates extra-renal granuloma-associated 1α-hydroxylase activity and 1,25$OH_2$D synthesis (**Figure 9**). A longer treatment duration and measures of functional alveolar-capillary recruitment at exercise may be necessary to detect significant changes in lung function.

**Figure 9. A hypothesis of reciprocal feedback control of immunomodulatory granuloma 1α-hydroxylase synthesis**. Low serum [25OHD] curtails substrate-dependent antigen-stimulated granuloma 1α-hydroxylase activity, reducing 1,25$OH_2$D synthesis and anti-inflammation (*left loop*). Repletion of serum 25OHD (**Rx**) permits continued 1,25$OH_2$D synthesis and effective anti-inflammation, which in turn down-regulates granuloma 1α-hydroxylase activity in a feedback loop that eventually lowers serum [1,25$OH_2$D] (*right loops*).



***Summary of main points:***

- Antigens entering the body via multiple portals that cannot be readily eliminated may stimulate Th1 cell-mediated immune response leading to granuloma formation.
- Activated granuloma immune cells produce both pro- and anti-inflammatory mediators and signaling molecules that collectively balance several functional goals:
    - maintain granuloma integrity for effective antigen sequestration
    - detoxify or eliminate sequestered antigens
    - minimize collateral tissue damage
- Imbalance of these immune processes may weaken the granuloma "*border wall*", allowing antigen escape and reactivation of inflammation, causing clinical relapse in chronic sarcoidosis as in other granulomatous diseases.
- The diversity of antigen sources and frequently unrecognized exposure, varied genetic/environmental predisposition, nonspecific definition of sarcoidosis as a diagnosis by exclusion, lack of specific biomarkers and absence of a robust animal model, contribute to the slow evolution of mechanistic understanding and lack of specific curative therapy for this condition.
- Optimal clinical evaluation should emphasize a thorough review of potential infectious, occupational, environmental and lifestyle factors with each patient, followed by appropriate steps for avoidance of further exposure where possible.
- Vitamin D plays a mechanistic role in the evolution and perpetuation of granulomatous inflammation. It is generally safe to replete vitamin D stores in sarcoidosis patients with vitamin D deficiency; calcium balance should be monitored during treatment. Larger scale studies are needed to examine the anti-inflammatory potential of repletion on sarcoid inflammation.

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