Reappraisal of Prevailing Premises in Sarcoidosis

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By

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This effort is dedicated to the guidance provided *in absentia* by the penetrating analyses of JG Scadding, and C Munro.

Human experience, which is constantly contradicting theory, is the great test of truth.
—Samuel Johnson

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PREFACE

MY SARCOIDOSIS ODYSSEY

My investigative interest in sarcoidosis was provoked and sustained by three events that acted as inflection points: As a practicing pulmonologist, I naturally shared an interest in this curious disorder. My informal observation of its natural history, which, at the time, reflected 10-years of practice at Kaiser Permanente, NW Region, differed materially from published reports. For example, I could not recall a single instance of fatality (or hospitalization) attributable to sarcoidosis. The contemporary edition of Harrison's, Principles of Internal Medicine and the sectional leader of a 1980's, annual meeting of American Thoracic Society each cited a 10% sarcoidosis mortality. The disparity provoked my curiosity and led me to inquire about a possible investigative interest at the Center for Health Research, Kaiser Permanente, Northwest Region (CHR, KPNW). The Center furnishes community-based health care information based on data compiled from medical records sourced from the serviced health maintenance population. Eager to demonstrate the utility and value of community-based health care research, Mitchel Greenlick, PhD, the CHR director, in response to my inquiry, encouraged me to pursue my interest, and provided funding, staff support and asked Richard Johnson, PhD, an experienced researcher, to furnish guidance. Among 86 persons identified with sarcoidosis in the serviced KPNW population observed long-term, we found no instances of fatality or hospitalizations attributable to sarcoidosis. I coauthored an article with Dr. Johnson's mentoring, Course and Prognosis of Sarcoidosis in a Nonreferral Setting . . . (The title paid homage to Sones' and Israel's seminal1960 article) and submitted it to The American Journal of Medicine, which accepted it within one week.[1] The response was extremely gratifying: we received more than 100 reprint requests. This encouragement constituted the first inflection point. It kindled a previously latent investigative spirit, which led me to reevaluate other aspects of sarcoidosis (and other pulmonary disorders), often with the resources and guidance of CHR.

Professor Izumi's 1994 article, demonstrating the long-term effect on the course of sarcoidosis imposed by elective corticosteroid therapy (CST), furnished the second inflection point. He reported on 185 asymptomatic, largely stage I individuals, one-third of whom received CST for a variety of non-therapeutic indications (e.g., to clear up radiographic abnormalities for employment). Izumi furnished clear evidence that it imposed harm evidenced by an adverse clinical and radiographic outcome long-term in the treated vs. the untreated controls.[2] In our 1985 paper, I had ascribed our favorable experience vs. published series, presumptively, to adverse selection for referral to reporting tertiary care settings. In response to Izumi's disturbing findings, I systematically reviewed the sarcoidosis mortality literature and reported the effect of long-term CST, which corroborated Izumi's conclusion. I added the observation that the harm imposed by CST was confined to persons with recent-onset sarcoidosis.[3]

The third inflection point was generated by a conversation with Dr Colin Munro at the 1986 American Thoracic Society meeting, in which, in response to my queries, he clarified and expanded on his novel investigative findings, which provided an evidence-based reversal of the prevailing sarcoidosis paradigm, from a systemic granulomatous disease of unknown etiology to a fallback granulomatous syndrome due to inefficient cell-mediated immune function.[4] This conceptual framework replaced a granulomatous response to an unidentifiable causal agent with an immunological deficit as the fundamental disorder underlying sarcoidosis. The ramifications of the proposed genesis provided a fruitful and coherent accounting for numerous enigmatic and seemingly paradoxical observations characterizing sarcoidosis.[5]

Like paroxysmal nocturnal hemoglobinuria (of which it is said that more people study it than have it!), sarcoidosis inspires far more published research—30K items returned in a Medline search for "sarcoidosis," 36K for "emphysema," and 300K for "lung cancer"—than appears justified by either its incidence or its morbidity/mortality. This reflects, I believe, its baffling, enigmatic character, which resembles no other disorder. Its fundamental nature—autoimmune disease? hyperimmune response? infection? aberrant immunological response? —remains uncertain. Its etiology remains unknown despite more than a century of the most arduous and varied investigative efforts. Many features remain inscrutable. For example, more than 90% of cases involve the lungs, leading investigators to infer that it is caused by a respired agent. However, in contrast to infectious respiratory diseases which incite a granulomatous response, e.g., tuberculosis and histoplasmosis, its radiographic pattern is

symmetrical and its progressive evolution, typically retrograde, from hilar adenopathy to pulmonary involvement. Its immunological features defy ready explanation. For example, why would sarcoidosis, defined as a multisystem noncaseating granulomatous disorder of unknown etiology, be confined in some instances to the liver, skin or central nervous system? What accounts for the local immune hyperactivation and peripheral anergy, the paradoxical divergence between the intense pulmonary immunological response and the cutaneous anergy manifested by a lack of response to delayed type hypersensitivity agents? Why is the defining response granulomatous? Is sarcoidosis a disease (sui generis) or a syndrome? What criteria are useful in making the distinction? What accounts for the secular increase in sarcoidosis mortality? As there is no evidence of pre-existing hypersensitivity to any component of Kveim suspension in subjects with sarcoidosis, what, precisely, does a positive (granulomatous) response at four-six weeks signify?

To the extent possible, I will endeavor to furnish evidence-based responses to these questions. For those aspects of sarcoidosis in which I cannot help resolve the issues, I offer the sentiments of David Hilbert (1842-1943), head of the Department Of Mathematics, University of Göttingen, widely regarded as the foremost mathematician of his time, who famously enunciated 23 fundamental unsolved problems which he considered the outstanding challenges in the new century at the 1900 annual International Conference of Mathematicians:

> If we do not succeed in solving a . . . problem, the reason frequently consists in our failure to recognize the more general standpoint from which the problem before us appears only as a single link in a chain of related problems. This conviction of the solvability of every . . . problem is a powerful incentive to the worker. We hear within us the perpetual call: There is the problem. Seek its solution." [6] "*Wir müssen wissen, wir werden wissen*.

My views on this subject have been largely shaped by the penetrating insights of Professors JG Scadding and C Munro and by my correspondence with the latter.

Notes

[1] Reich JM, Johnson RE. Course and prognosis of sarcoidosis in a nonreferral setting: analysis of 86 patients observed for ten years. *Am J Med.*1985;78:61-67.
[2] Izumi T. Are corticosteroids harmful to sarcoidosis—a conclusion drawn from a retrospective study on the chest radiographic prognosis of 185 asymptomatic patients with pulmonary sarcoidosis followed up for more than ten years. *Sarcoidosis*.1994;11(Supp 1):119-122.
[3] Reich JM. 2003 Adverse long-term effect of corticosteroid therapy in recent-onset sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis*. 20(3):227-234.
[4] Munro CS, Mitchell DN, Poulter LW, Cole PJ. Immunological processes active in developing Kveim responses differ in normal and sarcoidosis subjects. *Amer Rev Respir Dis*. 1986;132(Supp):A244
[5] Reich JM Anomalies in the dominant sarcoidosis paradigm justify its displacement. *Immunobiology.* 2017 222(4):672-675. DOI: 10.1016/j.imbio.2016.12.005
[6] Hilbert D. Mathematische Probleme. *Göttinger Nachrichten.* 1900: 253-297. English translation: Joyce D. http://babbage.clarku.edu/~djoyce/hilbert/problems.html#note1.

CHAPTER ONE

DEFINITION

Sarcoidosis can be defined as a disorder of unknown etiology, characterized histologically by noncaseating epithelioid granulomas involving various organs or tissues, with symptoms dependent on the site and degree of involvement. The most recent, updated definition was provided in the 1999 Joint Statement of the ATS/ERS/WASOG expert panel:

> Sarcoidosis is a multisystem disorder of unknown cause. It commonly affects young and middle-aged adults and frequently presents with bilateral hilar adenopathy; pulmonary infiltration, ocular and skin lesions. The liver, spleen, lymph nodes, salivary glands, heart, nervous system, muscles, bones and other organs may also be involved. The diagnosis is established when clinico-radiograpic findings are supported by histological evidence on non-caseating epithelioid cell granulomas. Granulomas of known causes must be excluded. Frequently observed immunological features are depression of cutaneous delayed type hypersensitivity and a heightened Th1 immune response at sites of disease. Circulating immune complexes along with signs of B-cell hyperreactivity may also be found. The course and prognosis may correlate with the mode of onset, and the extent of the disease. An acute onset with erythema nodosum or asymptomatic bilateral hilar adenopathy usually heralds a self-limiting course., whereas an insidious onset, especially with multiple extra-pulmonary lesions, may be followed by relentless, progressive fibrosis of the lungs and other organs.[1]

The earlier, similar, but less elaborate definition adopted at the Seventh International Conference on Sarcoidosis in 1975—"Sarcoidosis is a multisystem granulomatous disorder of unknown etiology, most commonly affecting young adults and presenting most frequently with bilateral hilar adenopathy . . ."— was rejected by Scadding and Mitchell on the basis that the lengthy ". . . description was intended to stand in

place as a definition;" ". . . that it provided no way in which agreement might be reached in a case in which informed observers disagree; and that it lacked a proviso that changes of a specific type must be widely disseminated." The authors recommended its replacement by a morbid anatomical definition: "Sarcoidosis is a disease characterized by the formation in all of several affected organs or tissues of epithelioid-cell tubercles, without caseation though fibrinoid necrosis may be present at the centres of a few, proceeding either to resolution or to conversion into hyaline fibrous tissue." They added: "Since there is no agreement concerning the etiology of sarcoidosis, nor indeed whether sarcoidosis is an etiologically homogeneous group, no reference to etiology can be made in the definition." This definition eliminates plausible etiological candidates (cancer, histoplasmosis, tuberculosis) from *a priori* exclusion. An additional benefit of excluding the requirement of unknown etiology from the definition, emphasized by the authors, is that: ". . . sarcoidosis may represent an unusual reaction to an agent or agents already known and normally causing a well-recognized disease, but difficult to demonstrate in the unusual manifestation of sarcoidosis."[2] The definition proposed by Scadding and Mitchell eliminates the anomaly of persistent failure, despite the most varied and arduous efforts, to ascertain *the* etiology of sarcoidosis: Like the unsuccessful 19th century searches for *caloric, aether* and *phlogiston*, 20th century efforts to identify "*sarcoidogen*" have been futile because, under the terms of this definition, it does not exist. The traditional descriptive definition of sarcoidosis is inherently unsatisfactory because it fails to characterize its fundamental nature.

Scadding observed that the definition of a disease progresses according to increased knowledge from an initial clinical-descriptive, through pathological, to an etiological basis.[3] I will adopt the operational (*i.e.,* morbid-anatomical, pathological) definition advanced by Scadding and Mitchell. Under this definition, "sarcoid like" vs. sarcoidosis is a distinction without a difference. Following Scadding's usage, where a plausible etiology has been identified, the term can be attached as a modifying adjective or noun e.g., "beryllium sarcoidosis" or "sarcoidosis due to tuberculosis."

Notes

[1] Hunninghake GW, Costabel U, Ando MH, et al. ATS/ERS/WASOG statement on sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis*. 1999;16(2):149-173.

[2] Scadding JG, Mitchell DN Definition. In: *Sarcoidosis*, 2nd ed. I985 University Press, Cambridge, UK:13-35.
[3] Scadding JG. Principles of definition in medicine. *Lancet*.1959;1(7068):323-325.

CHAPTER TWO

FUNDAMENTAL NATURE

Characterization of granuloma immunogenesis, its defining element, is a useful means of comprehending the fundamental nature of sarcoidosis. A T-cell helper type-1 (T_H1) response, sarcoidosis granulomas can be considered a fallback to a primitive immunological alternative that evolves following failure of efficient cell-mediated immunity to completely eliminate an intracellular antigen, e.g., M. tuberculosis. Its principal cellular constituents are CD4+ lymphocytes and macrophages. Grunewald et al.,[1] and Lynch et al.[2] furnish a detailed description of the cellular and cytokine components and their interaction. Chen and Moller[3] provide a critical assessment of causal candidates.

Kveim-induced granulomas resemble those of sarcoidosis microscopically, in cellular composition, and phenotype as assessed by monoclonal antibodies.[4,5,6,7,8,9] The early response to a validated Kveim suspension provides a means of assessing the sequence of immunologic events that result in the production of non-caseating epithelioid granulomas.[10] Biopsy-assessed at 28- to 42-days, a granulomatous response to Kveim reagent furnishes a model of sarcoidosis. Its genesis may therefore be considered representative of the sarcoidosis paradigm in which the cellular and cytokine components recapitulate those of the disease. O'Connor and Fitzgerald clearly framed the fundamental question about the conceptual nature of sarcoidosis:

> Assuming an antigenic inciting agent . . . current evidence suggests that the majority of affected individuals mount an efficient and controlled response, resulting in the elimination of antigen and the effective curtailment of the initial response. The prolonged nature of this response (up to 2 years) most likely reflects an inherent resistance to degradation by the antigen, enabling it to persist within the tissue and granuloma for some time. In a proportion of individuals, effective elimination of the inciting agent(s) and/or curtailment of the initial response do not occur and chronic disease ensues. The lack of an efficient response in the latter individuals may be

> due to: (a) exposure to higher doses of inciting antigens or exposure over a longer period of time than in individuals in whom the response resolves; (b) an inability, as a result of genetic or other factors to mount an adequate T-cell mediated response; (c) a defective regulation of the initial response; or a combination of all three of these factors.[11]

Kveim

Classification of sarcoidosis as a disease *sui generis* vs. a syndrome like erythema nodosum with a well-defined clinical presentation and distinctive pathology of varied etiology rests on the distinctive clinical and histological features, oligoclonality of the CD4+ response[12] and frequent positivity of the Kveim response in sarcoidosis. However, positive Kveim responses wane in persons with longstanding sarcoidosis and are less frequently positive in persons with advanced stages in contrast to what one might expect were it a marker of an immunological response. The origin of the suspension (tissue), evolutionary time scale (four- to six-weeks) and histology of a positive response (granulomatous vs. dense lymphocytic infiltration) distinguish it from that in delayed type hypersensitivity (DTH) tests e.g., Mantoux. The features of the Kveim test closely resemble the Mitsuda lepromin test, which is based on the intradermal injection of a suspension of lepromatous tissue containing (killed) causal acid-fast bacilli. The Mitsuda test does not distinguish between normals and individuals with known leprosy. Instead, it correlates with the clinical pattern of disease: those with the tuberculoid form (granulomatous, paucibacillary) test positive; those with the lepromatous (multibacillary) form test negative. This distinction is reflected in the cytokine response. Employing polymerase chain reaction (PCR) amplification of messenger RNA extracted from lepromatous tissue, Yamamura et al. reported that IL-2 and IFNγ (the principal T_H1 cytokines) were most evident in the resistant (granulomatous) form; and IL-4, 5 and 10 (T_H2 cytokines) predominated in the susceptible (lepromatous) form.[13] By analogy, this suggests the possibility that the Kveim reaction detects a special form of tissue reactivity, not a response to a specific antigen or infectious agent.

A positive response to validated Kveim suspension has been assumed to reflect hypersensitivity to some component of the sarcoidosis-spleen-derived agent. The unexplained features associated with this formulation are:

1) No such component has been identified.[10,14]
2) The reasons for selectivity (*i.e.*, specificity) of a "good" suspension are unknown.[10]
3) Efforts to develop an *in vitro* Kveim test (analogous to the beryllium lymphocyte proliferation test) have been unsuccessful.
4) The frequency of positive responses declines with the duration of sarcoidosis.[10]
5) The prevalence of positive Kveim responses in normal persons--0.7 to 2%--far exceeds the prevalence of sarcoidosis.[10]
6) The observation that a high proportion of healthy, young, British adults who failed to convert their tuberculin test following repeated BCG immunizations proved to be Kveim-positive supports the view that a positive Kveim response is a marker of the inability to develop delayed type hypersensitivity (DTH).[15]

Munro et al. employed the test as a model of sarcoidosis in Kveim-positive sarcoidosis subjects vs. normals in a brilliantly conceived sequence of experiments intended to generate an understanding of the genesis of systemic granulomas by immunohistochemically defining the early cellular immunological events that eventuate in a granulomatous response in persons with sarcoidosis.[16] Initially, they evaluated the 48-hour response to intradermal injections of 10-units of tuberculin (Mantoux) and of validated Kveim suspension. Positive tuberculin responses, signifying intact DTH, are characterized by dense, dermal, lymphocytic infiltration. Kveim suspension at 48-hours failed to elicit this response in either normals or Kveim-positive sarcoidosis patients; their cellular components were indistinguishable. The investigators concluded that: ". . . in comparison with healthy controls there is no evidence of pre-existing hypersensitivity to a component of Kveim suspension in subjects with sarcoidosis; neither is there any other manifestation of changed immunological reactivity unique to them at this stage." They further suggested that ". . . subsequent differences between sarcoid and normal subjects in the development of granulomas in the Kveim response may therefore relate to the different handling of the foreign material by the cells affected, rather than to differences in the early, non-specific recruitment of the cells to the test site."[17]

The authors then compared the 11- and 18-day (*i.e.,* three to four weeks prior to maturation) response in healthy controls, Kveim-negative, and Kveim-positive sarcoidosis subjects. The majority of healthy controls and Kveim-negative sarcoidosis subjects responded with features characteristic of DTH reactions: a dense perivascular infiltrate of mononuclear cells

composed of T-cells ("T" designates lymphocytes that arise and mature in the thymus) of helper and suppresser types, with markers of activation (Tac+; Leu9+), and dendritic (Langerhans) cells (OKT6+; RFD1+) with strong HLA-DR expression. Of 13 Kveim-positive sarcoidosis patients, 1 developed a comparable response. The remaining 12 developed a more gradual response characterized by close associations of phagocytic macrophages and helper T-cells, some of which were also Tac+; notably, dendritic cells were absent. The authors inferred that the systemic granulomas characterizing sarcoidosis represented a default to an immunologically more primitive and less efficient response as a consequence of an undefined deficiency in cell mediated immunity. In this paradigm, the exuberant systemic granulomatous response (SGR) and DTH anergy—the "immune paradox" of sarcoidosis—are dual manifestations of the same inefficiency.[17]

Prevailing View vs. Alternative Hypothesis

The prevailing view of sarcoidosis (PV) is that the SGR reflects a genetically-conditioned response to an unidentified antigen(s). The alternative (Munro) hypothesis (HA) is that the characterizing SGR is a distinctive form of reactivity, an immunological fallback reflecting an inefficient cellular immune response. The PV conceptualizes sarcoidosis as a disease *sui generis;* HA, as a syndrome. The corollaries imposed by these alternative hypotheses generate testable, corroborative, "if-then" observations, referred to as "affirming the consequence." Accepting either paradigm means rejecting the alternative; it supports but does not affirm the unrejected paradigm.

If the PV is true, then one would expect: 1) an identifiable causal agent; 2) greater intensity of the immune response would be a marker of disease severity manifested by an adverse prognosis and a consequent need for treatment; 3) sustained suppression of the response, *e.g.*, with corticosteroid therapy (CST), would have a long-term favorable effect by preventing lethal or disabling pulmonary fibrosis; 4) the occurrence of sarcoidosis would not be materially influenced by cellular immune dysfunction unless profound.

If the HA is true, then one would expect: 1) a multiplicity of plausible causative candidates; 2) greater intensity of the response would be a marker of favorable prognosis and the absence of a requirement for treatment; 3) suppression of the (inefficient) immune response would have an adverse long-term effect by hindering resolution; 4) the propensity to

develop sarcoidosis would be exhibited by individuals with a variety of cellular immune dysfunctions. Additionally, one might hope to find direct evidence of cellular immune dysfunction in otherwise healthy persons with sarcoidosis who lack an apparent cause of immune dysfunction.

The rationale justifying displacement of a PV is worth considering: Kuhn described the conditions required for fundamental changes in natural science in *The Structure of Scientific Revolutions.*[18] His inferences were based on historical observations drawn largely from physics (Newton vs. Einstein), chemistry (Priestly vs. Lavoisier) and astronomy (Ptolemy vs. Copernicus). He found that a PV paradigm crisis arose when data from nature failed to conform with the dominant epistemology and when anomalies and violations of paradigm-induced expectations were experienced. Kuhn noted that, in each instance, cumulative examples subverting the tradition of scientific practice led to the sense that something was fundamentally wrong. He emphasized that the revolution in scientific thought was invariably transformative, not cumulative; the supplanting paradigm did not augment the previous theory; it displaced it. He likened its effect to a gestalt-like transformation e.g., the image-ground reversal in the well-known, rabbit-ears/duck-beak exemplar. He further pointed out that theory choice depended not on demonstrable "truth," a philosophically elusive objective, but on practical, empirical criteria: 1) which theory better fit the facts, *i.e.*, explained the available evidence; 2) offered comparative simplicity, *e.g.*, Copernicus (*De revolutionibus orbium coelestium*) vs. Ptolemaeus (*Almagest;*< Gk, *al magiste*, the greatest); 3) its promise as a guide to future research (fruitfulness); 4) its ability to resolve some otherwise unresolvable, outstanding problem; and 5) its preservation of a large part of previously accrued problem-solving ability. Additional desirable paradigm values were: accuracy, predictive ability, self-consistency, plausibility, and compatibility with other theories. The dominant criterion for theory-choice was the demonstrated ability to set up and to solve puzzles presented by nature.

Affirming the Consequence

1. Identification of *the* causal agent:

Despite more than a century of the most arduous and methodologically varied efforts, the etiology of sarcoidosis remains unknown. Its status is well characterized in the concluding paragraph of this recent review in which the authors' conclusion is consistent with the HA:

> The inability to identify a single "cause" of sarcoidosis, as well as the wide variability of disease course and manifestations, suggests that sarcoidosis may represent a heterogeneous spectrum of disorders, caused by a complex interplay of a variety of host factors, infectious processes, and non-infectious environmental exposures that results in a final common pathway to systemic granulomatous inflammation. A plausible hypothesis is that multiple different antigens, when introduced to a host with a susceptible genetic background and appropriate immunologic milieu, may be capable of inducing this aberrant immune response.[19]

A multiplicity of plausible etiological candidates for non-caseating epithelioid SGRs have been advanced, e.g., tuberculosis, neoplasia, histoplasmosis and drugs that suppress the cellular immune response, particularly immune checkpoint inhibitors. Some of these responses have been characterized as "sarcoid-like reactions" by adherents of the view that sarcoidosis is a disease,[20] although their SGRs are indistinguishable from those of sarcoidosis. None of these candidates have proven acceptable as either *an* or *the* etiology.

The provisional classification of sarcoidosis as a disease (vs. a syndrome), rests in part on the response to validated Kveim reagent, which however, lacks an identifiable antigen. Efforts to develop an *in vitro* Kveim test (analogous to the beryllium lymphocyte proliferation test), employing a T-cell response, enabling the identification of the putative antigen, have been unsuccessful thus far.

The PV consequence is not affirmed.

2. Greater intensity of the response would be a marker of disease severity reflected by adverse prognosis and need for treatment:

Individuals exhibiting a brisk granulomatous response have a favorable outcome:

Patients with an acute onset of stage I disease with erythema nodosum, who are known to have a highly favorable prognosis, characteristically exhibit high-intensity lymphocytic alveolitis (assessed with bronchoalveolar lavage--BAL).[21,22] Conversely, persons with high-intensity lymphocytic alveolitis at any stage exhibit more favorable outcomes.[23,24] This observation led Haslam to advance the hypothesis that, "Patients with more efficient inflammatory responses may be better able to eliminate an unknown agent or antigenic stimulus in sarcoidosis."[24]

The PV consequence is not affirmed.

3. Sustained suppression of the SGR would have a long-term favorable effect:

Cyclosporine-A, a fungal metabolite, was considered an ideal therapeutic candidate for sarcoidosis because it specifically suppresses the granulomatous response by inhibiting nuclear factor of activated T cells (NFAT), which leads to a reduction in IL-2, IFNγ, and CD40L, the principal cytokines promoting granuloma genesis. Contrary to expectation, in a controlled, randomized trial limited to persons with progressive pulmonary shadowing, 58% of the subjects who received combined treatment with cyclosporin-A plus prednisone improved vs. 67% of those who received prednisone alone. Among 16 subjects who exhibited a favorable response, 5 of the 7 combined treatment recipients relapsed post-treatment vs. 2 of the 9 prednisone recipients.[25]

Milburn et al.[26] demonstrated that CST in sarcoidosis down-regulates the T_H1 response (granulomatous inflammation followed by resolution) in favor of the T_H2 (pulmonary fibrosis) response. In metanalysis of referral settings (RS), in which CST was provided to 41% of their patients, cumulative sarcoidosis mortality (4.8%) was 10-fold that in population-based (PBS) settings (0.5%), in which CST was provided to 6%. Correction for adverse selection (as indicated by the proportion with stages III, IV) reduced the mortality differential to 6-fold. Stage-normalized mortality was strongly correlated with the proportion treated with CST in the RS.[27] It seems likely that this marked mortality differential has been tacitly ascribed to adverse selection for referral to RS. However, no RS report has documented adverse selection by furnishing a comparison of the pulmonary functional status of their subjects to a reporting PBS. Scadding was the sole author from a RS to furnish information re. disease severity or duration in their reports. He reported that, " . . . many [all stage IV] patients were referred to me after prolonged periods of observation by other physicians because of an unfavorable course."[28] It is noteworthy that the proportion of patients with stage III, IV sarcoidosis in his series (47%) was more than threefold that of the case-weighted mean (15%) of the remaining six tabulated RS series. In an unpublished metanalysis of five competent controlled trials studies of CST in early stage II and III disease,[29,30,31,32,33] long-term adverse outcomes—radiographic deterioration, progressive multisystem sarcoidosis —were consistently higher (range of odds ratios: 2.0, 6.9) in the treated cohorts and deaths due to sarcoidosis were seen only in CST recipients.

The PV consequence is not affirmed.

4. The occurrence of sarcoidosis, widely considered a hyperimmune response, will not be materially influenced by modestly impaired cellular immune function:

4.a Common variable immunodeficiency Disorder(s) (CVID):

Up to 50% of persons with CVID have a deficiency in T lymphocytes in association with their hypogammaglobulinemia. Granulomatous disease in CVID is associated with diminished numbers of myeloid and plasmacytoid dendritic cells.[34,35] Fasano et al. reported the occurrence of sarcoidosis in 8 of 80 patients (10%) with CVID in the combined case registries of Johns Hopkins University and Children's Hospital of Philadelphia. All eight had chronic sarcoidosis, and T-lymphocyte deficiency was identified in the seven in whom subset characterization was reported. This 10% prevalence is 7-fold the estimated lifetime risk of acquiring ascertainable sarcoidosis in a screened Scandinavian population (a high incidence ethnicity).[36] The authors found 22 additional cases of the association in a literature survey.[37] Gathmann[38] and Resnick[39] reported a similar prevalence of SGR in larger CVID populations.

The PV consequence is not affirmed.

4.b Acquired immunodeficiency syndrome (AIDS):

Gomez et al. reported 12 instances in which sarcoidosis developed in individuals with AIDS. In 7, the CD4+ lymphocyte count was only modestly reduced. In the 5 who developed sarcoidosis during immune reconstitution following highly active antiretroviral therapy (HAART), the onset of sarcoidosis coincided with CD4+ repletion to normal or near-normal levels.[40] One might interpret these events as follows: AIDS-induced CD4+ lymphocyte depletion impaired normal cellular immune processing in a fashion similar to that seen in CVID; that, as a consequence, an undefined antigen(s), which, under normal lymphocytic function would have been eliminated, induced a SGR. This scenario became evident in AIDS patients with modest CD4+ depletion and in severely CD4+ depleted individuals only following HAART-induced CD4+ repletion.

The PV consequence is not affirmed.

4.c Drug-induced immune suppression:

Chopra et al. undertook a systematic analysis of drug classes in which numerous instances of SGR have been reported. Tumor necrosis factor alpha (TNFα) is one of the cytokines upgraded in sarcoidosis.[1] TNFα antagonists (etanercept, adalimumab and infliximab) have frequently been cited in the genesis of "sarcoidosis-like reactions."[41,42] Curiously, this drug class has been employed therapeutically for sarcoidosis.

Immune checkpoint inhibitors such as CTLA4-, PD1- and PDL1-blocking antibodies are known to generate "sarcoidosis-like reactions".[1,43] Unlike TNF antagonists, they enhance the immune response to malignant neoplasms by (confusingly) countering the neoplasm-induced, immune blockade. A plausible surmise for the mechanism by which they generate SGR is that, by suppressing the immune-response blocking actions of CTLA4, PD1, and PD1L, they enable cytotoxic lymphocytes to attack malignant cells with the consequent generation and release of abundant tumor antigens.

The PV consequence is not affirmed.

These findings and observations are consistent with HA, and are consonant with clinical observations .The unifying view of sarcoidosis as an etiologically-heterogeneous, immunologically-mediated syndrome as the central abnormality, not a disease (*sui generis*), serves to eliminate the anomalies, paradoxes, puzzles, and violations of expectations associated with PV: It holds that the defining SGR reflects a default to a more primitive and inefficient immunological response attributable to a deficit in efficient cellular immune processing. It thus accounts for its frequent development in persons with CVID and the propensity with which recipients of TNF suppressants and persons with AIDs (during immunological reconstitution) develop sarcoidosis. It elucidates the nature of the "immune paradox." The same deficiency in cellular immune processing that defaults to a granulomatous response (clinically and exemplified in the Kveim response) is manifested by the inability to generate a DTH response to tuberculin. It abolishes definitional shortcomings, eliminating spurious semantic distinctions between "sarcoidosis," pseudo sarcoid," "sarcoid reaction," and "sarcoid-like." It accounts for failure to identify both the etiology of sarcoidosis and the antigenic component of the Kveim suspension as well as the inability to produce an *in vitro* Kveim test. It provides a plausible explanation for the consistent observation that response intensity was positively correlated

with favorable outcomes and that, conversely, its suppression often has an unfavorable long-term effect. Additionally, one might speculate that the unanticipated protective effect of cigarette smoking is attributable to ongoing stimulation and enhancement of cellular immune processing by antigens contained in cigarette-smoke.[44]

Proposed Immunological Mechanisms

1. Influence of HLA II:

Under the assumption that the propensity to acquire sarcoidosis is dependent, in part, on the efficiency with which certain antigen classes are presented to or processed by CD4+ lymphocytes, there would be an expected variance in incidence or course in persons possessing certain HLAII alleles. Berlin et al. found both to be the case. The authors matched the HLA haplotypes of 122 Scandinavian patients with sarcoidosis against a healthy group of 250 control subjects: HLA-DR17 was twice as frequent in the former than the latter; and HLA-DR146 and HLA-DR152 were each associated with chronic disease.[45] On the basis of reports of familial clustering and the varying prevalence of sarcoidosis in different populations, McGrath et al. suggested that genetic predisposing host factors conveyed a susceptibility to develop a SGR to one or more microbes behaving in a non-infectious fashion. Their review of its association with the major histocompatibility complexes illustrated its influence on both the incidence and course of sarcoidosis.[46]

2. Influence of dendritic cells:

Dendritic cells (DC) are characterized by their potent ability to induce T cell proliferation, their high expression of MHC class II peptides, their facility at blood-borne migration in and out of tissue and their migration to lymph nodes. Their principal function is to capture, process and present antigens to and activate naïve T cells located in lymph nodes. They are of three general types: myeloid ("conventional"), plasmacytoid and Langerhans. Myeloid dendritic cells (mDC), the DC most directly involved in sarcoidosis, are derived from hematopoietic precursor cells. They are abundant at barrier sites, i.e., intestines, skin and lungs. They possess a versatile component of receptors for complement, Fc, C-type lectins, and, additionally, the ability to take up nonspecific antigens by means of macropinocytosis. They respond to bacterial stimuli by ligation with Toll-like receptors (TLR) that recognize bacterial cell wall components of peptidoglycan/lipoteichoic acid (TLR2) and

lipopolysaccharide (TLR4). Ligation of TLR2/TLR4 increases the expression of tumor necrosis factor (TNF) and upregulates MHC II and molecules that promote T cell activation. They constitutively produce IL-12, a T_H1 T cell-polarizing cytokine found at high levels in sarcoidosis BAL, which is augmented by co-culture with IFNγ. IL-12 amplifies its own response through induction of more IL-12 release from antigen presenting cells and up-regulation of IL-12b expression on activated T cells ("self-amplifying scenario"), further polarizing them toward the T_H1 lineage. IFNγ , produced by T_H1 T cells is the most highly expressed cytokine in sarcoidosis BAL fluid. T_H2 T cells do not develop in the presence of IFNγ.[47]

Lommatzsch et al. found a unique increase in CD1a-negative mDC employing four-color flow cytometry analysis of BAL cellular constituents. In addition, they observed altered expression of costimulatory molecules (increased CD80 and decreased CD86 expression) on their mDCs.[48] (CD80 and CD86 are costimulatory molecules expressed on antigen-presenting DCs to the receptor, CD28, on the T-cell.)

Mathew et al. hypothesized that diminished DC function might be the source of decreased DTH responses to recall antigens in affected persons. They reasoned that cutaneous anergy could be the consequence of abnormal antigen uptake or presentation or a defect in the effector arm—lymphocytes involved in the DTH response. In a series of immunological assessments, they isolated, phenotyped and assessed the function of ex-vivo blood mDC, plasmacytoid dendritic cells (pDC) and lymphocytes in normals and individuals with active, untreated sarcoidosis and demonstrated that sarcoidosis-derived mDC exhibited an impaired ability to generate T-cell proliferation vs. normals as judged by their diminished ability to incite an allogeneic mixed lymphocyte response (MLR). Moreover, they found evidence of a quantitative clinical counterpart: greater impairment in mDC function correlated with both impaired DTH response to Candida antigen and with advanced radiographic stage, a marker of disease severity. They reported that pDC derived from sarcoidosis subjects incited a normal allogeneic MLR. The number of circulating mDC and pDC were similar to normals. Functional analysis of MLR employing sarcoidosis-derived lymphocytes and control (normal) mDC showed a normal response. Costimulatory and maturation markers in DC of persons with sarcoidosis were up-regulated. T helper-1 (TH1) cytokines—IFNγ , IL-6, TNFα and IL-12—were present in increased amounts. They concluded that the diminished allogeneic immune response was due to isolated mDC functional attenuation and that the function of

sarcoidosis-derived lymphocytes was intact. Reflecting the generally accepted definition of what the presence of a granuloma signifies and thus echoing Munro's interpretation, they stated: “Granuloma formation occurs when the cellular immune response fails to eliminate the antigenic stimuli . . ..”[49]

Ten Berge et al. reported the opposite findings: mDCs in BAL from sarcoidosis patients were increased in number when compared with healthy controls; mDCs purified from BAL of sarcoidosis patients induced T cell proliferation and differentiation and did not show diminished immune reactivity. Additionally, immunohistochemical analyses revealed increased numbers of mature CD86+ DCs in granuloma-containing airway mucosal biopsies.[50]

Zaba and coworkers reviewed the role of dendritic cells in the pathogenesis of sarcoidosis. They noted that mDC are twice as rich in sarcoidosis BAL than in BAL of normals, a finding specific for sarcoidosis. These mDCs were phenotypically immature as reflected in their decreased expression of maturation marker CD83 and co-stimulatory CD86 and that sarcoidosis lung DCs were less able to induce T cell proliferation than normal lung DCs. They reported that mDC recovered from peripheral blood of sarcoidosis patients exhibited reduced function compared with those retrieved from peripheral blood in control subjects and suggested that blunted end organ cellular immunity may contribute to sarcoidosis pathogenesis. They further suggested that: “Decreased DC function is a potential mechanism for sarcoidosis-induced anergy, and may suggest that reduced cellular immunity at the end organs is responsible for disease perpetuation rather than resolution.”[47]

Uslu and co-workers demonstrated that dendritic cell vaccination, intended to augment the immunological response to metastatic cutaneous melanoma, had a notable effect: In 4 of 249 treated individuals, the vaccine generated a sarcoidosis response accompanied by a greater than 4-year freedom from metastasis or progression.[51]

3. CD4+ anergy:

Oswald-Richter et al. demonstrated that CD4+ anergic responses to polyclonal T-cell antigen receptor (TCR) stimulation were present peripherally and within the lungs of sarcoid patients. Consistent with prior observations, they found spontaneous release of IL-2 in sarcoidosis bronchoalveolar lavage CD4+ Tcells.

> However, in contrast to spontaneous hyperactive responses reported previously, the cells displayed anergic responses to polyclonal TCR stimulation. The anergic responses correlated with diminished expression of the Src kinase Lck, protein kinase C-u, and NF-kB, key mediators of IL-2 transcription. Although T regulatory (Treg) cells were increased in sarcoid patients, Treg depletion from the CD4+ T-cell population of sarcoidosis patients did not rescue IL-2 and IFN-γ production, whereas restoration of the IL-2 signaling cascade, via protein kinase C-u overexpression, did. Furthermore, sarcoidosis Treg cells displayed poor suppressive capacity indicating that T cell dysfunction was a global CD4+ manifestation. Analyses of patients with spontaneous clinical resolution revealed that restoration of CD4+ T_H1 and Treg cell function was associated with resolution. Conversely, disease progression exhibited decreased T_H1 cytokine secretion and proliferative capacity, and reduced Lck expression.[52]

Summary

The PV is rejected. The cumulative evidence justifies displacing the prevailing paradigm in favor of the parsimonious, unifying, alternative paradigm. The inference that what we designate as "sarcoidosis" is a syndrome attributable to cellular immune dysfunction, not a disease, is supported by the multiplicity of agents reported to infrequently generate an SGR indistinguishable from sarcoidosis. The view that sarcoidosis is etiologically heterogeneous is supported by Thomas and Hunninghake, who wrote, " . . . the disease might be triggered by different agents . . . the factor that would determine the development of the disease we recognize as sarcoidosis would be the host's immune response to the inciting agent" [italics added].[53] and in Cullinan's summary of the epidemiological evidence in which he suggested that sarcoidosis is ". . . an idiosyncratic response to one or more relatively common environmental agents."[54] Under this formulation, it is erroneous to dismiss a causal candidate because it is not uniformly present. The formulation that a SGR is a fallback to a more primitive response attributable to a variety of cellular immune deficiencies is supported by its frequent appearance in individuals with congenital, infectious and drug-induced cellular immune dysfunctions. Inefficient dendritic cell function appears the most likely underlying cause of sarcoidosis in persons lacking an otherwise apparent cause of impaired cellular immunity.

The HA is falsifiable by the identification of a universally present, confirmed-causative agent, which would imply that sarcoidosis is a disease, not a syndrome. It is reciprocally reinforced by the adverse impact of immunosuppression in recent-onset (presumably T_H1) disease.[55] For example, the far more frequent provision of CST in RS vs. PS (cited above), provided to suppress the granulomatous immune response with the expectation of preventing lethal pulmonary fibrosis, appears to account for their far higher sarcoidosis mortality.

Notes

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