CHAPTER 3

Aetiologies of sarcoidosis

L.S. Newman

Correspondence: L.S. Newman, Division of Environmental and Occupational Health Sciences, Dept of Medicine, National Jewish Medical and Research Center, 1400 Jackson St, Room G212, Denver, Colorado 80206, USA. Fax: 1 3033981983; E-mail: newmanl@njc.org

Why is the aetiology of sarcoidosis unknown?

One would think that in the nearly 130 yrs since its initial description, someone would have uncovered the root cause of sarcoidosis. This failure speaks to the probable complexity of the problem from both an exposure and genetic perspective, as discussed later. Since its earliest descriptions, sarcoidosis has been suspected to be an infectious disease and yet, to date, while many different microbes have been indicated, none has been convincingly proven to be causative. Interestingly, in the same century, the causes of many idiopathic disorders have been revealed, most of which were due to infectious agents. These include, among others, swamp fever (*Plasmodium falciparum*), anthrax (*Bacillus anthracis*), consumption (*Mycobacterium tuberculosis*), gastric ulcers (*Helicobacter pylori*) and Whipple's disease (*Tropheryma whippelii*). In most of these other cases, Henle-Koch postulates could be fulfilled (table 1). Several generations of sarcoidosis researchers have learned that fulfilment of these postulates is not an easy matter. This is because not all organisms can be isolated or cultivated, ignorance remains of the true spectrum of both pathogenic and nonpathogenic microbes, some microbes may be endogenous to the host, and some colonised microbes may cause pathology through their antigenic or adjuvant properties, not because of infection, *per se*. Hence, despite many attempts to discover a microbial pathogen, it has been difficult to fulfil Koch's postulates. Lingering concerns are left that either the right conditions have not been used to find and culture the organism, that the cause is not microbial, or the disease called "sarcoidosis" is made up of more than one disorder, each of which may have a different cause [1, 2].

There may be other reasons why the aetiology of sarcoidosis remains obscure. 1) Case definition: a careful review of the medical literature demonstrates that researchers have used highly variable, imprecise and inconsistent case definitions [4]. This may improve through the application of recent guidelines for defining sarcoidosis [5]. A more systematic and rigorous methodology will be needed in sarcoidosis in clinical research. 2) Case ascertainment: differences in how study subjects are recruited and enrolled introduce bias and make it difficult to draw comparisons among groups of patients and among published studies. 3) Disease heterogeneity: the wide range of clinical patterns seen in sarcoidosis [6] raises the possibility that sarcoidosis a) might not be one disease, b) might be triggered by more than one aetiologic agent, or c) that a single agent produces different effects based on host factors, such as genetics. 4) Indefinite time of disease onset: epidemiology is at its best when the onset of disease can be defined precisely. As discussed later, even though there appears to be time-dependent clustering of sarcoidosis in some studies, the latency between exposure to an inciting agent and the onset of disease is unknown. This makes it difficult to determine if, or when, a previous exposure triggered illness. 5) Absence of sensitive and specific diagnostic tests: the diagnosis of sarcoidosis is dependent on a constellation of clinical findings and the

Eur Respir Mon, 2005, 32, 23–48. Printed in UK - all rights reserved. Copyright ERS Journals Ltd 2005; European Respiratory Monograph; ISSN 1025-448x. ISBN 1-904097-22-7.

Table 1. – Paradigms for establishing causation *versus* association

Paradigm	Elements	Comments and limitations
Henle-Koch postulates	Isolation of pathogen from disease host	Limited ability to isolate known organisms Many unknown organisms
	Growth of organism in pure culture	Growth conditions uncertain for many microbes.
	Reproduce characteristics of disease by inoculating susceptible host	Organism might cause granulomatous disease because of microbial antigenicity, not infection. Causative organisms can be "endogenous" in genetically susceptible individual.
Bradford HILL [3] criteria	Strength of association	Greater relative risk favours causation Cause-and-effect not fully excluded just because association is weak. Also consider how often exposures occur and do not produce disease
	Consistency	Repeated observation of association between exposure and disease: by different researchers, in different places, circumstances and times Best when similar results reached in different ways (*e.g.* prospectively and retrospectively)
	Specificity	Show that the exposure is associated with a limited group of individuals, in particular environment, with particular clinical phenotypes of disease and that there is no association between that exposure and other diseases. Certain agents do cause more than one disease Diseases may have more than one cause. In fact, multicausation may be more common than single causation
	Temporal relationship	Show that exposure occurs prior to onset of disease Problematic for "slow" diseases in which it is not known when the disease began
	Biological gradient	Dose-response curve for exposure-disease Difficult to quantitatively measure environment Implies knowledge of intensity and duration of exposure Dose-response in sarcoidosis might not be a linear relationship because of genetic susceptibility or because response is immunological
	Plausibility	Biologically plausible that agent can cause granulomas Not essential, but helpful for making the case for causation. Presumes that the mechanism is understood and that the biological effect of a particular exposure is known
	Coherence	The data should not seriously conflict with generally known facts of the natural history and biology of the disease
	Experiment	If an intervention prevents disease, it strengthens the case for causation (*e.g.* if removal of exposure prevents or reduces frequency of illness, or if a treatment cures illness)
	Analogy	Suspected exposure is similar to other agents known to cause granulomatous disease (*e.g.* a metal, a mycobacterium)

exclusion of known causes of diseases that imitate it [5]. There are no diagnostic tests of high sensitivity or specificity for sarcoidosis. Even the Kveim-Siltzbach test, which possesses granuloma-causing attributes, is negative in a sizeable fraction of cases. The consequence is under-recognition, misdiagnosis, and misclassification of disease. Even though most current case definitions require the presence of noncaseating granulomas, this pathological finding is not pathognomonic. 6) Surrendering to the "idiopathic": when clinicians and researchers see sarcoidosis patients, their mindset is to consider the case to be idiopathic. As a result, they often do not aggressively pursue the causes of granulomatous diseases beyond routine cultures and stains for infectious diseases. 7) Limited tools for detecting relevant exposures: apart from available culture methods, mineral analysis of tissue, *in situ* staining for microbes or microbial antigens, and a few immunological assays that help separate sarcoidosis from other diseases like chronic beryllium disease (CBD), hypersensitivity pneumonitis and infection, the repertoire of tools for identifying prior environmental exposures is limited. In-roads are being made in the use of proteomics to identify microbial antigens in sarcoidosis patients, but this is still in its infancy and will need to be linked with other techniques to establish aetiology [7–9]. Epidemiological methods may help to identify environmental risk factors, as discussed later, but have thus far only helped narrow the search for a cause. Immunological studies suggest that antigens are important in pathogenesis, but the complexities of identifying antigen in disease by immunological and biochemical methods is as daunting as the epidemiology and microbiology. 8) Genetic and other host factor complexity: it is clear that people differ in their susceptibility to sarcoidosis. Genetic factors are associated with particular patterns of disease (clinical phenotype), disease risk, and with disease severity and progression [10–16]. Host factors, such as tobacco use, modify disease risk as well [17]. As a consequence, even if a specific environmental factor could be identified, it is probable that it will be confounded or interact with the individual's genetics and their habits in conferring risk. There are good reasons for hypothesising that sarcoidosis is caused by environmental antigens in genetically susceptible individuals. Both the lungs and skin, two common target organs for sarcoidosis, are regularly in contact with environmental antigens; studies of sarcoidosis immunopathogenesis strongly suggest that the disease is an overexuberant response to antigens, as discussed elsewhere in this Monograph [18–24]. There are many potential environmental antigens that can induce sensitisation, an adoptive, cell-mediated immune response responsible for the development of granulomas [25–27]. As summarised in table 2, such environmental factors cause a variety of granulomatous diseases that mimic sarcoidosis, including: CBD due to inhalation of and sensitisation by beryllium, and other metal-induced granulomatous lung diseases (aluminum, titanium, zirconium) [28]; hypersensitivity pneumonitis due to organic and inorganic antigens; and infections, such as tuberculosis, atypical mycobacteria, and fungi, amongst others [2, 5]. Inorganic dusts and fibres, such as talc, silica, glass fibres, and man-made mineral fibres can be considered another subclass of environmental exposures that induce sarcoidosis-like illness, possibly by inducing a less specific, innate immune response which might promote adoptive immune responses to available antigens [29–32]. The list of agents capable of inducing granulomatous responses in experimental animals is even longer, including mycobacteria, avian proteins, fungal spores, acanthamoeba, schistosome eggs, propionobacterium, carrageenan, brucella, and leishmania, amongst others [27].

Implications of epidemiologic studies of sarcoidosis aetiology

A number of epidemiological studies of sarcoidosis have been conducted over the past 50 yrs in efforts to narrow the field of possible aetiological agents. An increasingly

Table 2. – Granuloma-forming disorders and potential aetiologies for sarcoidosis

Category	Subcategory	Examples
Infections	Fungi	*Histoplasma* spp., *Aspergillus* spp., *Coccidioides* spp.
	Protozoa	Toxoplasma, Leishmania
	Metazoa	Schistosoma
	Spirochetes	*Treponema pallidum*
	Mycobacteria	*Mycobacterium tuberculosis, M. leprae*, NTM
	Bacteria	*Yersinia* spp., *Brucella* spp., *Borrelia* spp., Propionibacteria
	Viruses	*Epstein-Barr virus*, Herpes, Rubella, Measles, Cytomegalovirus, *Coxsackie B* virus,
Neoplasms	Carcinoma, Sarcoma	Malignant nasal granuloma. Malignancy associated granulomas
Metals		Beryllium, aluminium, titanium, zirconium
Inorganic dust		Silica, talc, man-made mineral fibres, starch, silicone
Organic dust	Hypersensitivity pneumonitis	Farmer's lung, bird fanciers' lung, suberosis, bagassosis, hot tub lung
	Hypothesised	Pine tree pollen, Clay
Immune disorders	Idiopathic	Sarcoidosis, Crohn's disease, primary biliary cirrhosis, giant cell arteritis, hypogammaglobulinaemia, idiopathic hepatic granulomas, common variable immune deficiency
Vasculitides	Idiopathic	Wegener's granulomatosis, Churg-Strauss, Lymphomatoid granulomatosis, Bronchocentric granulomatosis, polyarteritis nodosa
Other	Leukocyte oxidase deficiency Blau's syndrome	Chronic granulomatous disease of childhood

NTM: nontuberculous Mycobacteria.

coherent picture starts to emerge when these studies are taken in aggregate. Whilst no one study proves the cause, several conclusions can be drawn that help point the way to specific hypotheses meriting further investigation. The prevailing view from these studies is that sarcoidosis occurs as a consequence of exposure to one or more environmental agents interacting with genetic factors [2, 5, 33–35]. The challenge will be to identify and link such environmental factors with genetic susceptibility.

Clustering of disease has been well described in sarcoidosis, and should ultimately help guide us toward aetiology. At very least, any newly suspected causative agent must plausibly explain why some forms of clustering occur.

Age-specific clustering

There is a marked predilection for disease to develop in early adulthood, with the disease being notably rare in children and early teens [36] and rare in the elderly beyond the age of 70 yrs [37–39]. This observation might suggest that exposure to the aetiological agent(s), whether they are antigenic or infectious, may first occur about the time that individuals reach working age, raising speculation about the contribution of occupational exposures.

Race-specific clustering

This disease appears to occur more commonly among African Americans than among Caucasians [38–41]. Paradoxically, clustering also occurs among individuals of northern European descent, especially with acute forms of disease, such as Löfgren's syndrome. Data suggest that disease severity and prognosis also varies by race and ethnicity. Studies of the genetics of acute sarcoidosis have begun to suggest a strong genetic determinant in the latter group that may account for some of these differences [42–44]. However,

consideration should be given to differences in host factors and environmental factors that may impact on exposures and gene expression as well.

Sex-specific clustering

Although studies vary in their findings with regard to male/female rates of sarcoidosis, most suggest a slightly higher rate in females. In the only population-based incidence study of sarcoidosis in the USA, HENKE *et al.* [45] observed similar age-adjusted incidences in the two groups (5.9/100,000 person-yrs for males and 6.3/100,000 person-yrs for females). However, interestingly, they also noted an increase in incidence between 1946 and 1975 for females. HENKE *et al.* [45] hypothesised that this increase in sarcoidosis among females may have been due to earlier less aggressive case finding for females compared with males. An alternative hypothesis is that large numbers of females entered the workforce over this time period, where they may have encountered environmental antigens that induce sensitisation and disease.

Seasonal clustering

Numerous studies, have observed a predilection for sarcoidosis to become clinically apparent in winter and early spring, peaking in spring months [46–52]. If it is assumed that the latency between exposure to the causative agent and development of sarcoidosis-related symptoms is in the order of a few weeks to a few months, as is the case in animal models [27], it seems likely that exposure may first occur in many cases in the late fall to early spring. It is attractive to speculate that increased contact with the aetiological agent, whether the agent is infectious or antigenic, occurs when people spend more time in closed, confined spaces at work or at home during winter months. One might speculate that sarcoidosis is a type of building-related illness, resulting from sensitisation to airborne antigens or an infectious agent(s) (so-called bioaerosols), much like the building-related illnesses hypersensitivity pneumonitis, humidifier fever, Pontiac fever, and legionellosis [35, 53, 54].

Geographic clustering

Not all studies of the geographic clustering of sarcoidosis agree [41, 55–57]. Despite the discrepancies, methodological problems, case and control selection biases, diagnostic access biases, and reporting biases in these studies, the preponderance of published data suggests that this disease occurs more commonly in geographically distinct regions [41, 59–61]. This geographic distribution has promoted much speculation and a large number of studies that examined factors in the meteorology and soil [40, 56, 62–66], plants, pine pollen, and proximity to forests [61, 63–70], water supply [40], use of firewood [40, 63–66], and exposure to farm animals and pets [40, 63–66], amongst others. Past studies have noted a clustering in parts of the country where there is more lumbering activity [67, 71]. In particular, a study by DUNNER and WILLIAMS [71] suggested that sarcoidosis cases occurred twice as often where lumbering and wood milling was a principal industry. More recently, KAJDASZ *et al.* [72] confirmed that geography is associated with sarcoidosis risk as in the example of Atlantic coast clustering in South Carolina, Charleston, USA. Resurrecting the pine pollen hypothesis, GRIPENBACK *et al.* [73] in Sweden have recently reported increased accumulation of lung T-lymphocytes and eosinophils after pinewood dust exposure. A Case Control Etiologic Study of Sarcoidosis (ACCESS) [35] found no association with lumber or wood dust exposures, wood use in

the home or at work, in a study of 706 sarcoidosis cases and matched controls; however, that study recruited controls on the basis of telephone number prefix or, in some cases, zip code of the cases, thus matching for geography and possibly for regional environmental factors, such as forests and wood use as well. Taken in aggregate, most of the studies that have tried to use the geographical distribution of disease as a means of unearthing the cause of sarcoidosis have either not been confirmed by subsequent investigations, or have lacked biological plausibility and not been pursued further. Most have not considered antigens that are commonly associated with firewood, lumber and wood milling that are known to cause granulatomous disease in the form of hypersensitivity pneumonitis. These include thermophilic bacteria and fungi, such as *Merulium lacrymans*, *Aspergillus* spp., *Penicillium* spp., and *Trichosporon cutaneum*. For example, an interesting analogy can be drawn between sarcoidosis and "summer-type hypersensitivity pneumonitis" in Japan [74]. In that granulomatous disease, recurrent seasonal respiratory and systemic symptoms occur with familial clustering, and with the onset coinciding with occupancy of homes containing damp and decayed wood and woven straw mats contaminated with *T. cutaneum* [74–76]. Thus, from a hypothesis generating standpoint, the past sarcoidosis literature should be considered carefully for the possibility that the associations with forests, lumbering, wood milling and wood burning are surrogates for the sensitising antigens they harbour.

Rural clustering

A number of past studies have associated sarcoidosis with rural residence, birthplace, or time spent in rural regions [37, 41, 56, 67, 68, 77, 78]. In 1961, Buck *et al.* [63–66], for example, conducted an extensive case-control study of the rural hypothesis. Cases, as compared with controls, were more likely to have been born in rural areas, than to have lived at any time in rural areas prior to diagnosis, and to have spent more cumulative time in a rural residence. Unfortunately, this investigation used a relatively small study population, lacked precision in its definition of disease, took one-third of its control subjects from an urban venereal disease, and was subject to survivor, recall, and reporting bias. More recently, Kajdasz and co-workers [59, 72] have confirmed the rural risk and have investigated aspects of rural life that play a role, including exposure to wood stoves and fireplaces. In aggregate, these investigations suggest that sarcoidosis occurs more frequently in rural rather than urban areas. In the ACCESS study, the present author observed an elevated odds ratio (OR) among individuals who worked in agriculture and with having lived in a small town (<50,000 population) in childhood [35]. Thus, the rural risk is of interest, although inner city populations clearly develop disease as well [38].

Spatial clustering

Apart from geographic clustering, a number of studies have examined the tendency for sarcoidosis to occur in individuals who have close physical contact to one another or to a common location within a community. For example, Parkes *et al.* [60] and Hills *et al.* [79], in their 1987 case-control study of residents on the Isle of Man, UK, observed that 40% of the 96 sarcoidosis cases reported prior contact with a person known to have sarcoidosis, compared with 1–2% of controls. Of these contact-pairs, 14 occurred in the same household, only nine of which were blood relatives. A total of 19 pairs came in contact with one another at work, two were next-door neighbours, and 14 were noncohabitating friends [60, 79]. From an infectious disease perspective, these studies of space- and time-clustering of sarcoidosis may suggest that sarcoidosis is a communicable

disease [60, 79]. The same data, when viewed from an occupational/environmental medicine perspective, suggest that these cases might have shared a common environmental exposure in the home or work that induced a hypersensitivity response. Unfortunately, these Isle of Man studies examined only a very limited list of occupational titles and gave limited consideration to the home environment. Intriguingly, they did observe that 18.8% of the sarcoidosis cases were healthcare workers (especially nurses), a rate significantly higher than for controls (4.2%) [60]. This particular finding has raised concern that such studies may be subject to a diagnostic access bias, *i.e.* those with better access to healthcare may be more likely to be diagnosed, and thus counted in research studies, than those less connected to the healthcare system [80, 81]. Other reports of husband-wife occurrences of sarcoidosis fuel the hypothesis that shared environment may hold the key to finding aetiologies of sarcoidosis [82, 83]. In the future, prospective studies are needed to examine the frequency with which sarcoidosis clusters occur among individuals with shared work and home environments, and include more detail characterisation of these environments.

Familial clustering

Numerous studies have focused on the propensity of sarcoidosis to occur in families, amongst same-sex parent-child pairs, mother-child pairs, same-sex siblings and in monozygotic twins [39, 63–66, 82, 84–86]. One recent study suggests that familial clustering of sarcoidosis is more common among African Americans than Caucasians [87]. It is interesting to note that in the ACCESS study, Rybicki *et al*. [39] estimated familial relative risk (RR) in 10,862 first- and 17,047 second-degree relatives of 706 sarcoidosis cases. Siblings had the highest risk (OR=5.8; confidence interval=2.1–15.9), with other significant elevations of avuncular, grandparental and parental risk. White cases had a markedly higher familial RR than African Americans (OR=18.0 *versus* 2.8; p=0.098). McGrath *et al.* [88] have reported a sibling RR in a primarily White population of between 36 and 73. Although a more sophisticated genetic linkage analysis is underway, using variable number of tandem repeat DNA markers and PCR [89], the past genetic analyses of families with several affected members suggest that the heritable risk is complex and polygenic [13, 90–99]. Even if there proves to be genetic risk factors for sarcoidosis, it is likely that development of disease will also be contingent upon exposure to appropriate environmental antigens [18, 33]. Interestingly, the family studies have almost exclusively focused on genetic explanations, with little attention given to the families' members shared environment. A notable exception is the recent study by Kucera *et al*. [34], in which occupational data were collected from 921 African Americans in 273 sibships identified through a sarcoidosis case. The findings of that study are discussed later.

Industry and Occupational Associations

Some of the studies discussed above suggest the possibility of shared exposure to granuloma-inducing antigens at work [55, 60, 65–67, 71]. Until recently, very few studies had systematically investigated the occupational and environmental exposures of sarcoidosis patients. As with the other epidemiological studies described earlier, research addressing the possible role of industry or occupation are subject to recall, referral and selection biases, often have used imprecise case definitions, lacked controls or used inappropriate controls, and employed survey methodology that was incomplete or inadequate for defining coexisting or pre-morbid work-related risk factors [33]. Additionally, most of such studies have focused only on the occupations and industries held at the time of diagnosis rather than

systematically examining occupational history for the entire period preceding diagnosis. Nonetheless, three recent papers that were derived from the ACCESS study [100, 101], plus a study conducted in South Carolina [72] and one that examined occupational risk factors in African-American families [34] have helped advance the notion that occupational and environmental factors contribute to sarcoidosis risk.

Historically, in 1959, CUMMINGS *et al*. [67] examined the case records of 1,194 of a total of 1,700 patients seen in USA veteran's hospitals who were diagnosed between 1949 and 1954. In this large case series, there was no indication of case definition, no validation of diagnosis by review of radiographs or histopathology, and no control group. The geographic distribution was determined by identifying the county of birth. CUMMINGS *et al*. [67] observed a clustering of cases in 50 communities in the USA. In 40 out of the 50 communities, lumbering or wood milling were listed as the principal local industry. The authors concluded that this may be a "lead worth following".

Two other studies of USA veterans collected occupational exposure data retrospectively from discharge records in veteran's hospitals [63–66, 71]. In the study by DUNNER and WILLIAMS [71], a single occupation was obtained by reviewing the medical records of 500 sarcoidosis patients. The job titles were compared with those of a control group of hospitalised veterans without sarcoidosis. Without presenting any actual data, DUNNER and WILLIAMS [71] indicated that there were more post office workers and mechanics among the veterans with sarcoidosis than in the control group. KELLER [55] performed a retrospective review of charts from 420 USA male veterans from 1960–1964. The study matched 420 cases for age, sex, race, and the veteran's hospital from which they were diagnosed. Details of the method by which an occupation title was assigned were not provided, and no work histories were obtained apart from the job title found in the chart. Despite these shortcomings, KELLER [55] observed three statistically significant differences: 8.3% of sarcoidosis cases were professionals compared with 3.8% controls; 3.8% were sales workers, compared with 1.2% of controls, and sarcoidosis occurred at lower frequency among labourers (17.1% in sarcoidosis cases *versus* 28.6% for controls in the African-American veteran subgroup; 14.3% *versus* 21.4% overall). These data suggested and that certain occupations may confer increased risk and others reduced risk for sarcoidosis. It is intriguing to note, for example, that labourers, who often perform outdoor work and work at multiple different sites, had lower risk of being diagnosed with sarcoidosis (OR=0.5), again fuelling speculation about the association of indoor environments and sarcoidosis. In the case-control study by BUCK and co-workers [63–66], the authors collected data on work in lumbering and farming industries, finding no differences between 62 cases and controls. In addition to the studies by PARKES *et al.* [60] and HILLS *et al.* [79], showing an increased frequency of nurses and healthcare professionals with sarcoidosis, EDMONDSTONE [102] observed that 24 out of 156 cases of sarcoidosis from a London hospital (UK) were hospital workers (15.4%), including 16 nurses. The rate was compared to population census figures from 1981 showing that only 1.6% of the relevant population were nurses.

The ACCESS study [35] recruited 706 newly diagnosed, pathology-proven cases of sarcoidosis and age-, raced- and sex-matched controls from 10 USA academic centres. Interviewers administered questionnaires containing questions regarding occupational and nonoccupational exposures, including a full chronology of jobs and industries for each individual that were held for 6 months or longer at any time prior to diagnosis. Subjects reported history of an exposure, as well as where the exposure had occurred (home, work, or both) and duration of exposure ($<$ or $>$1 yr). The ACCESS study [35] observed positive associations between sarcoidosis and specific occupations, including agricultural employment, jobs raising birds, jobs in automotive manufacturing, middle/secondary school teaching, and physicians. A review of cases suggested that the bird exposure cases were not typical of hypersensitivity pneumonitis. The finding of physician

risk was suspected to be spurious, driven by the possibility that physicians without sarcoidosis would have been more likely to not agree to be control subjects. Exposures that were positively associated with sarcoidosis risk included insecticide use at work, and work environments with mould/mildew exposures, which the ACCESS study authors [35] speculated would have represented environments with possible exposures to microbial bioaerosols. Multivariable modelling observed statistically elevated ORs for work in areas with musty odours, with occupational exposure to insecticides, and a decreased OR for those who ever smoked cigarettes. This study notably did not find a single, predominant risk factor for sarcoidosis. While the ORs were elevated for a number of factors, in general these associations were weak [35].

In a more comprehensive analysis of the standardised industry codes (SIC) and standardised occupational codes (SOC) for each job held by the ACCESS cases and controls, BARNARD *et al.* [100] reported positive associations including employment in retail industries selling building materials, hardware, garden supplies, and mobile homes (by SIC). Study subjects who worked in these industries were three times more likely to be a sarcoidosis case than a control. Subjects who reported work in industries with exposure to industrial organic dusts (by SIC), including work in an industry producing aerosolised plant material in its raw (*e.g.* wood) or manufactured (textiles, paper, agricultural chemicals) form, were likewise more likely to be cases than controls. Work in both elementary and secondary school settings (by SIC) and as an educator (SOC combination) were positively associated with sarcoidosis. Interestingly, several industries and occupations were negatively associated with sarcoidosis status. Occupations in personal service (*e.g.* baggage porters and bellhops, welfare service aides, childcare workers (except in private households), and personal service occupations) were negatively associated with sarcoidosis (by SOC). Subjects reporting employment in industries manufacturing electrical energy (SIC), providing social and rehabilitation services (SIC), as well as a combination variable of work in childcare provision (SIC) were less likely to be sarcoidosis cases than controls. The ACCESS SIC/SOC data analysis also suggests that there may be differences in risk factors for sarcoidosis by race and exposure. For example, Caucasian sarcoidosis cases were more likely than African Americans with sarcoidosis to have worked in industries with exposures to metal dust/fumes or with exposure to industrial organic dust [100].

A somewhat different task was taken by the ACCESS research group in the study by KREIDER *et al.* [101], in which the sarcoidosis cases were subdivided by clinical phenotype. Hypothesising that different sarcoidosis clinical phenotypes may be associated with different exposure risks, KREIDER *et al.* [101] categorised 716 sarcoidosis cases into two groups: 1) pulmonary-only disease (311 cases, 43%) and 2) systemic disease (with or without pulmonary involvement; 407 cases, 57%). Of the systemic cases, 376 (92.3%) had lung involvement in addition to at least one other organ. For their analysis, location/duration variables were created for each exposure that incorporated both location (*e.g.* metal dusts at home, work or both) and duration of exposure (*e.g.* metal dusts for $<$ or $>$1 yr). The location/duration exposures were created because of concerns about exposure misclassification, potentially diluting the ability to detect real associations because of inclusion in the same category of subjects with relatively trivial exposures with those strong and long exposures. All information on exposures was collected and categorised prior to the determination of organ involvement, thus avoiding potential information bias. Logistic regression was used to examine associations of candidate exposures with clinical phenotype [101].

The study by KREIDER *et al.* [101] demonstrated that exposures to wood burning, agricultural organic dust, or military service are associated with sarcoidosis, in which disease is present in the lungs only. The number of subjects in the military service category was small and, thus, may be an unstable estimate of risk. Methodologically, this paper is

conceptually important, because it strongly suggests that differentiation of sarcoidosis subjects on the basis of clinical phenotypes will demonstrate that these subgroups may have unique environmental exposure associations. Self-defined race may also play a role in the determination of the effect of certain exposures on disease phenotypes [101].

Using a questionnaire instrument that was highly derivative of the ACCESS questionnaire, KUCERA *et al.* [34] found positive associations between sarcoidosis cases and certain occupations and industries for African Americans with sarcoidosis as compared with unaffected siblings. Although the number of cases per exposure category was small, these investigators observed that individuals who worked in occupations with potential metal exposures or in workplaces with high humidity, water damage or musty odours (again suggestive of a microbial-rich environment) may be at increased risk for sarcoidosis. KUCERA *et al.* [34] appropriately noted that the complexity of occupationally related exposures makes it difficult to identify specific agents based on job titles alone, a critique that applies to most of the environmental epidemiological research to date.

Also, using a questionnaire instrument and study design that was similar to that employed in ACCESS, KAJDASZ *et al.* [59] followed up on an earlier observation of geographic risk for sarcoidosis hospitalizations among African Americans from South Carolina, USA. A number of exposures were found by univariable analysis to be associated with sarcoidosis risk, including use of wood stoves, fireplaces, nonpublic water supplies (*e.g.* wells), and living or working on a farm. Dose-response gradients were found for exposure to wood stoves and use of fireplaces in both univariable and multivariable logistic regression models. The article suggested less emphasis for agricultural occupation, and greater importance of wood use in explaining the rural association in South Carolina [59]. Notably, when this rural-based study is taken in context with the other investigations described above, the data suggest that local environmental conditions may lead us to appreciate different aetiological triggers for sarcoidosis.

Occupational case clusters that suggest environmental aetiology for sarcoidosis

A number of investigators have identified clusters of sarcoidosis cases that suggest a possible occupational association. STEWART and DAVIDSON [103] reported on a cluster of sarcoidosis cases occurring in two sisters and two unrelated social contacts, including one sister's employer. However, this study did not address any occupational or environmental hypotheses. KERN *et al.* [104] reported on the occurrence of a cluster of three sarcoidosis cases among 57 fire-fighters who had previously apprenticed together in Rhode Island, USA. Other examples of environmental or occupational clustering of recent note include a study of New York City (NY, USA) fire-fighters [105] and an excess incidence among Navy enlisted males from 1971–1993 [106, 107]. It is not known with what frequency occupational clusters of sarcoidosis are missed due to insufficient clinical investigation of the work-relatedness of patient symptoms, but such clusters have historically offered important insights into the aetiology of idiopathic diseases [108–110].

A number of studies are illustrative of two important concepts: 1) in some instances sarcoidosis is really a misdiagnosed case of another environmental antigen-induced disorder; and 2) environmental exposures can cause sarcoidosis.

Example 1

An outbreak of cases of sarcoidosis was identified in an automotive manufacturing plant in which metal parts where being machined using a semi-synthetic oil coolant

called a metal working fluid [39, 111]. Clinically and pathologically indistinguishable from sarcoidosis, these cases were all linked to workplace exposure. Such metalworking fluids become contaminated with a variety of bacteria, including mycobacteria and endotoxin [112, 113]. Analogously, mycobacterially-contaminated water sources can create aerosols that, when inhaled, produced sarcoidosis-like illness [114–116]. Clinically, the patients respond to removal from exposure and corticosteroids, not to antimicrobial therapy.

Example 2

In 1979, an African-American male employee of a nuclear weapons facility developed hilar adenopathy, diffuse nodular interstitial infiltrates on chest radiograph, hepatic enzyme elevations, and had noncaseating granulomas on lung biopsy. Clinically and histologically, he was diagnosed with sarcoidosis. After a period of 5 yrs, through the use of blood and bronchoalveolar lavage (BAL) beryllium lymphocyte proliferation testing, the patient was recognised as a sentinel case of CBD in a company that manufactured nuclear weapons parts. This led to the systematic investigation of the plant and workforce, and the discovery of endemic sarcoidosis-like disease due to beryllium among the current and retired workforce that helped produce nuclear weapons [117–123]. Some of the CBD cases first carried the diagnosis of sarcoidosis, given the strong similarities between the two conditions [124–126]. In hindsight, the initial mistake of calling this nuclear weapons worker's disease "sarcoidosis" is not surprising, and continues to occur in many industries [127]. For example, FIREMAN *et al.* [126] in Israel asked patients in their sarcoidosis clinic if they had been exposed to metals. Those who said "yes" were offered blood beryllium lymphocyte proliferation tests. Approximately 6% of "sarcoidosis" patients were found to have CBD [126].

Example 3

In March 1989, a 24-yr-old lifeguard developed persistent cough, chest tightness, progressive dyspnoea on exertion, eye irritation and headache without fever. Symptoms worsened toward the end of his work shift, persisted through the evening, resolved by the next morning, but recurred. The patient developed hypoxaemia and a chest radiograph showing diffuse interstitial opacities consistent with Scadding stage III sarcoidosis. BAL showed marked T-helper cell (CD4+)-predominant lymphocytosis. Transbronchial biopsies showed multiple noncaseating granulomas read by the pathologist as consistent with sarcoidosis. This patient became the index case for an epidemic of sarcoidosis-like illnesses related to inhalation of bioaerosols at an indoor swimming pool [128]. The pool had many water spray features that generated a six-fold increase in airborne respirable particles and an eight-fold increase in endotoxin levels in the air, raising the hypothesis that the sprays may have disseminated an antigenic bioaerosol [128]. From two sequential investigations, a total of 33 symptomatic lifeguards had biopsy- or lavage-proven granulomatous lung disease. Gram-negative bacterial colonisation was found in the water sprays, predominately *Pseudomonas* spp., although there remains uncertainty about the actual causative agent in this particular environment. This investigation demonstrates that illnesses, which are clinically and pathologically indistinguishable from sarcoidosis, can occur in clusters with a common environmental exposure in both space and time. Similar stories have been described in relation to mycobacterially-contaminated hot tubs as discussed earlier.

Example 4

A patient with occupational exposure to glass fibres developed a sarcoidosis-like illness [31]. This case led DRENT *et al*. [32] to review records of 50 sarcoidosis cases seen from 1996–1999. In 14 cases, patients had reported an occupational history of exposure to either glass fibres or both. In six of the 12 biopsies, DRENT *et al*. [32] were able to review by electron microscopy with energy dispersive x-ray microanalysis, a number of minerals were detected, including silica, aluminum, and magnesium, which are all elements of man-made mineral fibres. Fibre deposits were found to be associated with granulomas, leading the authors to conclude that these fibres elicit a sarcoidosis-like granulomatous response.

Notably, in each of these four examples, the patients (index cases) initially carried the diagnosis of sarcoidosis based on both the pathologists' readings of lung or lymph node histology, and clinical assessment of experienced physicians caring for these individuals. These cases met current definitional criteria for sarcoidosis [5], including aetiology unknown, until investigators were able to identify an aetiologic agent or a common point source of exposure. Such cases suggest that when sarcoidosis cases are suspected by clinicians, evaluation must include careful consideration of the home and workplace, possibly including a combination of environmental sampling, epidemiologic assessment of other exposed individuals, biological monitoring, and careful case series description. With this sentinel health event follow-back model [129], the confusion about sarcoidosis aetiology may be reduced by identifying an already known cause of granulomatous disease cases in that environment or at least identifying common exposure circumstances that can lead to further hypotheses about possible new aetiological agents for sarcoidosis (fig. 1).

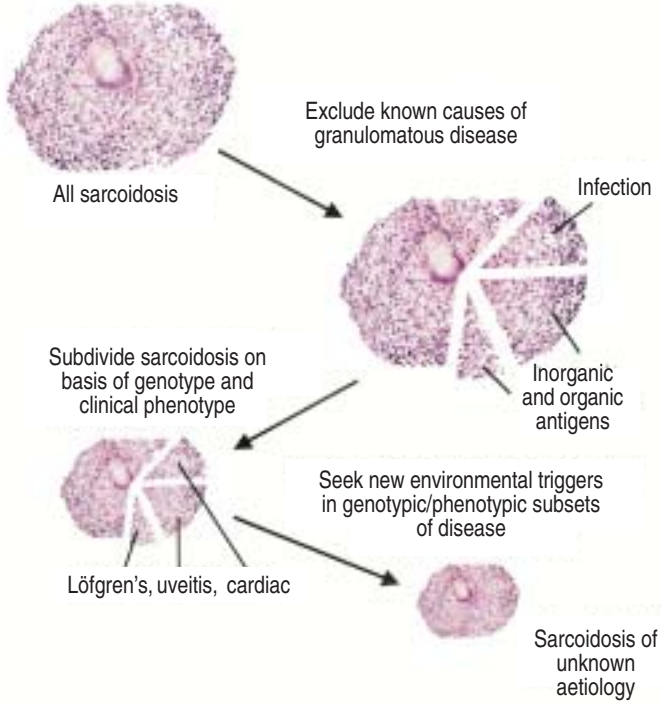

Fig. 1. – Proposed schema for approaching the aetiologies of sarcoidosis.

In order to address the cause or causes of sarcoidosis, first consideration should be given to distinguishing between granulomatous disorders of known aetiology from those that are idiopathic. At present, some cases of so-called "sarcoidosis" are due to known environmental agents. Using available clinical and research tools, it is important to thoroughly exclude known infectious agents (such as mycobacteria), organic antigens (such as thermophiles and fungi that produce granulomatous pneumonitis) and inorganic antigens (such as beryllium and other metals). If sarcoidosis has more than one cause because the disorder itself is a heterogeneous collection of disorders, the next step will be to refine the phenotypes. It is possible to capitalise on what is now known both clinically and genetically to separate subsets of "sarcoidosis". For example, there is now ample evidence to suggest that Löfgren's syndrome is a separate disease, based on its genetics, immunology, clinical phenotype and behaviour. The same might be said for sarcoidosis that predominantly presents with uveitis, as seen in Japan, and possibly cardiac sarcoidosis. By purifying the clinical phenotype and genotype, thus reducing sarcoidosis heterogeneity, it will be easier to then examine the possible environmental causes of each of those separate conditions, be they microbial or not (fig. 1).

Evidence for a microbial cause

For a century, microbial pathogens have been leading suspects as the cause of sarcoidosis. The search continues because, although no infectious agent has been consistently cultured from sarcoidosis specimens or even consistently detected using ribosomal RNA markers, there are clinical and epidemiological features of sarcoidosis that suggest an infectious origin. For example, there is evidence for transmissibility of sarcoidosis. "Donor-acquired sarcoidosis", in which sarcoidosis develops in naive transplant recipients who have received tissues or organs from donors who were known or suspected to have active sarcoidosis has been documented [130, 131]. Inversely, sarcoidosis has recurred in nonaffected allografts after lung transplantation [132]. Sarcoidosis-like granulomas develop in animals that have received tissue from sarcoidosis patients [133–136]. When human tissue containing sarcoidal granulomas was homogenised and then injected into mice, granulomas formed 15 months later [137]. In that study the effect was destroyed by autoclaving, by freezing at -20°C, and by radiation exposure.

Kveim antigen, which is a protein extract from the lymph node or spleen of sarcoidosis patients, elicits an oligoclonal T-cell response in patients with sarcoidosis and produces granuloma-like infiltration of skin [138]. A further study has shown that nonviable BAL cells harvested from patients with sarcoidosis and carefully filtered cause granuloma formation when injected intracutaneously into these patients [139]. Although the active agent in the Kveim antigen has not been identified and Kveim antigen has not been shown to contain bacterial DNA [140], a recent study by Song *et al*. [7] has detected mycobacterial antigens (mKatG) in sarcoidosis tissues, as well as their antibodies in some cases, fuelling new speculation of a mycobacterial aetiology.

Sarcoidosis granulomas have been examined for evidence of foreign matter and especially for structures that resemble microbial elements. Structures resembling leptospiral organisms have been identified by microscopy from the BAL fluid of patients with sarcoidosis [141]. Similar structures have been seen in epithelioid cells within granulomas in sarcoidosis and in familial granulomatous disease [142, 143], although they also bear strong resemblance to altered platelets and have not been proven to be infectious organisms [144]. Abnormal structures by electron microscope examination seen in white cells associated with sarcoidosis granulomas have been

identified as possible mycoplasma [145]. Recent immunohistochemical and electron microscopic examinations by EISHI *et al*. [9] has suggested propoiniobacterium antigen-specific antibody staining as well. Thus, the book remains open on the nature of the unusual ultrastructural elements found in some sarcoidosis granulomas.

Additional logic for an infectious cause stems from the fact that other granulomatous diseases are often induced by infectious organisms (table 2). These include mycobacteria, herpes viruses, histoplasmosis, treponematosis, sporotrichosis, coccidiomycosis, schistosomiasis, listeria, *Rhodococcus* spp., *Cladophialophora* spp., and the agent of Whipple's disease [146–151]. Epidemiological lines of evidence include the reports suggesting that sarcoidosis can occur through close interpersonal contact [60, 152] and in "clusters", such as that described by KERN *et al*. [153] among previously cohabitating fire-fighters. As discussed earlier, the epidemiological findings of ACCESS results suggest that environments favourable to the production of bioaerosols are associated with elevated sarcoidosis risk [35]. In that study, it was hypothesised that the environments favourable to the production of bioaerosols, whether infectious or antigenic, would be associated with sarcoidosis. Occupational exposures to musty odours were associated with sarcoidosis risk in the multiple logistic regression model. Most fungi exude volatile organic compounds during active growth, causing the "musty" or "mouldy" odour associated with fungal contamination [75, 154], and may reflect microorganism presence even when there is no visible growth [155, 156]. Additionally, ACCESS observed that sarcoidosis cases were more likely to report central air conditioner use in the home. Several studies have found symptoms to be associated with central air conditioning with or without humidification [75, 157, 158]. The ACCESS results, taken in context with several previous studies, add to mounting epidemiological evidence linking microbial bioaerosols to sarcoidosis risk. Many of the microbes that have been suggested as possible causes of sarcoidosis, or of diseases mimicking sarcoidosis, grow readily in standing water. Opportunities to aerosolise particulate antigen and/or infectious agents may result in the inhalation, pulmonary deposition, and immune response to such particles. In a recent study [59], sarcoidosis-related hospitalisations were concentrated in proximity to the South Carolina coastline. Previous studies have shown a predilection for sarcoidosis in coastal states [41, 56, 57]. In a study by ROSE *et al*. [128], the lifeguards who developed granulomatous pneumonitis were exposed to bioaerosols in an indoor leisure swimming centre. In a study of African-American siblings, KUCERA *et al*. [34] observed that siblings with sarcoidosis were more likely to report indoor exposures to high humidity, water damage, or musty odours than were their unaffected siblings. Additionally, clusters of granulomatous pneumonitis mimicking sarcoidosis have been described in relation to occupational exposure to microbially-contaminated metal working fluids in the automotive/metal machining industry [159]. The study by KUCERA *et al*. [34] similarly reported elevated sarcoidosis risk associated with metal machining and metalworking.

Proposed microbial candidates have included mycobacteria, human herpes virus, retroviruses, *Chlamydia pneumoniae*, *Borrelia burgdorferi*, and *Rickettsia Helvetica*, amongst others. However, none of these agents has yet been proven to be the pathogenic cause of sarcoidosis. Koch's postulates have not been fulfilled, despite major efforts to prove microbial cause, and especially the intense pursuit of mycobacteria [8, 160]. Culture and histiological staining of sarcoidosis tissues does not reveal mycobacteria, although two recent studies that have detected mycobacterial antigens. DRAKE *et al*. [8] detected mycobacterial rRNA or *rpoB* sequences in 60% of sarcoidosis tissues, but not in controls [8]. Consistent with this finding, SONG *et al*. [7] identified mycobacterial catalase-peroxidase mKatG and *mycobacterium tuberculosis* 16S rRNA DNA in a subset of sarcoidosis tissues using *in situ* hybridisation. Although such studies have found 16s rRNA for mycobacteria in pathologic tissue, the studies to date, when taken in

composite, are inconclusive, in part because of design limitations, such as definition of cases, selection of controls, details of experimental methodology, and lack of reproducibility of results in other laboratories using similar methods. Some investigators have been unable to demonstrate mycobacterial DNA in sarcoidosis lesions [160–166], whereas others have amplified mycobacterial DNA of various species [167–173].

Propionibacterium spp. have been implicated by Eishi *et al*. [9]. The original observation was that *Propionibacterium acnes* could be frequently isolated from patients with sarcoidosis [174]. Ishige *et al.* [175] reported that 15 out of 15 sarcoidosis tissue samples were positive for propionibacterial (*P. acnes* or *P. granulosum*) rRNA by PCR analysis, compared with only three patients positive for mycobacterial DNA; conversely, two out of 15 tissue samples from patients with tuberculosis were positive for propionibacteria and 15 out of 15 positive for mycobacteria. These results were extended in a study involving 259 patients from five centres from Japan, Italy, Germany and England. Propionibacterial DNA was detected in all but two of 108 sarcoidosis lymph node samples compared with *M. tuberculosis* DNA that was found in 0–9% of these samples. Propionibacterial DNA was detected in 0–60% of *M. tuberculosis* or control samples [9]. Using *in situ* hybridisation, Yamada *et al*. [176] localised Proprionibacterium 16S rRNA primarily to areas outside of granuloma formation in sarcoidosis and tuberculosis tissues. These results will require and await confirmation by other investigators in independent studies. As exciting as these studies have been, the majority of the work has been conducted in a single centre; moreover, there have been limitations in the selection, blinding and matching of controls and the organisms appear to be rather ubiquitous, challenging the theory of causation. In sum, to date, while traditional and PCR-based methods have been attempted in the search for microbial cause, there remains insufficient proof. Genetic analysis serves as an alternative means of identifying a putative infectious agent when histology and culture fail. Despite being inconclusive to date, PCR-based approaches, in principle, still hold great promise for detecting infectious agents in sarcoidosis tissue specimens. PCR was used to identify the aetiological agents of bacillary angiomatosis (*Bartonella henselae*) [177], Whipple's disease (*Tropheryma whippelii)* [178] and to demonstrate that the agent of severe acute respiratory syndrome is a novel coronavirus [179]. PCR has the advantage of circumventing the need for culturing an organism, with sensitivity that theoretically can approach one genome copy. An excellent example of the advantage of genetic analysis of pathologic tissue involves *Tropheryma whippelii,* the agent of Whipple's disease. Histological examination of specimens from patients with Whipple's disease revealed small gram-positive rods that appear as diastase-resistant intracytoplasmic inclusions on periodic acid-Schiff staining [180]. Cultures were unable to isolate this organism. PCR analysis was performed on patients with Whipple's disease [178], using broad-range bacterial primers to identify the proposed causative agent of Whipple's disease. The diagnosis of Whipple's disease was confirmed in peripheral blood by a genetic assay [181], and by 2000, this organism was cultured from the aortic valve of a patient with endocarditis due to *Tropheryma whippelii* [182]. In principle, this same approach should still be pursued in the microbial search for the sarcoidosis "holy grail".

Conceptual framework: from Henle-Koch to Bradford Hill

As described earlier, emerging technologies, such as genetic probes and immunological assays, offer great potential for helping to discovering the causes of sarcoidosis. However, despite the potential, there remain several nagging concerns and obstacles to establishing causation.

First, there are persuasive clinical, immunological and genetic reasons to think that sarcoidosis is not one disease, but rather a family of diseases. One of the most compelling examples is the acute form of Löfgren's syndrome, in which the clinical phenotype, patient demographics, T-cell immunological and human lymphocyte antigen (HLA) genetic polymorphism data all strongly suggest that this is a separate disease, distinct from other more chronic forms of sarcoidosis. It may share a common pathologic consequence (granulomas), but is otherwise quite unique. If sarcoidosis is a heterogeneous "family" of granulomatous disorders, efforts to find a single cause will prove fruitless, until investigators focus their aetiological investigations on carefully defined clinical/immunological/genetic subsets of patients.

Secondly, even if the cause were microbial, the mechanism leading to granuloma formation might not depend on a single organism, but rather require the interaction of more than one immunopathogenic moiety, such as the presence of factors that promote innate immunity (adjuvant properties) plus the presence of an antigen, which itself might or might not be microbial in origin. There is ample evidence in the immunology literature to suggest that "two hits", both innate and adoptive immunity, are at play in sarcoidosis [18]. As such, it is conceivable that more than one organism, or possibly an organism plus an environmental or endogenous source of adjuvant, is required for pathogenicity.

Thirdly, science is regularly humbled by the diversity of microbial species and by the relative ignorance of their number, name, and potential health effects. In other words, if the cause of sarcoidosis is microbial, there is a strong possibility that it could be an as-yet undiscovered and unnamed organism. Even more likely, there is a strong possibility that more than one organism may be able to cause sarcoidosis. By way of example, the literature on nontuberculous mycobacteria regularly discovers new mycobacteria, some of which: 1) have been shown to cause forms of granulomatous disease producing a sarcoidosis-like illness in workers exposed to contaminated metal working fluids; or which 2) have not yet been found to cause disease but which may, in time, be linked to idiopathic disease. Here, help may come from the work of those who laboriously probe the environment and human tissues for markers of new organisms.

Fourthly, detection of a genetic or immunological "fingerprint" does not, in and of itself, necessarily prove causation. If Henle-Koch postulates are strictly relied upon, then genetic analyses, including 16s ribosomal RNA or various *in situ* labelling of granulomas, will fall short of proving causation. Koch's postulates stipulate that the organism must be isolated from diseased hosts, grown in pure culture, and reproduce characteristics of disease when introduced into susceptible hosts. The postulates only apply if one is looking for an infectious agent. Science can and will continue to use the tools of immunology and genetics to narrow the list of microbial "suspects" and to then focus its attention on how to fulfill Koch's postulates. For example, in Whipple's disease, results of 16s rRNA PCR guided investigators to delve deeper into specific techniques for culturing and immunologically marking actinomycetes in tissue and blood of affected individuals. This may yet prove fruitful in sarcoidosis, but for the time being, sarcoidosis researchers will continue to work with the nagging concerns that not all organisms are known, not all can be isolated or cultivated, that the growth and proliferation of microbes might not always be necessary for them to be antigenic and induce granuloma formation, and that the cause might not be microbial.

If sarcoidosis is not caused by an infection, how can investigators hope to establish the causal link between an environmental factor or factors and disease? Clearly, one must employ a different paradigm for establishing causation, one that does not assume that all disease has infectious cause and that does not attempt to fulfil Koch's postulates. In the field of occupational and environmental medicine, this question is addressed routinely. A wide range of environmental toxins, from tobacco smoke to beryllium dust, has been established as the causes of disease, without the benefit of

Koch's postulates. Sarcoidosis researchers may benefit from using this more epidemiologic conceptual framework, maybe by merging the tools of epidemiology with those of immunology and genetics.

The conceptual framework for establishing causation due to noninfectious, environmental agents was most eloquently and clearly outlined 40 yrs ago by Bradford HILL [3], in an address entitled "The environment and disease: association or causation". As summarised in table 1, A. Bradford Hill, then a Professor Emeritus of Medical Statistics at University of London (London, UK), described nine "viewpoints" from which to study association "before we cry causation". Bradford HILL [3] was quick to point out that although the nine viewpoints summarised in table 1 may each contribute to the ability to establish cause and effect, "none can be required as a *sine qua non*". What they can do, with greater or lesser strength, is to help us to make up our minds on the fundamental question: is there any other way of explaining the set of facts before us, is there any other answer equally, or more likely than cause and effect? It was his belief that researchers cannot "usefully lay down some hard-and-fast rules of evidence that must be obeyed before we accept cause and effect", but that the use of these nine criteria would help to clarify thinking about cause.

Several of the Bradford HILL [3] criteria bear special comment in the context of sarcoidosis research, to help illustrate the challenges faced in proving, epidemiologically, the cause of sarcoidosis. 1) Specificity of the association presents a particular challenge, because most forms of pathology, be it cancer, fibrosis, or granuloma, are more likely to have more than one cause. 2) Demonstrating that a temporal relationship exists between exposure to a factor prior to the onset of illness is problematic for sarcoidosis research because it can only be guessed at when the disease process started in an individual or group. Sarcoidosis can present insidiously or dramatically, however, in either case, precise knowledge of when the disease started is lacking. Likewise, many exposures that are capable of producing granulomatous disease range widely in the latency between when the individual was first exposed and when they manifested their illness. For example, the range in time between first exposure to beryllium and the development of CBD stretches from 2 months to >40 years. By analogy, if there is an environmental cause of sarcoidosis, prior exposure could have occurred at almost any time prior to illness. 3) Bradford HILL's [3] criterion of biological gradient is also problematic for an illness such as sarcoidosis. Even if one were to know the cause of sarcoidosis, it is likely to be difficult to measure the dose of prior exposure that resulted in disease. It is likely to be modified by genetic susceptibility of the host and to require immunological sensitisation to occur in a step that precedes onset of disease. The dose-response curves for antigen sensitisation and for immune-mediated diseases are likely to be nonlinear. Thus, epidemiological studies of exposure×disease risk in sarcoidosis will face greater challenges than for those diseases in which the exposure obeys a linear cause-and-effect model. Fortunately, the immunological properties of any agent that causes sarcoidosis will allow investigators to use Bradford HILL's [3] criteria of experimental evidence to great advantage. Unlike many environmentally caused disorders, in sarcoidosis it should be possible, in fact required, to experimentally test a putative exposure for its ability to induce a disease-specific antigenic response. In the same way that the beryllium lymphocyte proliferation test is used to prove granulomatous disease is CBD and not sarcoidosis, any agent that is thought to cause sarcoidosis should lend itself to one or more demonstration of antigen-specific, disease-specific response, such as the antigen-specific humoral response, antigen-specific lymphocyte proliferation, cytokine production, or skin test reactivity. Experiments that test the antigenicity of microbial peptides or of self, found in the HLA binding-groove of sarcoidosis antigen-presenting cells, are being conducted in several laboratories in an effort to help make this causal link experimentally. As suggested by Bradford HILL [3], such experiments will need to be

integrated with the other eight criteria as part of the set of evidence for causation for this perplexing illness.

Summary

The cause or causes of sarcoidosis remain unknown. This chapter addresses some of the reasons for the collective ignorance, discusses clues that can be derived from past research literature, reviews the strengths and weaknesses of the prevailing candidates, including microbes, and proposes a conceptual framework for addressing causation. With increasing numbers of studies supporting a putative role for biological agents in sarcoidosis pathogenesis, this review critically examines the body of evidence toward the goal of making reviews, such as this one, someday seem quaint and obsolete.

Keywords: Aetiology, environment, occupational, sarcoidosis.

References

1. du Bois RM, Goh N, McGrath D, Cullinan P. Is there a role for microorganisms in the pathogenesis of sarcoidosis? *J Intern Med* 2003; 253: 4–17.
2. Newman LS, Rose CS, Maier LA. Medical progress: Sarcoidosis. *N Engl J Med* 1997; 336: 1224–1234.
3. Bradford Hill A. The environment and Disease: association or causation? *Proc R Soc Med* 1965; 58: 295–300.
4. Yamamoto M, Sharma OP, Hosoda Y. Special Report: The 1991 descriptive definition of sarcoidosis. *Sarcoidosis* 1992; 9: Suppl. 1, 33–34.
5. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med* 1999; 160: 736–755.
6. James DG. Clinical picture of sarcoidosis. *In*: Schwartz M, King TE Jr, eds. Interstitial Lung Disease, 2nd Edn. St. Louis, Mosby Year Book, 1993; pp. 159–178.
7. Song Z, Marzilli L, Greenlee BM, *et al.* Mycobacterial catalase-peroxidase is a tissue antigen and target of the adaptive immune response in systemic sarcoidosis. *J Exp Med* 2005; 201: 755–767.
8. Drake WP, Pei Z, Pride DT, Collins RD, Cover TL, Blaser MJ. Molecular analysis of sarcoidosis tissues for mycobacterium species DNA. *Emerg Infect Dis* 2002; 8: 1334–1341.
9. Eishi Y, Suga M, Ishige I, *et al.* Quantitative analysis of mycobacterial and propionibacterial DNA in lymph nodes of Japanese and European patients with sarcoidosis. *J Clin Microbiol* 2002; 40: 198–204.
10. Foley PJ, Lympany PA, Puscinska E, Zielinski J, Welsh KI, du Bois RM. Analysis of MHC encoded antigen-processing genes TAP1 and TAP2 polymorphisms in sarcoidosis. *Am J Respir Crit Care Med* 1999; 160: 1009–1014.
11. Iannuzzi MC, Maliarik MJ, Poisson LM, Rybicki BA. Sarcoidosis susceptibility and resistance HLA-DQB1 alleles in African Americans. *Am J Respir Crit Care Med* 2003; 167: 1225–1231.
12. Rossman MD, Thompson B, Frederick M, *et al.* HLA-DRB1*1101: a significant risk factor for sarcoidosis in blacks and whites. *Am J Hum Genet* 2003; 73: 720–735.
13. Schurmann M, Reichel P, Muller-Myhsok B, Schlaak M, Muller-Quernheim J, Schwinger E.

Results from a genome-wide search for predisposing genes in sarcoidosis. *Am J Respir Crit Care Med* 2001; 164: 840–846.
14. Rybicki BA, Maliarik MJ, Poisson LM, *et al.* The major histocompatibility complex gene region and sarcoidosis susceptibility in African Americans. *Am J Respir Crit Care Med* 2003; 167: 444–449.
15. Foley PJ, McGrath DS, Puscinska E, *et al.* Human leukocyte antigen-DRB1 position 11 residues are a common protective marker for sarcoidosis. *Am J Respir Cell Mol Biol* 2001; 25: 272–277.
16. Grutters JC, Sato H, Pantelidis P, *et al.* Increased frequency of the uncommon tumor necrosis factor -857T allele in British and Dutch patients with sarcoidosis. *Am J Respir Crit Care Med* 2002; 165: 1119–1124.
17. Maier LA. Is smoking beneficial for granulomatous lung diseases? *Am J Respir Crit Care Med* 2004; 169: 893–895.
18. Moller DR, Chen ES. Genetic basis of remitting sarcoidosis: triumph of the trimolecular complex? *Am J Respir Cell Mol Biol* 2002; 27: 391–395.
19. Moller DR. Involvement of T cells and alterations in T cell receptors in sarcoidosis. *Semin Respir Infect* 1998; 13: 174–183.
20. Grunewald J, Janson CH, Eklund A, *et al.* Restricted V alpha 2.3 gene usage by CD4+ T lymphocytes in bronchoalveolar lavage fluid from sarcoidosis patients correlates with HLA-DR3. *Eur J Immunol* 1992; 22: 129–135.
21. Forman JD, Klein JT, Silver RF, Liu MC, Greenlee BM, Moller DR. Selective activation and accumulation of oligoclonal V beta-specific T cells in active pulmonary sarcoidosis. *J Clin Invest* 1994; 94: 1533–1542.
22. Forrester JM, Wang Y, Ricalton N, *et al.* TCR expression of activated T cell clones in the lungs of patients with pulmonary sarcoidosis. *J Immunol* 1994; 153: 4291–4301.
23. Klein JT, Horn TD, Forman JD, Silver RF, Teirstein AS, Moller DR. Selection of oligoclonal V beta-specific T cells in the intradermal response to Kveim-Siltzbach reagent in individuals with sarcoidosis. *J Immunol* 1995; 154: 1450–1460.
24. Moller DR. Cells and cytokines involved in the pathogenesis of sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 1999; 16: 24–31.
25. Semenzato G, Agostini C. Immunology in sarcoidosis. *In*: Schwarz MI, King TE Jr, eds. Interstitial Lung Disease, 2nd Edn. St. Louis, Mosby Year Book, 1993; pp. 127–158.
26. O'Connor CM, FitzGerald MX. Speculations on sarcoidosis. *Respir Med* 1992; 86: 277–282.
27. Boros DL. Mechanisms of granuloma formation in the lung. *In*: Daniele RP, ed. Immunology and immunologic diseases of the lung. Boston, Blackwell Scientific Publications, 1988; pp. 263–287.
28. Newman LS. Metals that cause sarcoidosis. *Semin Respir Infect* 1998; 13: 212–220.
29. Schauble TL, Rich EA. Lymphocytic alveolitis in a crematorium worker. *Chest* 1994; 105: 617–619.
30. Gysbrechts C, Michiels E, Verbeken E, *et al.* Interstitial lung disease more than 40 years after a 5 year occupational exposure to talc. *Eur Respir J* 1998; 11: 1412–1415.
31. Drent M, Kessels BL, Bomans PH, Wagenaar SjSc, Henderson RF. Sarcoid-like lung granulomatosis induced by glass fibre exposure. *Sarcoidosis Vasc Diffuse Lung Dis* 2000; 17: 86–87.
32. Drent M, Bomans PH, Van Suylen RJ, Lamers RJ, Bast A, Wouters EF. Association of man-made mineral fibre exposure and sarcoid-like granulomas. *Respir Med* 2000; 94: 815–820.
33. Bresnitz EA, Strom BL. Epidemiology of sarcoidosis. *Epidemiol Rev* 1983; 5: 124–156.
34. Kucera GP, Rybicki B, Kirkey KL, *et al.* Occupational Risk Factors for Sarcoidosis in African-American Siblings. *Chest* 2003; 123: 1527–1535.
35. Newman LS, Rose CS, Bresnitz EA, *et al.* A case control etiologic study of sarcoidosis: environmental and occupational risk factors. *Am J Respir Crit Care Med* 2004; 170: 1324–1330.
36. Gordis L. Sarcoidosis. *In*: Epidemiology of chronic lung diseases in children. Baltimore, The Johns Hopkins University Press, 1973; pp. 53–78.

37. Cooch JW. Sarcoidosis in the United States Army, 1952 through 1956. *Am Rev Respir Dis* 1961; 84: Suppl., 103–108.
38. Rybicki BA, Major M, Popovich JJ, Maliarik MJ, Iannuzzi MC. Racial differences in sarcoidosis incidence: A 5-year study in a health maintenance organization. *Am J Epidemiol* 1997; 145: 234–241.
39. Rybicki BA, Iannuzzi MC, Frederick MM, *et al.* Familial aggregation of sarcoidosis. A case-control etiologic study of sarcoidosis (ACCESS). *Am J Respir Crit Care Med* 2001; 164: 2085–2091.
40. Terris M, Chaves AD. An epidemiologic study of sarcoidosis. *Am Rev Respir Dis* 1966; 94: 50–55.
41. Sartwell PE, Edwards LB. Epidemiology of sarcoidosis in the U.S. Navy. *Am J Epidemiol* 1974; 99: 250–257.
42. Spagnolo P, Renzoni EA, Wells AU, *et al.* C-C chemokine receptor 2 and sarcoidosis: association with Lofgren's syndrome. *Am J Respir Crit Care Med* 2003; 168: 1162–1166.
43. Grunewald J, Wahlstrom J, Berlin M, Wigzell H, Eklund A, Olerup O. Lung restricted T cell receptor AV2S3+ CD4+ T cell expansions in sarcoidosis patients with a shared HLA-DRbeta chain conformation. *Thorax* 2002; 57: 348–352.
44. Berlin M, Fogdell-Hahn A, Olerup O, Eklund A, Grunewald J. HLA-DR predicts the prognosis in Scandinavian patients with pulmonary sarcoidosis. *Am J Respir Crit Care Med* 1997; 156: 1601–1605.
45. Henke CE, Henke G, Elveback LR, Beard CM, Ballard DJ, Kurland LT. The epidemiology of sarcoidosis in Rochester, Minnesota: A population-based study of incidence and survival. *Am J Epidemiol* 1986; 123: 840–845.
46. Putkonen T, Hannuksela M, Mustakallio KK. Cold season prevalence in the clinical onset of sarcoidosis. *Arch Environ Health* 1966; 12: 564–568.
47. Poukkula A, Huhti E, Lilja M, Saloheimo M. Incidence and clinical picture of sarcoidosis in a circumscribed geographical area. *Br J Dis Chest* 1986; 80: 138–147.
48. James GD. Erythema nodosum. *BMJ* 1961; 1: 853–857.
49. Selroos O. The frequency, clinical picture and prognosis of pulmonary sarcoidosis in Finland. *Acta Med Scand* 1969; 503: Suppl., 3–73.
50. Badrinas F, Morera J, Fité E, Plasencia A. Seasonal clustering of sarcoidosis. *Lancet* 1989; 2: 455–456.
51. Panayeas S, Theodorakopoulos P, Bouras A, Constantopoulos S. Seasonal occurrence of sarcoidosis in Greece. *Lancet* 1991; 338: 510–511.
52. Wilsher ML. Seasonal clustering of sarcoidosis presenting with erythema nodosum. *Eur Respir J* 1998; 12: 1197–1199.
53. Kreiss K. The epidemiology of building-related complaints and illness. *In*: Cone JE, Hodgson MJ, eds. State of the Art Reviews: Problem Buildings: Building-Associated Illness and the Sick Building Syndrome. Philadelphia, Hanley and Belfus, Inc., 1989; pp. 575–592.
54. Burge HA. Indoor air and infectious disease. *In*: Cone JE, Hodgson MJ, eds. State of the Art Reviews: Problem Buildings: Building-Associated Illness and the Sick Building Syndrome. Philadelphia, Hanley and Belfus, Inc., 1989; pp. 713–721.
55. Keller AZ. Hospital, age, racial, occupational, geographical, clinical, and survivorship characteristics in the epidemiology of sarcoidosis. *Am J Epidemiol* 1971; 94: 222–230.
56. Gentry JT, Nitowsky HM, Michael JM. Studies on the epidemiology of sarcoidosis in the United States: The relationship to soil areas and to urban-rural residence. *J Clin Invest* 1955; 34: 1839–1856.
57. Israel H. Influence of race and geographical origin on sarcoidosis. *Arch Environ Health* 1970; 20: 608–609.
58. Michael M Jr, Cole RM, Beeson PB, Olson BJ. Sarcoidosis: preliminary report on a study of 350 cases with special reference to epidemiology. *Am Rev Tuberc* 1950; 62: 403–407.
59. Kajdasz DK, Lackland DT, Mohr LC, Judson MA. current assessment of A rurally linked exposures as potential risk factors for sarcoidosis. *Ann Epidemiol* 2001; 11: 111–117.

60. Parkes SA, Baker SBdC, Bourdillon RE, Murray CRH, Rakshit M. Epidemiology of sarcoidosis in the Isle of Man–1: A case controlled study. *Thorax* 1987; 42: 420–426.
61. Horwitz O. Geographic epidemiology of sarcoidosis in Denmark. *Am Rev Respir Dis* 1961; 84: Suppl., 130–134.
62. Comstock GW, Keltz H, Sencer DJ. Clay Eating and Sarcoidosis: A controlled study in the state of Georgia. *Am Rev Respir Dis* 1961; 84: Suppl., 130–134.
63. Buck AA, Sartwell PE. Epidemiologic investigations of sarcoidosis: II. Skin sensitivity and environmental factors. *Am J Hyg* 1961; 74: 152–173.
64. Buck AA. Epidemiologic investigations of sarcoidosis: I. Introduction; material and methods. *Am J Hyg* 1961; 74: 137–151.
65. Buck AA, McKusick VA. Epidemiologic investigations of sarcoidosis: III. Serum proteins; syphilis; association with tuberculosis; familial aggregation. *Am J Hyg* 1961; 74: 174–188.
66. Buck AA. Epidemiologic investigations of sarcoidosis: IV. Discussion and summary. *Am J Hyg* 1961; 74: 189–202.
67. Cummings MM, Dunner E, Williams JH Jr. Epidemiologic and clinical observations in sarcoidosis. *Ann Intern Med* 1959; 50: 879–890.
68. Douglas AC. Sarcoidosis in Scotland. *Am Rev Respir Dis* 1961; 84: Suppl. 5 Pt 2, 143–147.
69. Nobechi K. Sarcoidosis in Japan. *Am Rev Respir Dis* 1961; 84: Suppl. 5 Pt 2, 148–152.
70. Uehlinger EA. Epidemiology of sarcoidosis in Switzerland. *Am Rev Respir Dis* 1961; 84: Suppl. 5 Pt 2, 153.
71. Dunner E, Williams JH Jr. Epidemiology of sarcoidosis in the United States. *Am Rev Respir Dis* 1961; 84: Suppl. 5 Pt 2, 163–168.
72. Kajdasz DK, Judson MA, Mohr LC Jr, Lackland DT. Geographic variation in sarcoidosis in South Carolina: its relation to socioeconomic status and health care indicators. *Am J Epidemiol* 1999; 150: 271–278.
73. Gripenback S, Lundgren L, Eklund A, *et al.* Accumulation of eosinophils and T-lymphocytes in the lungs after exposure to pinewood dust. *Eur Respir J* 2005; 25: 118–124.
74. Kawai T, Tamura M, Murao M. Summer-type hypersensitivity pneumonitis: A unique disease in Japan. *Chest* 1984; 85: 311–317.
75. Rose C. Hypersensitivity pneumonitis. *In*: Rosenstock L, Cullen MR, eds. Textbook of Clinical Occupational and Environmental Medicine. Philadelphia, W.B. Saunders Co., 1994; pp. 242–248.
76. Rose CS, Newman LS. Hypersensitivity pneumonitis and chronic beryllium disease. *In*: Schwarz MI, King TE Jr., eds. Interstitial Lung Disease, 2nd Edition. St. Louis, Mosby Year Book, 1993; pp. 231–253.
77. Whitehead HG. Sarcoidosis: A manifestation of tuberculosis without allergy. *Bull Am Acad Tuberc Physicians* 1940; 4: 117.
78. Gundelfinger BF, Britten SA. Sarcoidosis in the United States Navy. *Am Rev Respir Dis* 1961; 84: 109–115.
79. Hills SE, Parkes SA, Baker SBdC. Epidemiology of sarcoidosis in the Isle of Man–2: Evidence for space-time clustering. *Thorax* 1987; 42: 427–430.
80. Newman AN, Taylor P. Cullinan, Sarcoidosis: in search of the cause. *Am J Respir Crit Care Med* 2004; 170: 1268–1269.
81. Hennessy TW, Ballard DJ, DeRemee RA, Chu CP, Melton LJ 3rd. The influence of diagnostic access bias on the epidemiology of sarcoidosis: a population-based study in Rochester, Minnesota, 1935–1984. *J Clin Epidemiol* 1988; 41: 565–570.
82. Scadding JG, Chappell AG, Sutherland I. Familial association in sarcoidosis: British Thoracic and Tuberculosis Association. *Tubercle* 1973; 54: 87–98.
83. Hosoda Y, Iwai K, Hiraga Y. Recent epidemiologic features of sarcoidosis in Japan. *In*: Williams WJ, Davies BH, eds. Eighth International Conference on Sarcoidosis and Other Granulomatous Diseases. Cardiff, Alpha Omega Publishing, Ltd., 1980; pp. 519–521.
84. Headings VE, Weston D, Young RC Jr, Hackney RL Jr. Familial sarcoidosis with multiple

occurrences in eleven families: a possible mechanism of inheritance. *Ann NY Acad Sci* 1976; 278: 377–385.
85. Sharma OP, Neville E, Walker AN, James DG. Familial sarcoidosis: a possible genetic influence. *Ann NY Acad Sci* 1976; 278: 386–400.
86. Nassif X, Valeyre D, Loiseau A, Battesti JP. Familial sarcoidosis: apropos of 22 families. *Ann Med Interne* 1985; 136: 611–614.
87. Harrington D, Major M, Popovich J, Maliarik M, Iannuzzi M. Familial sarcoidosis: analysis of 61 families. *Am Rev Respir Dis* 1993; 147: A91.
88. McGrath D, Daniil Z, Foley P, *et al.* Epidemiology of familial sarcoidosis in the UK. *Thorax* 2000; 55: 751–754.
89. Maliarik M, Kost J, Harrington D, *et al.* Genetic analysis of T cell receptor genes in familial sarcoidosis. *Am J Respir Crit Care Med* 1994; 149: A266.
90. Brackertz D, Kueppers F, Schwarb B. Histocompatibility (HL-A) antigens in sarcoidosis. *In*: Iwai K, Hosoda Y, eds. Proceedings of the Sixth International Conference on Sarcoidosis. Baltimore, University Park Press, 1972; pp. 235–238.
91. Hedfors E, Moller E. HL-A antigens in sarcoidosis. *Tissue Antigens* 1973; 3: 95–98.
92. Persson I, Ryder LP, Nielson LS. The LH-A7 histocompatibility antigen in sarcoidosis in relation to tuberculin sensitivity. *Tissue Antigens* 1975; 6: 50–53.
93. McIntyre JA, McKee KT, Loadholt CB, Mercurio S, Lin I. Increased HLA-B7 antigen frequency in South Carolina blacks in association with sarcoidosis. *Transplant Proc* 1977; 9: 173–176.
94. Turton CWG, Turner-Warwick M, Morris L. HLA in familial sarcoidosis. *In*: Williams WJ, Davies BH, eds. Eighth International Conference on Sarcoidosis and Other Granulomatous Diseases. Cardiff, Alpha Omega Publishing, Ltd., 1980; pp. 195–200.
95. Al-Arif L, Goldstein RA, Affronti LF. HLA antigens and sarcoidosis in a North American Black population. *In*: Williams WJ, Davies BH, eds. Proceedings of the Eighth International Conference on Sarcoidosis and Other Granulomatous Diseases. Cardiff, Alpha Omega Publishing, Ltd., 1980; pp. 206–212.
96. Newill CA, Johns CJ, Cohen BH. Sarcoidosis, HLA and immunoglobulin markers in Baltimore blacks. *In*: Chrétien J, Marsac J, Saltiel JC, eds. Proceedings of the Ninth International Conference on Sarcoidosis and Other Granulomatous Disorders. Paris, Pergamon Press, 1983; pp. 253–256.
97. Nowack D, Goebel KM. Genetic aspects of sarcoidosis: class II histocompatibility antigens and a family study. *Arch Intern Med* 1987; 147: 481–483.
98. Lenhart K, Kolek V, Bartova A. antigens associated with HLA sarcoidosis. *Dis Markers* 1990; 8: 23–29.
99. Luisetti M, Maartinatti M, Cuccia M, *et al.* HLA markers in sarcoidosis. *Sarcoidosis* 1991; 8: 177.
100. Barnard J, Rose C, Newman L, *et al.* Job and industry classification associated with sarcoidosis in a case control etiologic study of sarcoidosis. *J Occ Env Med* 2005; 47: 226–234.
101. Kreider ME, Christie JD, Thompson B, *et al.* Environmental exposures and the clinical phenotype of sarcoidosis. *Clin Chest Med* 2004; (In press).
102. Edmondstone WM. Sarcoidosis in nurses: is there an association? *Thorax* 1988; 43: 342–343.
103. Stewart IC, Davidson NM. Clustering of sarcoidosis. *Thorax* 1982; 37: 398–399.
104. Kern DG, Sherman CB, Neill MA, Wrenn DS. Serum neopterin elevations in firefighters. *Am J Respir Crit Care Med* 1994; 149: A408.
105. Prezant DJ, Dhala A, Goldstein A, *et al.* The incidence, prevalence, and severity of sarcoidosis in New York City firefighters. *Chest* 1999; 116: 1183–1193.
106. U.S. Sarcoidosis among Navy enlisted men, 1965–1993. *Morb Mortal Wkly Rep* 1997; 46: 539–543.
107. Jajosky P. Sarcoidosis diagnoses among U.S. military personnel: trends and ship assignment associations. *Am J Prev Med* 1998; 14: 176–183.
108. Fleming LE, Ducatman AM, Shalat SL. Disease clusters: A central and ongoing role in occupational health. *J Occup Med* 1991; 33: 818–825.

109. Figueroa WG, Raszowski R, Weiss W. Lung cancer of chloromethylether workers. *N Engl J Med* 1973; 28: 1096–1099.
110. Creech JL Jr, Johnson MN. Angiosarcoma of the liver in the manufacture of polyvinyl chloride. *J Occup Med* 1974; 16: 150–151.
111. Rose CS, Robins T, Harkaway P. Biopsy-confirmed hypersensitivity pneumonitis associated with exposure to metal-working fluids. *Morb Mortal Wkly Rep* 1996; 45: 606–609.
112. Shelton BG, Flanders WD, Morris GK. Mycobacterium sp. as a possible cause of hypersensitivity pneumonitis in machine workers. *Emerg Infect Dis* 1999; 5: 270–273.
113. Gordon T. Metalworking fluid – the toxicity of a complex mixture. *J Toxicol Environ Health A* 2004; 67: 209–219.
114. Embil J, Warren P, Yakrus M, *et al.* Pulmonary illness associated with exposure to Mycobacterium-avium complex in hot tub water. Hypersensitivity pneumonitis or infection? *Chest* 1997; 111: 813–816.
115. Khoor A, Leslie KO, Tazelaar HD, Helmers RA, Colby TV. Diffuse pulmonary disease caused by nontuberculous mycobacteria in immunocompetent people (hot tub lung). *Am J Clin Pathol* 2001; 115: 755–762.
116. Marras TK, Wallace RJ Jr, Koth LL, Stulbarg MS, Cowl CT, Daley CL. Hypersensitivity pneumonitis reaction to Mycobacterium avium in household water. *Chest* 2005; 127: 664–671.
117. Kreiss K, Mroz MM, Zhen B, Martyny J, Newman LS. Epidemiology of beryllium sensitization and disease in nuclear workers. *Am Rev Respir Dis* 1993; 148: 985–991.
118. Kreiss K, Wasserman S, Mroz MM, Newman LS. Beryllium disease screening in the ceramics industry. Blood lymphocyte test performance and exposure-disease relations. *J Occup Med* 1993; 35: 267–274.
119. Newman LS, Kreiss K, King TE Jr. Pathologic and immunologic alterations in early stages of beryllium disease. *Am Rev Respir Dis* 1989; 139: 1479–1486.
120. Mroz MM, Kreiss K, Lezotte DC, Campbell PA, Newman LS. Reexamination of the blood lymphocyte transformation test in the diagnosis of chronic beryllium disease. *J Allergy Clin Immunol* 1991; 88: 54–60.
121. Stange AW, Furman FJ, Hilmas DE. Rocky Flats beryllium health surveillance. *Env Health Perspect* 1996; 104: 981–986.
122. Stange AW, Hilmas DE, Furman FJ. Possible health risks from low level exposure to beryllium. *Toxicology* 1996; 111: 213–224.
123. Stange AW, Hilmas DE, Furman FJ, Gatliffe TR. Beryllium sensitization and chronic beryllium disease at a former nuclear weapons facility. *Appl Occup Environ Hyg* 2001; 16: 405–417.
124. Newman LS, Kreiss K. Non-occupational chronic beryllium disease masquerading as sarcoidosis: Identification by blood lymphocyte proliferative response to beryllium. *Am Rev Respir Dis* 1992; 145: 1212–1214.
125. Newman LS. Beryllium disease and sarcoidosis: Clinical and laboratory links. *Sarcoidosis* 1995; 12: 7–19.
126. Fireman E, Haimsky E, Noiderfer M, Priel I, Lerman Y. Misdiagnosis of sarcoidosis in patients with chronic beryllium disease. *Sarcoidosis Vasc Diffuse Lung Dis* 2003; 20: 144–148.
127. Infante PF, Newman LS. Beryllium exposure and chronic beryllium disease. *Lancet* 2004; 363: 415–416.
128. Rose CS, Martyny JW, Newman LS, *et al.* "Lifeguard lung": endemic granulomatous pneumonitis in an indoor swimming pool. *Am J Public Health* 1998; 88: 1795–1800.
129. Rutstein DD, Mullan RJ, Frazier TM, Halperin WE, Melius JM, Sestito JP. Sentinel health events (occupational): A basis for physician recognition and public health surveillance. *Am J Public Health* 1983; 73: 1054–1062.
130. Padilla ML, Schilero GJ, Teirstein AS. Donor-acquired sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2002; 19: 18–24.
131. Heyll A, Meckenstock G, Aul C, *et al.* Possible transmission of sarcoidosis *via* allogeneic bone marrow transplantation. *Bone Marrow Transplant* 1994; 14: 161–164.

132. Müller CBJ. Sarcoidosis recurrence following lung transplantation. *Transplantation* 1996; 16: 1117–1119.
133. Ikonomopoulos JA, Gorgoulis VG, Kastrinakis NG, Galanos AA, Karameris A, Kittas C. Experimental inoculation of laboratory animals with samples collected from sarcoidal patients and molecular diagnostic evaluation of the results. *In vivo* 2000; 14: 761–765.
134. Mitchell IC, Turk JL. Observations on the Kveim reaction using an animal model of granulomatous bowel disease. *Gut* 1991; 32: 159–162.
135. Kataria YP, Park HK, Mountain LD. Enhanced inflammatory response with histiocytes and epithelioid cells in rabbits to the intradermal injection of supernatants of cultured cutaneous sarcoidal granulomas. *Ann N Y Acad Sci* 1986; 465: 176–182.
136. Kooy R, Ruitenberg EJ, Sirks JL. Experience with a Dutch Kveim suspension in men and guinea pigs. *Ann N Y Acad Sci* 1976; 278: 717–721.
137. Teirstein AS. The Kveim-Siltzbach test. *Clin Dermatol* 1986; 4: 154–164.
138. Richter E, Kataria YP, Zissel G, Homolka J, Schlaak M, Muller-Quernheim J. Analysis of the Kveim-Siltzbach test reagent for bacterial DNA. *Am J Respir Crit Care Med* 1999; 159: 1981–1984.
139. Chemical and physical characteristics of the active principle of Kviem suspensions. *In*: V International Congress on Sarcoidosis, 1971. Prague, Czechoslovakia, Univ Karlova, 1971.
140. Ewer K, Deeks J, Alvarez L, *et al.* Comparison of T-cell-based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak. *Lancet* 2003; 361: 1168–1173.
141. Williams WR, Davies BH. Pseudo-leptospires in bronchoalveolar lavage fluid and blood cell cultures. *Br J Exp Pathol* 1986; 67: 167–170.
142. Wang N, Schraufnagel DE, Sampson MG. The tadpole-shaped structures in human non-necrotising granulomas. *Am Rev Respir Dis* 1981; 123: 560–564.
143. de Chadarevian JP, Raphael SA, Murphy GF. Histologic, ultrastructural, and immunocytochemical features of the granulomas seen in a child with the syndrome of familial granulomatous arthritis, uveitis, and rash. *Arch Pathol Lab Med* 1993; 117: 1050–1052.
144. Lawrence HJ, Greenberg BR. Autoimmune thrombocytopenia in sarcoidosis. *Am J Med* 1985; 79: 761–764.
145. Johnson LA, Edsall JR, Austin JH, Ellis K. Pulmonary sarcoidosis: could mycoplasma-like organisms be a cause? *Sarcoidosis Vasc Diffuse Lung Dis* 1996; 13: 38–42.
146. Bisaccia E, Scarborough DA, Carr RD. Cutaneous sarcoid granuloma formation in herpes zoster scars. *Arch Dermatol* 1983; 119: 788–789.
147. Su WP, Kuechle MK, Peters MS, Muller SA. Palisading granulomas caused by infectious diseases. *Am J Dermatopathol* 1992; 14: 211–215.
148. Ehlers S, Mielke ME, Hahn H. The mRNA-phenotype of granuloma formation: CD4+ T cell-associated cytokine gene expression during primary murine listeriosis. *Immunobiology* 1994; 191: 432–440.
149. Cho C, Linscheer WG, Hirschkorn MA, Ashutosh K. Sarcoid-like granulomas as an early manifestation of Whipple's disease. *Gastroenterology* 1984; 87: 941–947.
150. Southern JF, Moscicki RA, Magro C, Dickersin GR, Fallon JT, Bloch KJ. Lymphedema, lymphocytic myocarditis, and sarcoid-like granulomatosis. Manifestations of Whipple's disease. *JAMA* 1989; 261: 1467–1470.
151. Tintelnot K, von Hunnius P, de Hoog GS, Polak-Wyss A, Gueho E, Masclaux F. Systemic mycosis caused by a new Cladophialophora species. *J Med Vet Mycol* 1995; 33: 349–354.
152. Parkes SA, Baker SBdC, Bourdillon RE, *et al.* Incidence of sarcoidosis in the Isle of Man. *Thorax* 1985; 40: 284–287.
153. Kern DG, Neill MA, Wrenn DS, Varone JC. Investigation of a unique time-space cluster of sarcoidosis in firefighters. *Am Rev Respir Dis* 1993; 148: 4 Pt 1, 974–980.
154. Favata EA, Neill HM, Yang CS. Emerging microbial diseases of the indoor environment. *In*:

Couturier AJ, ed. Occupational and environmental infectious diseases. Beverly Farms, MA, USA, OEM Press, 2000; pp. 697–716.
155. Andersson MA, Nikulin M, Koljalg U, *et al.* Bacteria, molds, and toxins in water-damaged building materials. *Appl Environ Microbiol* 1997; 63: 387–393.
156. Verhoeff AP, van Wijnen JH, Brunekreef B, Fischer P, van Reenen-Hoekstra ES, Samson RA. Presence of viable mould propagules in indoor air in relation to house damp and outdoor air. *Allergy* 1992; 47: Suppl. 2 Pt 1, 83–91.
157. Finnegan MJ, Pickering C, Burge PS. The sick building syndrome: Prevalence studies. *BMJ* 1984; 289: 1573–1575.
158. Mendell MJ, Smith AH. Consistent pattern of elevated symptoms in air-conditioned office buildings: a reanalysis of epidemiologic studies. *Am J Public Health* 1990; 80: 1193–1199.
159. Rose C, Robins T, Harkaway P. Biopsy-confirmed hypersensitivity pneumonitis in automobile production workers exposed to metal working fluids-Michigan, 1994–1995. *Morb Mortal Wkly Rep* 1996: 606–609.
160. Mangiapan G, Hance AJ. Mycobacteria and sarcoidosis: an overview and summary of recent molecular biological data. *Sarcoidosis* 1995; 12: 20–37.
161. Richter E, Greinert U, Kirsten D, *et al.* Assessment of mycobacterial DNA in cells and tissues of mycobacterial and sarcoid lesions. *Am J Respir Crit Care Med* 1996; 153: 375–380.
162. Bocart D, Lecossier D, De Lassence A, Valeyre D, Battesti J-P, Hance AJ. A search for mycobacterial DNA in granulomatous tissues from patients with sarcoidosis using the polymerase chain reaction. *Am Rev Respir Dis* 1992; 145: 1142–1148.
163. Ghossein RA, Ross DG, Salomon RN, Rabson AR. Search for mycobacterial A DNA in sarcoidosis using the polymerase chain reaction. *Am J Clin Pathol* 1994; 101: 733–737.
164. Wilsher ML, Menzies RE, Croxson MC. *Mycobacterium tuberculosis* DNA in tissues affected by sarcoidosis. *Thorax* 1998; 53: 871–874.
165. Chao SC, Yan JJ, Lee JY. Cutaneous sarcoidosis among Taiwanese. *J Formos Med Assoc* 2000; 99: 317–323.
166. Vokurka M, Lecossier D, du Bois RM, *et al.* Absence of DNA from mycobacteria of the *M. tuberculosis* complex in sarcoidosis. *Am J Respir Crit Care Med* 1997; 156: Suppl. 3 Pt 1, 1000–1003.
167. Popper HH, Klemen H, Hoefler G, Winter E. Presence of mycobacterial DNA in sarcoidosis. *Hum Pathol* 1997; 28: 796–800.
168. Li N, Bajoghli A, Kubba A, Bhawan J. Identification of mycobacterial DNA in cutaneous lesions of sarcoidosis. *J Cutan Pathol* 1999; 26: 271–278.
169. Ikonomopoulos JA, Gorgoulis VG, Kastrinakis NG, *et al.* Sensitive differential detection of genetically related mycobacterial pathogens in archival material. *Am J Clin Pathol* 2000; 114: 940–950.
170. Klemen H, Husain AN, Cagle PT, Garrity ER, Popper HH. Mycobacterial DNA in recurrent sarcoidosis in the transplanted lung – a PCR-based study on four cases. *Virchows Arch* 2000; 436: 365–369.
171. Almenoff PL, Johnson A, Lesser M, Mattman LH. Growth of acid fast L forms from the blood of patients with sarcoidosis. *Thorax* 1996; 51: 530–533.
172. El-Zaatari FA, Naser SA, Markesich DC, Kalter DC, Engstand L, Graham DY. Identification of *Mycobacterium avium* complex in sarcoidosis. *J Clin Microbiol* 1996; 34: 2240–2245.
173. Moller DR. Identification of potential etiologic antigens in sarcoidosis. *J Clin Microbiol* 2001; 163: A214.
174. Homma JY, Abe C, Chosa H, *et al.* Bacteriological investigation on biopsy specimens from patients with sarcoidosis. *Jpn J Exp Med* 1978; 48: 251–255.
175. Ishige I, Usui Y, Takemura T, Eishi Y. Quantitative PCR of mycobacterial and propionibacterial DNA in lymph nodes of Japanese patients with sarcoidosis. *Lancet* 1999; 354: 120–123.
176. Yamada T, Eishi Y, Ikeda S, *et al. In situ* localization of Propionibacterium acnes DNA in lymph

nodes from sarcoidosis patients by signal amplification with catalysed reporter deposition. *J Pathol* 2002; 198: 541–547.
177. Relman DA, Loutit JS, Schmidt TM, Falkow S, Tompkins LS. The agent of bacillary angiomatosis. An approach to the identification of uncultured pathogens. *N Engl J Med* 1990; 323: 1573–1580.
178. Relman DA, Schmidt TM, MacDermott RP, Falkow S. Identification of the uncultured bacillus of Whipple's disease. *N Engl J Med* 1992; 327: 293–301.
179. Ksiazek TG, Erdman D, Goldsmith CS, *et al.* A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* 2003; 348: 1953–1966.
180. Fleming JL, Wiesner RH, Shorter RG. Whipple's disease: clinical, biochemical, and histopathologic features and assessment of treatment in 29 patients. *Mayo Clin Proc* 1988; 63: 539–551.
181. Lowsky R, Archer GL, Fyles G, *et al.* Brief report: diagnosis of Whipple's disease by molecular analysis of peripheral blood. *N Engl J Med* 1994; 331: 1343–1346.
182. Raoult D, Birg ML, La Scola B, *et al.* Cultivation of the bacillus of Whipple's disease. *N Engl J Med* 2000; 342: 620–625.