Granulomatous Infections: Etiology and Classification

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Granulomatous disorders are frequently due to a wide variety of infections. Over the past decade advances in molecular diagnostic techniques have allowed identification of organisms involved in granulomatous disorders that previously were of unknown etiology. On the basis of currently available information, granulomatous infections can now be classified in three categories. Group 1 infections are due to a well-recognized organism. Group 2 comprises infections due to organisms that have been recently identified in granulomas by molecular methods but are not readily isolated by conventional microbiological techniques. Group 3 consists of disorders for which the causal organisms have not yet been identified but are strongly suspected; further advances in diagnostic techniques will lead to reclassification of some of these disorders as group 2. This review describes the etiology, histopathologic features, and classification of granulomatous disorders, with an emphasis on those of groups 2 and 3.

Granuloma Formation

A granuloma can be defined as a focal, compact collection of inflammatory cells in which mononuclear cells predominate. Granulomas usually form as a result of the persistence of a nondegradable product or as the result of hypersensitivity responses [1]. An overlap of the two mechanisms occurs in most infectious diseases since microorganisms can serve both as foreign bodies and as antigens for immunologic responses. Normally granulomas are the result of protective mechanisms and form when acute inflammatory processes cannot destroy invading agents.

The granuloma forms by a stepwise series of events and is the end result of a complex interplay among invading organism, antigen, chemical, drug or other irritant, prolonged antigenemia, macrophage activity, T cell responses, B cell overactivity, circulating immune complexes, and a vast array of biological mediators [2]. Areas of inflammation or immunologic reactivity attract monocyte-macrophages, which may fuse to form multinucleated giant cells [3]. Further cellular transformation of macrophages to epithelioid cells may occur.

The granuloma is an active site of numerous enzymes and cytokines and, with aging, fibronectin and progression factors such as platelet-derived growth factor, transforming growth factor-β, insulin-like growth factor, and TNF-α. There is a close relationship between CD4+ T lymphocytes and activated macrophages showing increased expression of major histocompatibility (MHC) class II molecules. The T helper cells recognize protein peptides presented to it by antigen-presenting cells bearing MHC class II molecules.

The T cell induces IL-1 on the macrophage, and thereafter a cavalcade of chemotactic factors promote granuloma genesis [2, 4]. IFN-γ increases the expression of MHC class II molecules on macrophages, and activated macrophage receptors carry a crystallizable fraction of IgG to potentiate their ability to phagocytose [4]. The end result is the epithelioid granuloma, which progresses toward fibrosis under the impact of transforming growth factor and platelet-derived growth factor.

T cell subsets, macrophages, and other immune cells produce several cytokines, the amounts and types of which determine further subclassifications of granulomas. CD4+ T helper (Th) lymphocytes are necessary for the development of granulomas and are broadly classified into two functional types, Th1 and Th2 [5]. Th1-type cells produce IL-2, IFN-γ, and TNF-β upon stimulation with antigen and also participate in delayed-type hypersensitivity responses, while Th2-type cells make IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, cytokines which are important for development of B cells and eosinophilia.

There appears to be a complex relationship between Th1-type and Th2-type responses and infectious granulomas. Granulomas that synthesize predominantly Th2-type cytokines, such as those that form in response to parasite ova, make only small quantities of Th1-type cytokines chronically [6], whereas granulomas such as those of tuberculosis leprosy make large quantities of Th1-type cytokines and less of Th2-type lymphokines [7]. Granulomas of various infections may have different immunoregulatory mechanisms governing their formation and resolution [1, 2, 7].

Classification of Granulomatous Infections

Granulomatous disorders are frequently due to a wide variety of infections. Over the past decade advances in molecular diag-
Table 1. Classification of granulomatous infections and associated pathogens.

<table>
<thead>
<tr>
<th>Group number, type of causal agents</th>
<th>Granulomatous disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
</tr>
<tr>
<td>Well recognized</td>
<td>Tuberculosis, leprosy, Buruli ulcer, swimming pool (fish tank)</td>
</tr>
<tr>
<td>Mycobacteria</td>
<td>Granuloma</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Brucellosis, melioidosis, actinomycosis, nocardiosis, granuloma inguinale, listeriosis, tularemia</td>
</tr>
<tr>
<td>Spirochetes</td>
<td>Syphilis, pinta, yaws</td>
</tr>
<tr>
<td>Fungi</td>
<td>Mycoses (see table 3)</td>
</tr>
<tr>
<td>Protozoa</td>
<td>Leishmaniasis, toxoplasmosis</td>
</tr>
<tr>
<td>Nematodes</td>
<td>Visceral larva migrans (toxocariasis)</td>
</tr>
<tr>
<td>Trematodes</td>
<td>Schistosomiasis, paragonimiasis, fascioliasis, clonorchiasis</td>
</tr>
<tr>
<td>Chlamydia species</td>
<td>Lymphogranuloma venereum, trachoma</td>
</tr>
<tr>
<td>Rickettsia</td>
<td>Q fever (Coxiella burnetii infection)</td>
</tr>
<tr>
<td>Viruses</td>
<td>Infectious mononucleosis, cytomegalovirus infection, measles, mumps</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
</tr>
<tr>
<td>Recently recognized</td>
<td>Cat-scratch disease (Bartonella henselae infection)</td>
</tr>
<tr>
<td>Bacterium</td>
<td></td>
</tr>
<tr>
<td>Actinomyces</td>
<td>Whipple’s disease (Tropheryma whippelii infection)</td>
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<tr>
<td><strong>Group 3</strong></td>
<td></td>
</tr>
<tr>
<td>Suspected but not established</td>
<td></td>
</tr>
<tr>
<td>Measles virus</td>
<td>Crohn’s disease</td>
</tr>
<tr>
<td>Mycobacterium</td>
<td>Primary biliary cirrhosis</td>
</tr>
<tr>
<td>? (see table 4)</td>
<td>Sarcoidosis</td>
</tr>
<tr>
<td>Viral</td>
<td>Kikuchi’s disease</td>
</tr>
<tr>
<td>?</td>
<td>Chronic granulomatous disease of childhood</td>
</tr>
<tr>
<td>?</td>
<td>Langerhans granulomatosis</td>
</tr>
</tbody>
</table>

A wide range of infectious organisms cause the granulomatous diseases that fall into this category (table 1). These diseases share similar histologic features and have identifiable and distinct etiologic agents.

**Mycobacterial Infections**

Mycobacteria are a large group comprising *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Mycobacterium avium* complex, *Mycobacterium ulcerans*, *Mycobacterium marinum*, several opportunistic mycobacteria, and the mycobacteria from which BCG vaccine is produced. The nontuberculous mycobacteria produce a wide clinical spectrum of granulomatous disorders, summarized in table 2. Mycobacteria have been implicated in the etiopathogenesis of sarcoidosis and Crohn’s disease, and this is discussed in the section on group 3 disorders.

**Tuberculosis.** The etiologic agent of tuberculosis, *M. tuberculosis*, is clinically one of the most common microbial causes of granulomas [2,8]. The *M. tuberculosis* granuloma, classically characterized by the presence of central caseous necrosis, is referred to as a tubercle. Typically, there is a central area of amorphous caseating granular debris and loss of cellular detail, and acid-fast bacilli are present. This area is encircled by epithelioid cells, lymphocytes, histiocytes, fibroblasts, and occasionally Langhans’ giant cells. These histologic features of the granuloma are sufficiently characteristic to allow reasonably accurate diagnosis of tuberculosis [8].

While caseating granulomas are the classic finding in cases of tuberculosis, they are not always present; noncaseating granulomas may occur. When acid-fast bacilli are not identifiable in the granuloma, culture of the biopsy specimen may yield *M. tuberculosis*. PCR with use of primers specific for *M. tuberculosis* is currently under evaluation for the identification of this species [9] and may develop into an important tool for distinguishing it from other (nontuberculous) mycobacteria.

Immunosuppression may alter the classic histopathologic features of tuberculosis. A spectrum of histopathologic granulomatous tissue reactions are seen in patients infected with HIV [10]. Classic well-formed granulomas have been observed in individuals with early HIV disease whose CD4 cell counts have remained adequate. In patients with advanced HIV disease, the granulomas are less well formed, are more necrotic, and may contain abundant bacilli, which may be demonstrated by appropriate staining techniques as well as by culture of biopsy specimens [9–11].

**Leprosy.** Leprosy, or Hansen’s disease, is a chronic granulomatous disorder caused by *M. leprae*, an obligate intracellular bacterium that predominantly affects the skin and nerves [12]. First identified over a century ago by Armauer Hansen, the bacterium has defied all attempts to grow it in culture. Leprosy...
manifests as a spectrum of well-described clinical, pathological, and immunologic features [12].

At one end of the spectrum is lepromatous leprosy, the low resistance form with multiple lesions, extensive skin and visceral involvement, and diffuse infiltration of lesions with numerous leprotic bacilli. At the other end of the spectrum, tuberculoid leprosy, the highly resistant form, is characterized by one or few skin lesions and involvement of the nerve at the site of the lesion. Skin biopsies of patients with tuberculoid leprosy reveal a few acid-fast and alcohol-fast leprotic bacilli and a well-defined giant cell granuloma. Actual granulomatous invasion and destruction of dermal nerves, occasionally with caseous necrosis, sometimes occurs.

Within the dermis of patients with lepromatous leprosy, increased levels of Th2-type cytokines (IL-4, IL-5, and IL-10) and decreased levels of IL-2 and IFN-γ have been noted. In contrast, Th1-type cytokines predominate in tuberculoid lesions [7]. The diagnosis of leprosy is based on a combination of clinical and histologic findings. The paucity of acid-fast bacilli in the lesions of tuberculoid leprosy may sometimes make it difficult to distinguish them from other infectious and noninfectious granulomas, especially those of sarcoidosis and syphilis [12]. PCR for the detection of *M. leprae* in tissue lesions is being developed, and this may in the future allow more accurate diagnosis in such circumstances [13].

*Buruli ulcer.* *M. ulcerans* is the cause of chronic, relatively painless, cutaneous Buruli ulcers. The disease is most prevalent in Africa and Australia. The organism causes extensive undermined ulcers on the extensor surface of the extremities. The centers of the ulcers are necrotic, and the edges are undermined; the organisms are usually found at the periphery, where granulation tissue is most extensive [14]. While it is relatively easy to diagnose Buruli skin ulcers on the basis of clinical features and histologic findings, microbiological identification of the causal mycobacteria may sometimes be quite difficult, requiring long periods of culture.

Newer techniques such as gas-phase chromatography are becoming useful for identification of the acid-fast bacilli in low-count subcultures [15]. A report from West Africa [16] of a case of disseminated *M. ulcerans* infection that followed a snakebite and caused multifocal osteomyelitis illustrates the difficulties that are associated with identifying the organism by conventional microbiological techniques. Although repeated cultures were negative, the organism was identified as *M. ulcerans* by PCR amplification of the genes coding for 16S rRNA from biopsy specimens.

*Swimming pool (fish tank) granuloma.* *M. marinum* causes swimming pool (fish tank) granuloma [17]. Although the primary skin infection may be inconspicuous, the draining lymph nodes are extensively involved and caseous. A similar microscopic picture, with conspicuous plasma cell infiltration, is associated with granulomas due to other opportunistic mycobacteria. Fish tank granulomas develop in persons with minor abrasions who dip their hands in tropical fish tanks. Usually a solitary granuloma, nodule, or pustule forms, which may ulcerate or suppurate; however, multiple lesions may extend along the line of lymphatic vessels (in sporotrichotic infections).

Biopsy specimens that are cultured on Löwenstein-Jensen medium at room temperature yield pigmented mycobacterial colonies in 2–4 weeks. The response to treatment is variable and not dramatic. Antituberculous drugs, cotrimoxazole, and high doses of minocycline have been advocated.

A wide range of mycobacterial diseases occur in humans (table 2), and in some cases the clinical features and results of staining for acid-fast bacilli in granulomas may not reveal the identity of the causal organisms, which may also be difficult.
to isolate in culture. The development and application of molecular techniques such as PCR may in the future allow fairly accurate diagnosis [18].

Bacterial Infections

Brucellosis. Brucellosis (Malta fever) is a multisystem granulomatous disorder caused by small gram-negative, intracellular coccobacilli of the Brucella species [19]. The United Kingdom is one of 17 Brucella-free countries. It is more prevalent than its reported incidence in the United States, France, Spain, and Ireland [20]. Unpasteurized dairy products remain a common mode of transmission of this chronic granulomatous disorder among travelers to areas of endemcity. Raw camel’s milk, raw sheep’s milk, and goat’s milk, raw sheep’s and goat’s liver, and reindeer bone marrow have all been associated with transmission.

Diagnosis, which may be difficult by means of culture, is usually based on clinical features and serological and histopathologic findings [20]. Delayed-type hypersensitivity is believed to be important in the formation of the tissue granuloma, which limits the spread of the organism [21]. Epithelioid-cell granulomas may be found in the reticuloendothelial system, lung, brain, bone, joints, and soft tissue and are difficult to distinguish from granulomas due to other causes.

Necrotizing hepatic granulomas due to brucellosis have been described. In these cases, bacteriologic diagnosis may be difficult on the basis of blood or abscess pus cultures, although serology may be useful [22]. PCR tests for the identification of Brucella species are being developed [23, 24], and these may be useful tools for the diagnosis of brucellar granulomas.

Melioidosis. Melioidosis is caused by a gram-negative bacillus, Burkholderia pseudomallei. Acute melioidosis presents with protean clinical manifestations during which septicemia may occur, causing septic shock and multiorgan involvement. This may progress to a chronic suppurative phase, in which lung involvement, subcutaneous abscesses, lymphadenitis, and suppurative parotitis are common [25, 26]. Granulomatous osteomyelitis has also been described [27].

The lesions vary histologically from acute, chronic inflammation with a focal granulomatous component to a predominantly granulomatous picture. Caseous or purulent central necrosis is surrounded by epithelioid and giant cells and by fibrosis in lungs, lymph nodes, and bone. The presence of intracellular gram-negative bacteria within macrophages or giant cells and of similar extracellular bacilli may provide a clue to the diagnosis [28].

More-specific enzyme immunoassays are being developed for the detection of circulating antibody [29] or of B. pseudomallei antigen in the urine [30] of patients with melioidosis. PCR tests for the identification of B. pseudomallei have recently been described [31, 32], but these require field testing in areas of endemcity.

Treponemal Infections

The spirochetes include Treponema pallidum subspecies pallidum (the cause of syphilis), T. pallidum subspecies pertenue (responsible for yaws), and Treponema carateum (the causal agent of nonvenereal pinta in Latin America). All three are chronic granulomatous disorders that may cause diagnostic confusion with other granulomatous disorders [33]. Mucocutaneous lesions of secondary syphilis show a spectrum of histopathologic changes, ranging from minimal infiltration to granulomatous infiltration throughout the dermis [34]; granulomatous infiltrates show endothelial proliferation, with mononuclear cell infiltration.

The syphilitic gumma may present as a microscopic to grossly visible lesion, with an enclosing wall of histiocytes, plasma cell infiltrate, and necrotic center cells with intact cellular outlines. PCR to detect T. pallidum subspecies pallidum DNA in clinical specimens, including gumma biopsy material, appears to be highly sensitive and specific [35, 36].

Protozoan Infections

Leishmaniasis. Leishmaniasis refers to the spectrum of clinical disease caused by the protozoan Leishmania [37]. This parasite is transmitted by phlebotomine sandflies, and the disease presents in three clinical forms: cutaneous, mucocutaneous, and visceral. Amastigotes of Leishmania species live within macrophages and are usually seen as single or focal collections within macrophages in blood, aspirates of sternal marrow, liver or spleen specimens, or special culture medium.

Leishmaniasis has been extensively studied as a model infection for analysis of cell-mediated immunity. Granulomatous responses are a feature of cutaneous and mucocutaneous leishmaniasis. Cutaneous ulceration is characterized by a mononuclear cell infiltrate, with Th1-type responses emerging after weeks of infection. Resolution of the infection depends on an increase in the number of leishmania-specific CD4+ T cells of the Th1 subset [38], following which a granulomatous response with epithelioid and giant cells emerges.

The nodular stage of post-kala-azar dermal leishmaniasis is characterized by massive granulomas consisting of lymphocytes, plasma cells, and histiocytes, with numerous amastigotes of the species Leishmania donovani [39]. A preponderance of CD8+ T cells occurs in the lesion, in contrast to normal levels in the peripheral blood. A recent report described the use of PCR for identifying Leishmania aethiopica DNA in formalin-fixed and paraffin-embedded tissue specimens [40]: seven of the 40 skin biopsy specimens in which parasites were not found histopathologically were subsequently found to contain parasite DNA.

Toxoplasmosis. Toxoplasmosis refers to the clinical disease caused by the protozoan Toxoplasma gondii. Neonatal infection occurs during acute maternal infection in pregnancy. Acquired toxoplasmosis presents as a glandular, fever-like syn-
Table 3. Causes and immunopathologic features of granulomatous mycoses.

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Clinical condition</th>
<th>Immunopathologic feature(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptococcus neoformans</td>
<td>Cryptococcosis</td>
<td>Pneumonia, infarction, abscess, meningoencephalitis, granuloma, fibrosis</td>
</tr>
<tr>
<td>Candida species</td>
<td>Candidiasis</td>
<td>Abscess, necrosis, granuloma (mucocutaneous or multiorgan)</td>
</tr>
<tr>
<td>Sporothrix schenckii</td>
<td>Sporotrichosis</td>
<td>Granuloma (cutaneous, skeletal)</td>
</tr>
<tr>
<td>Histoplasma capsulatum</td>
<td>Histoplasmosis</td>
<td>Pneumonia, cavitation, granuloma</td>
</tr>
<tr>
<td>varieties</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus species</td>
<td>Aspergillosis</td>
<td>Necrotizing multisystem granulomas</td>
</tr>
<tr>
<td>Paracoccidioides brasiliensis</td>
<td>South American blastomycosis</td>
<td>Pneumonia, cavitation, granuloma, cutaneous plaques, nodules</td>
</tr>
<tr>
<td>Coccidioides immitis</td>
<td>Coccidioidomycosis</td>
<td>Granuloma, microabscess, pneumonia, chronic pulmonary or extrapulmonary disease</td>
</tr>
<tr>
<td>Blastomyces dermatitidis</td>
<td>Blastomycosis</td>
<td></td>
</tr>
<tr>
<td>Phialophora species</td>
<td>Chromoblastomycosis</td>
<td>Granuloma (cutaneous)</td>
</tr>
<tr>
<td>Pseudallescheria boydii,</td>
<td>Mycetoma</td>
<td>Granuloma (skin and subcutaneous tissue)</td>
</tr>
<tr>
<td>Madurella species</td>
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</tbody>
</table>

drome that rapidly resolves. The T. gondii trophozoites encyst and persist in nucleated cells as the cause of chronic, latent infection that can reactivate with immunosuppression and produce multisystem disease.

The histopathologic changes characteristically occur with a triad of features [41]: reactive follicular hyperplasia, epithelioid histiocytes, and monocytoid cells. Langhans' giant cells are not typically seen. Accurate diagnosis may be difficult in many cases. PCR is increasingly being used to detect T. gondii DNA in various clinical samples [42], and its application to identification of T. gondii DNA in tissue biopsy specimens is under investigation.

Fungal Infections

Fungal infections are common causes of granulomas worldwide. Granulomatous fungal infections can present as localized conditions or systemic illnesses (table 3). A wide range of medically important fungi can now be diagnosed by PCR [43]. Histoplasmosis and coccidioidomycosis are caused by dimorphic fungi that produce clinical disease resembling tuberculosis [44–46].

Histoplasma infections in the lungs of immunocompetent persons cause epithelioid-cell granulomas that undergo coagulative necrosis. Histologic differentiation from tuberculosis, sarcoidosis, and coccidioidomycosis may be possible by identification of thin-walled yeast forms in Grocott-Gomori methenamine–silver nitrate stains. In immunodeficient persons, histoplasmosis may be fulminant and is not associated with formation of epithelioid-cell granulomas: focal collections of mononuclear phagocytes containing yeasts are seen throughout tissues of the body, and the reticuloendothelial system becomes full of macrophages containing yeasts [46, 47].

Coccidioides immitis spores inhaled by immunocompetent persons induce a delayed-type hypersensitivity reaction. Most primary cases of coccidioidomycosis involving such patients are asymptomatic. Lung lesions may develop in some patients, in association with fever, chest symptoms, and erythema nodosum. A minority of cases progress to disseminated infection.

The primary and secondary granulomatous lung lesions due to C. immitis are similar to those due to Histoplasma species. C. immitis organisms are thick-walled, nonbudding spherules often filled with endospores. A neutrophil infiltrate may occur around the area of granulomatous reaction when the spherules rupture to release the endospores. In disseminated disease, the inflammatory response may be purely granulomatous, pyogenic, or mixed. Pyogenic lesions may dominate in immunosuppressed patients [46, 47].

Helminthic Infections

Several helminthic parasites can cause granulomas with pathological consequences. Of particular interest are schistosomiasis and visceral larva migrans.

Schistosomiasis. Schistosomiasis is caused by five main human-blood flukes of the genus Schistosoma: S. haematobium, S. mansoni, S. japonicum, S. intercalatum, and S. mekongi. These currently infect more than 200 million people. Diagnosis is made by identification of the species-specific characteristic ova in feces, urine, or tissues (usually rectal and liver biopsy specimens). During the chronic phase of the infection, the mature worms produce large numbers of ova, which become trapped in tissues and induce formation of granulomas [48], with chronic fibro-obstructive sequelae.

Granuloma formation is the result of a combination of factors: a foreign-body reaction to the deposited ova (which may
or may not be identifiable within the granuloma) and a cell-mediated delayed-type hypersensitivity reaction to antigenic determinants of the parasite [49]. The giant cells are of the multinucleate type, and the eosinophils are conspicuous. The ova contain living larvae that release a variety of toxic and antigenic substances through the egg wall, which itself is antigenic.

These substances induce and maintain the granulomatous response. The granulomatous response appears to be tightly regulated and involves development of Th1-type and Th2-type cytokine responses [50, 51].

Toxocarsis (visceral larva migrans). Dog and cat larval nematodes, especially *Toxocara canis* and *Toxocara cati*, may infect children and lead to the syndrome of visceral larva migrans [52]. Swallowed infected eggs emerge as larvae in the intestine, whence they pass to the liver, lungs, brain, and eye, producing eosinophilic granulomas in these different organs. This infection should be particularly suspected in children with hepatomegaly or miliary granulomas in liver biopsy specimens, miliary lung mottling, eosinophilia, and focal posterior uveitis or endophthalmitis. Liver granulomas may reveal part of the larvae with surrounding eosinophilia.

**Viral Infections**

*Measles.* The paramyxovirus family is a group of RNA viruses that includes the measles virus, which has been implicated in the etiology of idiopathic granulomatous disorders, sarcoidosis, and Crohn’s disease. The measles (rubeola) virus is spread by respiratory droplets and it multiplies within the upper respiratory tract epithelial cells, mononuclear cells, B and T lymphocytes, and macrophages. Viremia follows, which disseminates the virus throughout the body. T cell-mediated immunity that controls the infection develops and produces the measles rash.

The blotchy rash of measles consists of dilated skin vessels, edema, and a nonspecific mononuclear perivascular infiltrate [47, 53]. Lymphoid organs have marked follicular hyperplasia, large germinal centers, and randomly distributed multinucleate giant cells (Warthin-Finkeldey cells), which have eosinophilic nuclear and cytoplasmic inclusion bodies. In the lung, peribronchial and interstitial mononuclear infiltration occurs. The measles virus has been implicated in the etiopathogenesis of sarcoidosis and Crohn’s disease and is discussed in the section on group 3 disorders.

*Herpesviruses.* The herpes group of viruses are double-stranded DNA viruses that cause a spectrum of human disease. The Epstein-Barr virus is the etiologic agent of the clinical condition *infectious mononucleosis* and is transmitted by close contact, especially via saliva during kissing. It has also been implicated in the pathogenesis of several disorders such as Burkitt’s lymphoma, nasopharyngeal carcinoma, B cell lymphomas, and sarcoidosis.

Infectious mononucleosis in its benign form is characterized by fever, sore throat, lymphadenopathy, splenomegaly, and the appearance of atypical activated T cells in the peripheral blood. Further multisystem involvement may occur, leading to hepatitis, meningoencephalitis, and pneumonitis. Atypical lymphocytes are seen in the portal areas and sinusoids of liver biopsy specimens, and scattered isolated areas of focal parenchymal necrosis filled with lymphocytes may occur [47].

This histologic picture is difficult to distinguish from that of other types of viral hepatitis, such as hepatitis B, and from that of cytomegalovirus [54] infections. Specific diagnosis can be made by culture of the virus or identification of virus-specific DNA by PCR [55].

**Group 2 Granulomatous Disorders (Recently Recognized Causal Agents)**

**Cat-Scratch Disease**

Cat-scratch disease or fever is also known as benign lymphoreticulosis or regional granulomatous lymphadenitis [56]. It only occurs in humans, especially those who are scratched or bitten by kittens and then develop regional lymphadenitis proximal to the site of injury. Primary involvement is that of the lymph nodes, which first show lymphoid hyperplasia. Later, scattered granulomas with central areas of necrosis coalesce to form abscesses.

The histopathologic features of cat-scratch disease are not diagnostic and may be mistaken for tularemia, lymphogranuloma venereum, syphilis, brucellosis, atypical mycobacterial infections, fungal infections, and toxoplasmosis [47]. Warthin-Starry silver staining is used to detect *Bartonella henselae*, which may be present in the early phase of the disease. A skin test antigen has been made from lymph node pus. It is inoculated intradermally, and the degree of induration and erythema is measured at 48 hours.

The cat-scratch antigen skin test is positive for about 90% of patients who are clinically suspected of having the disease. This test will become redundant when techniques for amplifying specific nucleotide sequences with PCR come into general use. There is no well-recognized response to antibiotics, and recovery usually occurs without treatment.

Until recently, the causal agent remained controversial. Two organisms, *Afipia felis* and *B. henselae*, have been identified as potential candidates [57]. Substantial evidence is now accumulating that *B. henselae*, a small pleomorphic gram-negative bacillus, is the etiologic agent [58–60].

A PCR hybridization assay designed to amplify DNA from *B. henselae* and *A. felis* was used on pus aspirates from 89 skin test positive patients with cat-scratch disease. *B. henselae* DNA was found in 96% of samples, while no samples contained *A. felis* DNA [58]. An indirect fluorescent antibody assay for *B. henselae*—specific antibody has been described that appears to be sensitive for the diagnosis of cat-scratch disease [61].
Whipple’s Disease

George Hoyt Whipple of Johns Hopkins Hospital initially described a 37-year-old missionary who presented with fever, polyarthritis, and steatorrhea. His report was entitled “A Hitherto Undescribed Disease Characterized Anatomically by Deposits of Fat and Fatty Acids in the Intestinal and Mesenteric Lymphatic Tissues” [62]. Whipple’s disease is a chronic multisystem granulomatous disorder that affects middle-aged white males. It presents, as did Whipple’s patient, with fever, polyarthritis, weight loss, and diarrhea progressing to malabsorption.

Hepatosplenomegaly and generalized lymphadenopathy may be present, and biopsy of the lymph node, liver, or small intestine will reveal sarcoid granulomas, of which PAS stains will reveal foamy macrophages. The PAS-positive material corresponds with lysosomes containing bacilliform bodies. Electron microscopy reveals rod-shaped bacilli termed Whipple bacilli [63], Whipple’s associated bacterial organisms, or (more correctly) Tropheryma whippelii [64–66].

The nucleic acids extracted from an endoscopic biopsy specimen of the proximal small bowel of a patient with Whipple’s disease were subjected to amplification and nucleotide sequencing by PCR. The resulting PCR product from the bacterial 16S rRNA was then the subject of a computer database search for the rRNA sequences most similar to it. T. whippelii is an actinomycete that behaves like an intracellular pathogen and responds particularly well to antibiotics that enter cells easily. It is an erythrocyte-associated organism (like Bartonella bacilliformis, Babesia species, and Plasmodium species, which are also hemotropic). T. whippelii DNA has been detected by PCR examination of peripheral blood, pleural effusion cells [66, 67], biopsy tissue from the intestine [68], heart [69], or vitreotomy specimen of the eye [70].

The PCR primers that have been developed are specific for T. whippelii, and this technique is now one of the standard methods for establishing the diagnosis of Whipple’s disease [66, 67, 70]. The technique ensures a more specific diagnosis since the organism cannot be cultivated. Other data show that there may be a closely related second causative agent of Whipple’s disease [71, 72].

Group 3 Granulomatous Disorders (Infective Agent Strongly Suspected)

Primary Biliary Cirrhosis

This condition of progressive, nonsuppurative, granulomatous destruction of intrahepatic bile ducts has also been termed xanthomatous biliary cirrhosis, but its most accurate description is chronic nonsuppurative destructive cholangitis [73]. Liver cell necrosis may become superadded, later progressing to cirrhosis. Epithelioid granulomas associated with the bile ducts are found in about one-third of the cases of primary biliary cirrhosis (PBC). The condition has been likened to chronic graft-versus-host rejection, with similar structural changes in the bile, lacrimal, and pancreatic ducts, which have a high concentration of human leukocyte class II antigens on the epithelial surface.

PBC is distinguished by the fact that it predominates in women in the reproductive years of age and by the presence of serum mitochondrial antibodies. It is classified as an autoimmune disorder and is associated with other autoimmune conditions, including rheumatoid arthritis, systemic lupus erythematosus, scleroderma, Sjögren’s syndrome, and the CREST syndrome.

The etiology of PBC remains unknown. Two infectious agents have been postulated as possible trigger cofactors [74, 75]. The antigen specific for PBC serum is M2, a component of the pyruvate dehydrogenase complex of mitochondrial enzymes. There are similarities between mitochondrial components and Escherichia coli, and cross-reactivity between bile duct mitochondria and bacteria may be conceivable [73]. Mitochondrial antibodies cross-react with subcellular constituents or gram-negative intestinal or urinary tract organisms.

An increased incidence of gram-negative urinary tract infections in patients with PBC has been reported. Another infective association was suggested in a report from Barcelona that incriminated Mycobacterium gordonae. This ubiquitous organism causes granuloma formation, and serum antibodies are evident in PBC serum. Furthermore, there is cross-reactivity between this organism and the important PBC-defining M2 antigen. PCR has disclosed the presence of this mycobacterium in the tissues and bile of patients with PBC [75].

Sarcoidosis

Sarcoidosis is a multisystem disorder of unknown etiology [76]. An antigen (chemical or infectious organism)–driven, cell-mediated immune response leads to a cytokine cascade, to sarcoid granuloma formation, and eventually to sarcoid fibrosis. An intense search for an infectious cause has yielded many theories, yet none is conclusive (table 4).

In 1961, Edith Mankiewicz [77], of Montreal, reported that bacteriophages, lytic for mycobacteria, can be isolated with higher frequency from stool and resection specimens from patients with tuberculosis and sarcoidosis. Raised titers of phage-neutralizing antibodies were found in tuberculous patients infected with mycobacteria harboring mycobacteriophages but not in cases of sarcoidosis.

While mycobacteriophages are common to both diseases, the persistence of phages without antibody was the explanation given for the absence of M. tuberculosis in sarcoid tissue [78]. This observation led to further experiments in which guinea pigs were infected with tubercle bacilli alone or together with mycobacteriophages, and the histological responses (analyzed blindly) were of two dissimilar types. The first was caseous necrosis with the presence of acid-fast bacilli, and the second was a different response modified by the mycobacteriophages:
Table 4. Causal agents implicated in cases of sarcoidosis.

<table>
<thead>
<tr>
<th>Causal agents implicated</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Mycobacteria</td>
<td>[76-92]</td>
</tr>
<tr>
<td>Streptococci</td>
<td>[94]</td>
</tr>
<tr>
<td><em>Propionibacterium acnes</em></td>
<td>[88, 93]</td>
</tr>
<tr>
<td><em>Borrelia burgdorferi</em></td>
<td>[95, 96]</td>
</tr>
<tr>
<td><em>Mycoplasma</em> species</td>
<td>[97]</td>
</tr>
<tr>
<td><em>Nocardia</em> species</td>
<td>[98]</td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td></td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>[99]</td>
</tr>
<tr>
<td>Herpesvirus</td>
<td>[100, 101]</td>
</tr>
<tr>
<td>Rubella</td>
<td>[102]</td>
</tr>
<tr>
<td>Measles</td>
<td>[102]</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>[102]</td>
</tr>
<tr>
<td>Coxsackievirus B</td>
<td>[102, 103]</td>
</tr>
<tr>
<td>Retrovirus</td>
<td>[104]</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
</tr>
<tr>
<td>Beryllium</td>
<td>[105]</td>
</tr>
<tr>
<td>Zirconium</td>
<td>[105]</td>
</tr>
<tr>
<td>Pine pollen</td>
<td>[106, 107]</td>
</tr>
<tr>
<td>Peanut dust</td>
<td>[107]</td>
</tr>
<tr>
<td>Clay (ingestion)</td>
<td>[108]</td>
</tr>
</tbody>
</table>

The number of hybrids obtained with the sarcoid spleens (from which mycobacteria were neither seen on microscopy nor cultured) was 4.8 times higher than that obtained with normal spleens.

However, the results obtained by PCR are being fiercely contested by German [83], Scottish [84], and French [85] investigators. The findings of the German group contradict those of Saboor et al. [81], and the group concludes that mycobacterial DNA cannot be detected in tissues or bronchoalveolar lavage cells in cases of sarcoidosis. They use the technique to distinguish tuberculosis (which produces a positive result) from sarcoidosis. The Glasgow investigators [84] also failed to detect mycobacterial DNA in sarcoid lymph nodes. The French investigators [85], using PCR, rarely found DNA from *M. tuberculosis* in cases of sarcoidosis.

The literature for and against a mycobacterial cause of sarcoidosis is summarized in Table 5. The existence of mycobacterial sarcoidosis would validate several claims of infective or chemical causes of sarcoidosis: Scadding’s long-held views on tuberculous causation [86], a Swedish suggestion [87] of an interaction between mycobacteria and a virus, a Japanese theory [88] regarding *Propionibacterium* as the causal agent, and that of investigators in Houston [89] who isolated cell wall-defective spheroplasts of acid-fast mycobacteria from sarcoid skin biopsy specimens.

The controversy continues [90-93] and many different antigens have come under suspicion, including other bacteria [94-97], fungi [98], viruses [99-104], and chemicals [105-108]. Many attempts have been made but tissue culture has failed to uncover a virus. Elevated antibody responses to several viruses have been reported, but such responses may only reflect B-cell hyperreactivity rather than a causal relationship.

**Crohn’s Disease**

Crohn’s disease is a chronic granulomatous disorder affecting predominantly the gut, but it has multisystem involvement and variable clinical manifestations. The pathological changes may involve any parts and any layer of the alimentary tract. Transmural inflammation consists of chronic inflammatory cells with lymphoid aggregates scattered throughout. Noncaseating sarcoid-like granulomas may be present in all layers of diseased and unaffected tissue throughout the alimentary tract [109].

As with sarcoidosis, controversy prevails concerning a causal agent. Clinicopathologic studies at the Royal Free Hospital (London) demonstrated the presence of granulomatous vasculitis and vessel thrombosis, giving rise to focal microulcers. The investigations have shown early superficial mucosal changes and disruption of the capillary basement membrane preceding the formation of the micro ulcer [110]. These inflammatory changes may extend beyond the alimentary tract to the lung, causing pulmonary vasculitis, granulomatous interstitial lymphocyte infiltration, and interstitial fibrosis [111, 112].
### Table 5. Summary of the literature for and against a mycobacterial cause of sarcoidosis.

<table>
<thead>
<tr>
<th>Reference(s)</th>
<th>Year(s) of study</th>
<th>Location of study</th>
<th>Laboratory method used or feature analyzed (finding)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[77, 78]</td>
<td>1961–1964</td>
<td>Montreal</td>
<td>Reaction to mycobacteriophage infection</td>
</tr>
<tr>
<td>[79]</td>
<td>1964</td>
<td>Dallas</td>
<td>Presence of mycobacterial antibodies in serum (positive)</td>
</tr>
<tr>
<td>[80]</td>
<td>1971</td>
<td>Prague</td>
<td>Auramine-rhodamine staining, fluorescent microscopy (positive)</td>
</tr>
<tr>
<td>[87]</td>
<td>1972</td>
<td>Stockholm</td>
<td>Mycobacteria-virus interaction</td>
</tr>
<tr>
<td>[93]</td>
<td>1984</td>
<td>Tokyo</td>
<td>Culture of lymph nodes (positive for Propionibacterium acnes)</td>
</tr>
<tr>
<td>[89]</td>
<td>1988</td>
<td>Houston</td>
<td>Skin biopsy (cell-wall-defective, acid-fast bacilli noted)</td>
</tr>
<tr>
<td>[81]</td>
<td>1992</td>
<td>London</td>
<td>PCR (positive for mycobacterial DNA)</td>
</tr>
<tr>
<td>[82]</td>
<td>1992</td>
<td>London</td>
<td>Liquid-phase hybridization (positive for mycobacterial rRNA)</td>
</tr>
<tr>
<td>[83]</td>
<td>1992</td>
<td>Borstel, Germany</td>
<td>PCR (negative for mycobacterial DNA)</td>
</tr>
<tr>
<td>[84]</td>
<td>1992</td>
<td>Glasgow</td>
<td>PCR (negative for mycobacterial DNA)</td>
</tr>
<tr>
<td>[85]</td>
<td>1992</td>
<td>Paris</td>
<td>PCR (negative for mycobacterial DNA)</td>
</tr>
<tr>
<td>[90]</td>
<td>1993</td>
<td>London</td>
<td>PCR (positive for mycobacterial DNA)</td>
</tr>
<tr>
<td>[91]</td>
<td>1993</td>
<td>Ohio</td>
<td>ELISA (positive for antibodies to Mycobacterium paratuberculosis)</td>
</tr>
<tr>
<td>[92]</td>
<td>1993</td>
<td>Copenhagen</td>
<td>Western blotting (positive for antibodies to Mycobacterium tuberculosis)</td>
</tr>
</tbody>
</table>

Against this background is the conflict about whether the causal agent is *Mycobacterium paratuberculosis* [113], other mycobacteria [114], or the measles virus [115, 116]. Measles virus nucleocapsids have been detected in foci of granulomatous inflammation of the intestine in cases of Crohn’s disease [117]. It has been postulated that Crohn’s disease may be a result of chronic granulomatous vasculitis that develops in reaction to a persistent infection with measles virus within the vascular endothelium [118].

**Langerhans’ Cell Granulomatosis**

The term Langerhans’ cell granulomatosis refers to proliferative disorders of histiocytes, previously referred to as histiocytosis X [119]. It encompasses a group of disorders of unknown etiology characterized by granulomatous infiltration of the lungs, bone, skin, lymph nodes, and brain. The clinical conditions have been known by several names, based on the type of presentation, sites of involvement, rate of progression, and degree of associated immune dysfunction. They include eosinophilic granuloma, Letterer-Siwe disease, and Hand-Schüller-Christian disease. These are now considered to be different expressions for the same basic disorder, in which the proliferation of Langerhans’ cells results from disturbances in immunoregulation.

Langerhans’ histiocytes are bone marrow–derived monocyte-macrophage cells; they include Langerhans’ epidermal cells, Kupffer’s cells in the liver, osteoclasts, and alveolar macrophages. They are human leukocyte antigen–DR-positive functioning macrophages that present antigen to T cells and play a role in cell-mediated immunity. Unlike histiocytes, Langerhans’ cells can be stained immunohistochemically for S-100 protein and OKT-6.

Lung biopsy reveals a mixed cellular exudate, foam cells, eosinophils, and characteristic X bodies (Birbeck granules) in macrophages. Langerhans’ or X bodies are an ultrastructural feature in 90% of patients [120]. They are identical to the granules in Langerhans’ epidermal cells and consist of intracytoplasmic rod-, plate-, or cup-like pentalaminar structures.

The presence of these tennis racket–shaped ultrastructural Birbeck granules is diagnostic of the disorder. They have surface adenosine triphosphate activity identifiable by gold fluorescence. These diagnostic cells are readily found by bronchoalveolar lavage, and this technique may make lung biopsy unnecessary. It may also be a likely means of detecting a possible causal agent.

**Chronic Granulomatous Disease of Childhood (CGD)**

CGD comprises a genetically heterogeneous group of diseases that have in common the functional disorder of a multi-component enzyme system, NADPH oxidase, found in phagocytic cells [121–124]. This defect leads to severe infections, particularly with *Staphylococcus aureus*, *Serratia marcescens*, *Burkholderia cepacia*, and *Aspergillus* species. Organisms that lack catalase supply the neutrophil with the hydrogen peroxide for their own destruction. Thus, catalase-negative organisms such as pneumococci or streptococci present no problem because they are normally destroyed.
There are three autosomal recessive types, corresponding to mutations in p22<sub>phox</sub>, p47<sub>phox</sub>, and p67<sub>phox</sub>. The classic X-linked disorder (found in ~60% of cases), which affects the gene encoding p22<sub>phox</sub>, occurs in boys and is diagnosed in infancy, usually on the basis of an infection with a catalase-producing bacterium or fungus. The diagnosis is confirmed on the basis of a nitroblue tetrazolium test or other test showing defective superoxide production.

Hepatosplenomegaly is common and does not reflect active infection per se. Lymphadenopathy is often undetected, but when it is significant it usually reflects an active infection. Severe granulomatous problems can occur in association with CGD, such as obstruction of the gut and the genitourinary system. Weeping granulomatous skin lesions occur in the setting of an active wound, typically post-surgically. The histological appearance is marked by lipid-laden macrophages. While the etiology of CGD is genetic, there may be undetected pathogens responsible for some of the granulomatous complications of the disease.

**Orofacial Granulomatosis (Melkersson-Rosenthal Syndrome)**

This is a rare granulomatous disorder of the mouth and adjacent tissues, involving the oral mucosa, gum, lips, tongue, pharynx, eyelids, and skin of the face. Melkersson [125] described an association between facial edema and facial paralysis. Rosenthal [126] added the features of lingua plicata or scrotal tongue. Other clinical features include granulomatous cheilitis, edema of the gums and scalp, salivary gland dysfunction, vulvitis [127-129].

Biopsy of an involved area reveals a noncaseating granuloma. Unlike the findings in cases of sarcoidosis, there are no chest radiographic changes or uveitis, and the Kveim-Siltzbach skin test is negative. The etiology remains unknown.

**Kikuchi’s Disease**

This disorder was described in 1972 by a Japanese pathologist and is characterized by lymphadenitis with focal reticulum-cell hyperplasia, nuclear debris, and phagocytosis [130]. The lymphadenitis may be proliferative, necrotizing, or xanthomatous. Immunohistologic analysis shows that the predominant cells involved in the lymphadenitis are various types of histiocytes, plasmacytoid monocytes, and T cells; B cells are absent [131]. Clinically there is localized, tender, cervical lymphadenopathy with an upper respiratory tract prodomne.

Kikuchi’s disease was initially thought to occur mostly in women under the age of 30 years, although a recent study of 79 cases showed that the preponderance of cases involving females is not striking [131]. The disease runs a self-limiting course and is associated with a recurrence rate of 3%. Kikuchi’s disease has been recognized as a presenting feature of mixed connective-tissue disease and has been noted in association with adult Still’s disease and systemic lupus erythematosus, a finding leading to the hypothesis that Kikuchi’s disease and autoimmune rheumatic disorders may share a common etiology [132].

The disease occurs worldwide and has often been confused with toxoplasmosis, cat-scratch disease, tuberculosis, infectious mononucleosis, and non-Hodgkin’s lymphoma. The cause of the disease is not known. A viral etiology is strongly suspected on the basis of clinical features, although serological and ultrastructural studies have not yet identified an infectious agent [133].

**Conclusions**

Classification schemes are most useful when they provide insight into the purpose of events and the etiologic mechanisms that govern them. Granulomas are remarkably complex inflammatory foci that sequester organisms or other substances resistant to degradation. While a large number of granulomatous disorders are recognized, infections are clearly the most common underlying causes of granulomas in general.

It is apparent that granulomatous conditions of diverse etiologies share common histologic features, although the etiologic agent is not always identifiable. Although the histopathologic patterns in various infectious granulomas may be sufficiently different to prevent an accurate diagnosis, atypical presentations may necessitate identification of the specific etiologic agent by direct microscopic examination, culture, serology, or molecular detection.

In several granulomatous diseases the etiologic agent is difficult to identify by microscopic examination, culture, or serological means. The availability of PCR for the diagnosis of several infectious conditions has been of major importance in detecting the etiologic agents of granulomatous disease conditions that previously were considered to be of unknown etiology. In particular, PCR has been used successfully for detecting the bacteriologic causes of Whipple’s disease and cat-scratch disease.

Further applications of molecular techniques may pin down the microbiological etiology (if any) of several granulomatous disorders of group 3 (table 1), of which the etiology remains elusive.

**References**


