CHAPTER 6

Pathology

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From the pathological point of view, sarcoidosis is characterised by the formation of small, granular inflammatory lesions (granulomas). The word "granuloma" comes from the Latin word "granum," meaning grain or seed. This characteristic lesion of sarcoidosis is a discrete, compact, noncaseating epithelioid cell granuloma (fig. 1a and b).

The epithelioid cell granulomas consist of highly differentiated mononuclear phagocytes (epithelioid cells and giant cells) and lymphocytes (fig. 2a). The central portion of the granuloma consists predominantly of CD4+ lymphocytes, whereas CD8+ lymphocytes are mainly located in the peripheral zone [1–3]. A proportion of these CD4+ lymphocytes exhibit a "memory" phenotype, as defined by the expression of CD45RO, as well as proliferation and activation markers (Ki67, CD25) [3, 4]. Sarcoid granulomas may develop fibrotic changes that usually begin at the periphery and travel centrally, ending with complete fibrosis and/or hyalinisation. Granulomas may occasionally exhibit focal coagulative necrosis [1]. It has been suggested that necrotising sarcoid granulomatosis may be a variant of sarcoidosis [2]. On electron microscopy, well developed epithelioid cells show numerous cytoplasmic projections with frequent interdigitations. The morphology suggests a secretory function [1, 5]. These cytoplasmic inclusions, including basophilic calcifications (Schaumann's bodies) and lucent, birefringent calcium oxalate crystals, can be valued using polarising microscopy.

The morphological diagnosis of pulmonary sarcoidosis relies on three main findings: 1) the presence of tight, well-formed granulomas and a rim of lymphocytes and fibroblasts in the outer margin of granulomas; 2) perilymphatic interstitial distribution of

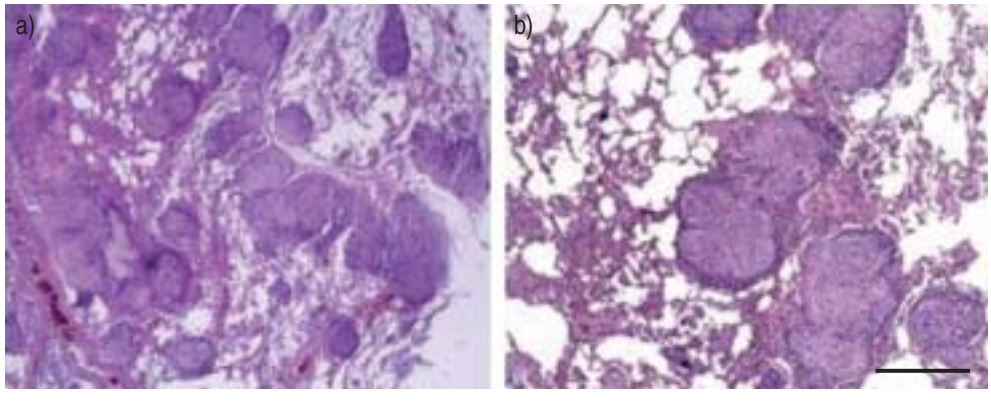

Fig. 1. – Pulmonary sarcoidosis: numerous large epithelioid granulomas are present, mainly located along lymphatic routes (a and b). Haematoxylin and eosin staining. b) Scale bar=10 μm.

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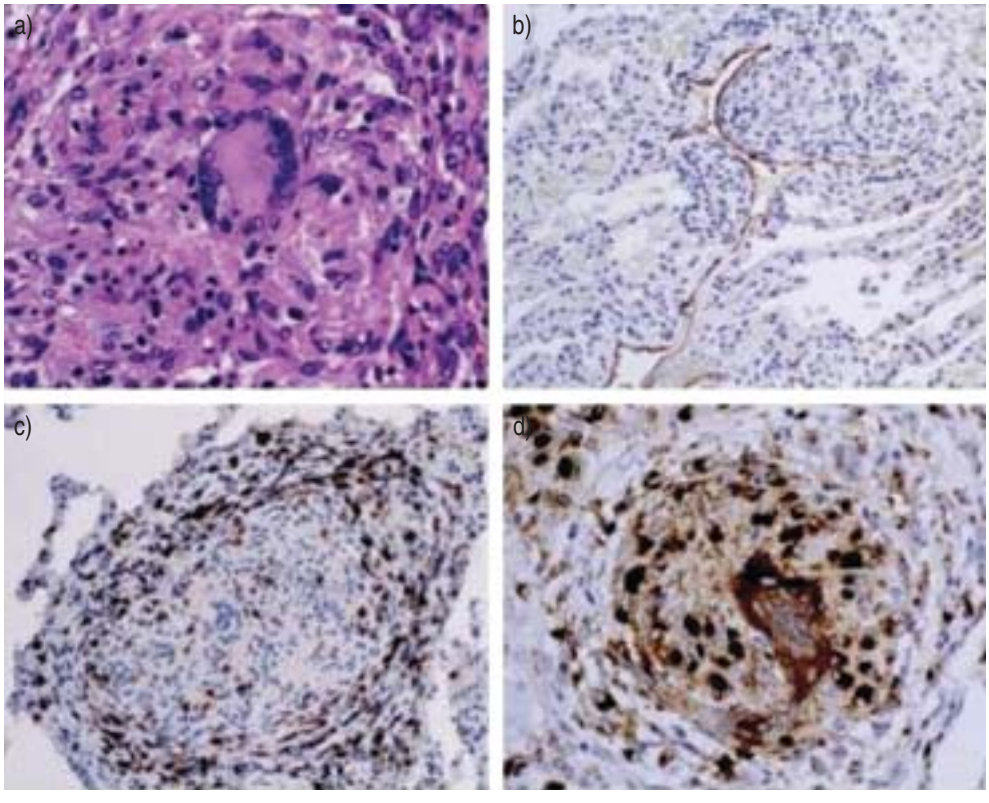

Fig. 2. – a) The morphology of granuloma is evidenciated at higher magnification, including numerous epithelioid macrophages, a giant cell and a minority of lymphocytes. b) The location of a granuloma near a lymphatic vessel is evidenciated by immunostaining for podoplanin (a marker of lymphatic endothelium). c) All lymphocytes associated to macrophages within a granuloma all T-cells, as defined by their expression of CD3. d) CD68 expression in epithelioid and giant cells.

granulomas (which allows transbronchial biopsies to be used as sensitive diagnostic tools; fig. 2b and c); and 3) exclusion of an alternate cause [6–7]. The granulomas are distributed along the pulmonary lymphatics, in the pleura and septa and along pulmonary arteries, veins, and bronchi. This distribution along lymphatic routes is one of the most helpful features in recognising sarcoidosis and in distinguishing it from other forms of granulomatous pulmonary disease. Small foci of fibrinoid necrosis may be seen in the granulomas. The extent of granulomas identified histologically often correlate with the changes on chest radiograph, but do not necessarily correlate with pulmonary function tests [5]. Lung tissue from patients with sarcoidosis demonstrated at an extrapulmonary site and normal chest radiographs often show scattered granulomas, either singly or as small confluent masses along lymphatic routes, too small to be radiographically visible, especially on conventional chest radiographs. More than half the cases of pulmonary sarcoidosis show histological involvement of pulmonary arteries or veins or both [1, 4, 8]. However, pulmonary hypertension caused by extensive vascular involvement in sarcoidosis is extremely rare [9–11]. More often, pulmonary hypertension in sarcoidosis is related to massive lung fibrosis and honeycombing, with loss of more than half the capillary bed. Small and large airways can also be involved by granulomas, and measurable airflow obstruction occurs in a small percentage of cases [12, 13], with segmental bronchial stenoses being a frequent cause. Granulomas in the mucosa and the

submucosa of large airways may be visible as multiple small nodules at bronchoscopy. The presence of granulomas in the pleura of patients with sarcoidosis is relatively common, although clinical evidence of pleural disease is quite unusual [14]. Fewer than 10% of patients have evidence of a pleural effusion, which may be small, asymptomatic, and transient, or massive and associated with significant symptoms [15]. KANADA *et al.* [16] described a case of sarcoidosis in which there was massive pleural thickening by granulomas and severe respiratory compromise.

Various other diseases can cause the formation of granulomas. In tuberculosis, the centres of the granulomas become so isolated that the cells probably die for a variety of causes, including oxygen shortage and accumulation of toxic substances produced by the macrophages and leukocytes that make up the granuloma (*e.g.* tumour necrosis factor). The dead tissue resembles a soft, cheesy substance, so the process is called caseation. In contrast, sarcoid granuloma is never caseating, although small foci of fibrinoid necrosis can occasionally be observed. Regional lymph nodes draining carcinomas can occasionally show the presence of noncaseating epithelioid cell granulomas (sarcoid reactions) with an average frequency of 4.4% [17–18]. In seminoma/disgerminoma, sarcoid-like granulomas can be present in the primary tumours in a significant number of cases, and can represent a diagnostic challenge in small mediastinal biopsies.

Epithelioid granulomas can be observed in Hodgkin's and non-Hodgkin's lymphomas. Biopsy specimens of liver and spleen obtained at laparotomy for the staging of Hodgkin's disease and non-Hodgkin's lymphomas show granulomas with an average frequency of 13.8% and 7.3% respectively [17–18]. A total of 15–20% of biopsy samples with granulomatous lesions have an undetermined aetiology. These patients have a disease process that has been named GLUS syndrome (granulomatous lesions of unknown significance) [19]. Immunohistologically, GLUS syndrome granulomas are B-cell positive, as are tumour-related sarcoid reactions and toxoplasmosis. However, granulomas in sarcoidosis and mycobacterial infection are B-cell negative [20].

Granulomas may or may not be found in the rare cases that progress to honeycombing [21]. In cases with marked airway involvement, bronchiectasis may develop that can represent a dominant feature of the honeycombing. Aspergilloma may be a late complication in patients with bronchiectasis and honeycombing [22]. If untreated, these lesions can become scarred.

Diagnostic approaches

The diagnosis of sarcoidosis needs a compatible clinical picture, histological demonstration of noncaseating granulomas and exclusion of other diseases capable of producing a similar histological or clinical picture. The presence of noncaseating granulomas in a single organ, such as skin, does not establish a diagnosis of sarcoidosis.

Radiology

The chest radiographic findings vary to a great extent: 5–10% of patients have normal chest radiographs despite the fact that granulomas in the lungs are identified histologically. One-third of all patients (40% of Black patients) have both pulmonary infiltrates and bilateral hilar lymph node enlargement. Another one-third have hilar or lymph node enlargement alone, which is unilateral in 5%. Pulmonary infiltrates may be reticular, reticulonodular, alveolar [23], or show a ground-glass appearance and are not necessarily bilateral [24]. Peripheral pulmonary infiltrates, similar to those of chronic eosinophilic pneumonia, can be occasionally observed in sarcoidosis [25]. Radiological

staging systems have been in use for many years and are still of value in pulmonary sarcoidosis. The chest radiographic evaluation of sarcoidosis was vastly improved by conventional and high-resolution computed tomography (CT) of the chest. CT allows identification of pathological changes not seen on conventional chest radiographs. These techniques show remarkably good correlation with gross and microscopic changes. There is a distribution of small nodules and irregular linear opacities along lymphatic routes, particularly bronchovascular bundles [26, 27].

Bronchoalveolar lavage

This technique was first used to evaluate patients with sarcoidosis in the mid 1970s. The initial results suggested a poorer prognosis for patients who had >28% lymphocytes in their bronchoalveolar lavage (BAL) cell differential. Subsequent studies have not confirmed these early results, and some results suggest that patients with a high lymphocyte count actually have a more favourable course. In BAL fluid, there is generally a moderate increase in the total number of cells, and an increased percentage (~15–60%) of CD4+ T-lymphocytes. As a consequence, the CD4+:CD8+ ratio will be high. There are also reports on alveolitis in sarcoidosis dominated by CD8+ cells [28], and neutrophils may accumulate in more advanced fibrosing cases [29]. If T-cell subpopulations are analysed, typically the CD4+:CD8+ ratio is >1.5, usually ranging from 3:1 to as high as 10:1. There may be prognostic significance to an elevated CD4+:CD8+ ratio in BAL fluid, but again the results of studies addressing this issue are conflicting. Several suggest that pulmonary function values and prognosis are inversely related to the ratio, whereas others suggest that radiographic improvement in pulmonary infiltrates is greater with a high CD4+:CD8+ ratio. These discrepancies may be due to inclusion of patients with acute onset disease, *i.e.* Löfgren's Syndrome, who typically have an elevated CD4+:CD8+ and a favourable prognosis. Subjects with more chronic disease may also have an elevated CD4+:CD8+ ratio but overall their prognosis is not as good as those with the acute onset of disease [29]. For now, the role of BAL in the management of sarcoidosis remains ill-defined. Although it cannot reliably diagnose this disease, it is helpful in assessing the presence of infection and other immunological diseases of the lung, such as chronic hypersensitivity pneumonitis, where the CD4+:CD8+ ratio is typically inverted. If there is an experienced BAL laboratory available to analyse the fluid, doing a BAL as part of the initial workup of a patient with suspected sarcoidosis is justified.

Transbronchial needle aspiration

Transbronchial needle aspiration (TBNA) is a minimally invasive bronchoscopic procedure that allows sampling of hilar and mediastinal lymph nodes in close contact with the airways. In a recent study, the authors assessed the value of TBNA in the diagnosis of sarcoidosis manifesting with intrathoracic lymphadenopathies (stages I and II), and compared its yield with that of transbronchial biopsy (TBB) [30]. The conclusions of this study suggest that a diagnostic approach combining TBNA and TBB is safe and effective in the setting of stage I and II sarcoidosis. It also confirmed the value of TBNA, with excellent diagnostic yields, especially in stage I of the disease. In particular, the combination of the two methods was associated with the highest diagnostic yield (93.7% overall sensitivity), and allowed significantly better results over both TBNA alone (93.7% *versus* 65.6%; p=0.011) and TBB alone (93.7% *versus* 62.5%; p=0.005).

Biopsy

Although the clinical and radiological features in some cases are so typical that a presumptive diagnosis of sarcoidosis can be made without histological confirmation, it is generally agreed that histological confirmation should be obtained before institution of steroid therapy [14]. TBB is the recommended procedure in most cases. Its diagnostic yield depends largely on the experience of the operator, ranging 40–90% when four to five lung biopsies are carried out [31–32]. TBBs show features compatible with the diagnosis of sarcoidosis in 65–80% of cases, with the highest positive rate in patients with both hilar lymphadenopathy and pulmonary infiltrates [33]. In one study it was found that four or more TBB specimens yielded a diagnostic rate of 90% [31]. TBB may yield positive results even in patients with normal chest radiographs. The use of a restricted panel of monoclonal antibodies, such as CD68+ and CD45RO, can significantly increase the sensitivity of histological recognition of sarcoid lesions in small, occasionally crunched, TBBs (fig. 2d) [34].

In the absence of the typical clinical circumstances, a diagnosis of sarcoidosis based solely on TBB alone is very risky, particularly if poorly formed or only a few granulomas are present. TBNA has been used to confirm the presence of granulomatous tissue in the sampled nodes and to complement TBB but it has the same limitations that apply to TBB alone. Bronchial mucosal biopsy may be performed during the same procedure; it is positive for noncaseating granuloma in 41–57% of patients with sarcoidosis [35–37]. A careful examination of the patient may disclose other possible sites for biopsy, such as skin, lips, or superficial lymph nodes. A granulomatous scar (a fresh granulomatous reaction on the site of an old scar) may be a very useful site for biopsy. It is not useful to biopsy erythema nodosum lesions since they will not show granulomas. Liver biopsy is rarely indicated, even if there is biochemical or clinical evidence of liver involvement. Finally, the diagnostic yield of biopsy by mediastinoscopy or video-assisted thoracoscopic (VTLB) or open lung biopsy [38] is reported to be >90%. VTLB has the advantage of permitting biopsy of both the lung and lymph nodes.

In conclusion, to provide histological confirmation of the disease, the bronchial mucosal biopsy should also be taken, since the histological demonstration of granuloma is possible in 40–60% of cases, even when the bronchial mucosa is grossly normal. When gross endoscopic findings, such as mucosal nodularity, oedema or hypervascularity are present, the yield of endobronchial biopsies may exceed 90% [39]. TBB through a fibreoptic bronchoscope is the recommended procedure in most cases [33]. The diagnostic yield is high, reaching 80–90% if $\geq$4–5 adequate samples are obtained [31]. Even in stage I disease, the yield may be 70–80% [40]. These bronchoscopic biopsy procedures may be combined with BAL and studies of lymphocyte subpopulations. Three independent groups have shown very similar values for the sensitivity and specificity of BAL CD4+:CD8+ ratios [41–43]. A ratio of >3.5 or 4.0 has a sensitivity of 52–59% and a specificity of 94–96%. These three studies reached a similar conclusion: in patients with a clinical picture typical of sarcoidosis, an elevated CD4+:CD8+ ratio in BAL may confirm the diagnosis and obviate the need for confirmation by additional biopsy [44]. It is important to note that in the study of WINTERBAUER *et al.* [42], TBB had a specificity of 89% for the distinction between sarcoidosis and other forms of diffuse lung disease, and was, therefore, no better than the CD4+:CD8+ ratio in this regard.

Differential diagnosis of various granulomatous disorders

Noninfectious environmental agents. It is assumed that sarcoidosis is caused by inhalation of airborne agents in susceptible persons triggering the inflammatory reaction.

The noninfectious environmental agents can elicit a granulomatous response with many features that are similar to sarcoidosis, *e.g.* beryllium (Be), aluminium dusts and zirconium (Zr) [45–47]. In particular, Berylliosis is an environmental chronic inflammatory disorder of the lung caused by inhalation of insoluble Be dusts and characterised by the accumulation of CD4+ T-cells and macrophages in the lower respiratory tract. In response to Be inhalation, noncaseating granuloma form and, eventually, fibrosis occurs. The immunopathogenic process is maintained by Be-specific lung CD4+ T-lymphocytes. Consistent with the disease immunopathology, these Be-specific T-cells have a T-helper type-1 phenotype producing interleukin-2 and interferon-gamma, the macrophage-activating cytokine driving the granulomatous reaction [48, 49]. However, De Vuyst *et al.* [45] in a case report suggests that also aluminium may cause granulomatous lung disease accompanied by a helper T-lymphocyte alveolitis, similar to that of berylliosis. Furthermore, it has been suggested in some subjects a special susceptibility responsible for the development of a granulomatous disease after Zr exposure occurs. In any case, Zr must be considered as another metal, besides Be, which can cause pulmonary and generalised granulomatosis [50]. Finally, Drent *et al.* [51] observed the association of sarcoid-like granulomatous reaction and occupational history of glass fibre exposure and that, in susceptible people, exposure to other man-made mineral fibres, such as rock wool, might be related to a chronic granulomatous disease similar to chronic Be disease. Histologically similar granulomatous disease has been associated with a variety of other metals including barium, cobalt, copper, gold, rare earth metals (lanthanides) and titanium [52].

Therefore, the accurate diagnosis of sarcoidosis depends on a stringent inquiry of potential exposures to both organic and inorganic antigens. Finally, the host itself has been considered a potential source of autoreactive granuloma-inducing antigens, although the possibility that sarcoidosis is an autoimmune disorder is not supported by any evidence. As granulomatous inflammation is the histological hallmark of sarcoidosis, investigators continue to improve and apply modern diagnostic tools in search for infectious agents, such as mycobacteria, which are known to induce a host granulomatous response [53]. To date, there is no evidence that sarcoidosis is caused by an infectious agent.

Infectious environmental agents. Infections are the commonest causes of disseminated granulomatous disease. For instance, cat scratch disease is due to *Bartonella henselae* and Whipple's disease due to *Tropheryma whippeli*. Infective causes are suspected but not yet established for sarcoidosis, Crohn's disease, primary biliary cirrhosis, Kikuchi's disease, Langerhans' granulomatosis, and chronic granulomatous disease of childhood. Granulomatous fungal infections mimic sarcoidosis worldwide. It is important to recognise or exclude fungi localised to one system or disseminated; in particular, granulomatous fungal meningitis needs to be distinguished from sarcoidosis by all available techniques.

Whipple's Disease is also a chronic multisystemic granulomatous disorder. There may be hepatosplenomegaly and generalised lymphadenopathy, but there is evidence for Whipple's disease also presenting with large pulmonary nodules and endobronchial lesions and for the absence of gastrointestinal features [54]. Furthermore, a case of systemic Whipple's disease with pericardial and pleural effusions and severe pulmonary hypertension has been described, where 3 months of antibiotic treatment produced a complete resolution, not only of the symptoms known to be associated with Whipple's disease (diarrhoea, arthralgia, pericardial and pleural effusions), but also of pulmonary hypertension [55]. Biopsy of lymph node, liver, or small intestine reveals foci of periodic acid-schiff (PAS) staining foamy macrophages in all sites. The PAS-positive material

within these histiocytes corresponds with lysosomes containing bacilliform bodies. Electron microscopy reveals rod shaped bacilli, termed Whipple bacilli or *T. whippelii* or Whipple-associated bacterial organisms [56]. The resulting PCR product from the bacterial 16S ribosomal DNA showed that Whipple bacilli were most likely to belong to the family of gram-positive bacteria of the rhodococcus, streptomyces and arthrobacter genera, and more weakly related to mycobacteria. PCR primers for *T. whippeli* now provide a helpful diagnostic technique.

Other causes of granulomatous inflammation. The family of vasculitic granulomatoses comprise Wegener's granulomatosis, necrotising sarcoidal granulomatosis, Churg-Strauss syndrome, lymphomatoid granulomatosis, polyarteritis nodosa, bronchocentric granulomatosis, giant cell arteritis, and systemic lupus erythematosus. They may occasionally be confused with sarcoidosis and hypersensitivity pneumonitis (extrinsic allergic alveolitis), so a careful clinico-pathological synthesis is essential

Finally, the commonest cause of granulomatous inflammation in the gastrointestinal tract is Crohn's disease. This reaction seems to centre on the blood vessels of the intestinal wall causing multifocal gastrointestinal infarction. There may be associated lung changes, including pulmonary vasculitis, granulomatous interstitial lymphocytic infiltration, alveolitis, and interstitial fibrosis. Alveolar macrophages may show an increased spontaneous superoxide anion production. An increase in CD4+ cells is found in BAL fluid and even in sputum. Serum antibody increases include anti-reticulin antibody, anti-saccharomyces cerevisiae antibody (ASCA), and p-anti-neutrophil cytoplasmic antibody (ANCA). There is concordance between ASCA and ANCA. ASCA occurs in up to 60% of patients, particularly with familial Crohn's disease, and ASCA is evident in 20% of first-degree relatives [57, 58].

Summary

Sarcoidosis is a chronic, multisystemic disorder of unknown cause characterised in involved organs by an accumulation of activated CD4+ T-cells and macrophages, noncaseating epithelioid cell granuloma, and tissue injury. The diagnosis is established when clinico-radiological findings are supported by histological evidence of noncaseating epithelioid cell granulomas. Granulomas of known causes and local sarcoid reactions must be excluded.

Keywords: Bronchoalveolar lavage, CD4+ lymphocytes, hilar lymph node enlargement, noncaseating granuloma, sarcoidosis, transbronchial needle aspiration.

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