

REVIEW

Immunological mechanisms in sarcoidosis

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Introduction

Sarcoidosis is a multisystem disorder of unknown aetiology characterized by tissue infiltration by mononuclear phagocytes and lymphocytes with associated granuloma formation. Sarcoidosis was originally described as a chronic granulomatous skin disorder, but the systemic nature of the disease was soon appreciated when a number of patients from the original case series subsequently developed granulomatous inflammation at other sites [1]. Since that time descriptions of its protean clinical manifestations, and the demonstration of granulomas in virtually every organ, have meant that sarcoidosis is seldom omitted from the differential diagnosis of any patient presenting with a multisystem disease. Almost 100 years of clinical observation provided clues to the aetiology of sarcoidosis, but it has been the application of cellular and molecular biological techniques over the last 20 years that has provided most information about disease pathogenesis. Exploring the importance of antigen triggering, genetic predisposition and intercellular signalling has not only enabled us to identify possible future therapeutic targets in sarcoidosis, but has also provided insights into the immunopathogenesis of other related granulomatous diseases.

Sarcoid granulomas have no unique histological features to differentiate them from immune granulomas caused by conditions such as Crohn's disease or hypersensitivity pneumonitis. As a result, in all but the most classical presentations, confusion can arise regarding the certainty of the diagnosis. The working definition of sarcoidosis currently used in clinical practice is that 'the diagnosis is established most securely when compatible clinical and radiological findings are supported by histological evidence of non-caseating epithelioid cell granulomas in one or more organs' [2]. Diseases causing immune granulomas share common immunological pathways, but there are also

important points of difference, making the clinical expression of sarcoidosis unique. In this review we will outline some of the current concepts in the immunopathogenesis of sarcoidosis and how they relate to aetiology, progression and resolution of the disease.

Aetiology

Clinical considerations

One of the earliest clinical observations in sarcoidosis was the recognition that there were geographical differences in prevalence. A review by Bauer *et al.* summarized the results of a number of surveys carried out in 24 different countries and found the prevalence to vary widely, ranging from 0.2 cases per 100 000 in the South American countries of Brazil and Uruguay, to 64 per 100 000 in Sweden [3]. Interpretation of geographical variation in incidence is difficult because results may be coloured by ascertainment bias. For instance, in the examples given above, the low risk of sarcoidosis in Brazil may be explained by lack of access to sophisticated diagnostic techniques and difficulty distinguishing sarcoidosis from tuberculosis that is endemic in the area. There is, however, considerable evidence based on reasonably standardized screening procedures supporting variation between countries. In a review of incidental presentations of sarcoidosis based on mass chest radiological screening up to 10-fold differences in prevalence were noted between countries, with nations furthest from the equator having the highest rates of attack [3].

Evidence of antigenic triggering causing MHC/T cell receptor interaction

It has been a long held theory that the geographical variation in prevalence can be explained by environmental exposure to an infectious agent. Emerging data from immunological studies lend weight to this concept. In one

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study the intradermal inoculation of an autologous bronchoalveolar lavage (BAL) cell preparation induced a cutaneous granulomatous response in patients with sarcoidosis, in a situation analogous to the Kveim reagent. Subsequent analysis has revealed that the activity of this material lies within membrane fragments of alveolar macrophages [4], suggesting that an inhaled 'sarcoid antigen' is presented to the immune system in the lower respiratory tract. In a separate study looking at mRNA transcripts of the T cell receptor (TcR) β -chain, du Bois *et al.* found that in T cells from BAL, there is down-regulation of cell surface TcR numbers and increase in TcR mRNA transcripts, consistent with antigen-specific T cell activation [5]. Importantly, this finding was not reproduced in peripheral blood lymphocytes, suggesting that alveolar T cells are activated locally and accumulate in response to the antigenic stimulus presented by alveolar macrophages.

A number of studies have demonstrated that there is an over-expression of particular variable regions of the α - and β -chains of the TcR [6] (Table 1). This skewing of the TcR repertoire is most pronounced in the lung, but also present in peripheral blood. Jones *et al.* demonstrated bias in variable gene usage on both the α - and β -chains of the TcR and also showed that the V-gene bias was specific for each patient, failing to demonstrate over-representation of the same gene families in different patients [7]. As well as T cell oligoclonality, consistent with the 'persisting antigen' hypothesis, there is a background polyclonal expansion of T cells using all the known V region genes. This conclusion was confirmed by studies of the T cell repertoire by Bellocq *et al.*, who found that the T cell clones expressing single V β genes largely used heterogeneous junctional sequences, but there was also evidence of some clonal expansion in that certain families had rearrangements of a single predominant length [8]. This is a pattern of T cell response seen with 'conventional', rather than 'super' antigens [9]. Taken together, these findings point to a local oligoclonal response to a 'conventional' antigen that is not cleared from the lower respiratory tract, with an associated polyclonal expansion of T cells.

Inhalation of beryllium can produce a granulomatous disease phenotypically indistinguishable from sarcoidosis and has proved to be a useful model for the study of granulomatous inflammation. Analysis of MHC class

Table 1 T cell receptor (TcR) over-expression reported at disease sites in sarcoidosis (see [6–8,13,43,51])

V α 2.3
V β 2,3,5,6,8,14,15,16,18,19
V γ 9
V δ 1

HLA-DP gene usage in berylliosis found that 97% of patients had at least one DPB1 allele with the glutamate amino acid at position 69, compared with 30% of the general population [10]. In addition, there is selection of multiple T cell clones with restricted TcR V gene usage, consistent with a T cell response to a conventional antigen [11,12]. It is likely that in genetically predisposed individuals beryllium presentation, possibly in association with an oligopeptide, by MHC class II DP-B molecules with glutamate at position 69, results in an antigen-specific T cell response. Although there is a greater variety of MHC class II molecules seen in sarcoidosis, Grunewald *et al.* showed that in HLA DR3(17), DQ2⁺ patients with sarcoidosis there is over-expression of T cells bearing the V α 2.3 α -chain of the TcR [13,14]. The association between MHC class II expression and expansion of particular T cell clones is not as tight in sarcoidosis compared with berylliosis, but they do appear to share similar immunopathogenic pathways.

Transmissible antigens

The similar pathological appearance and geographical distribution of sarcoidosis and mycobacterial infection has led many to suggest that the two diseases are associated. Studies employing bacterial culture, histochemistry and polymerase chain reaction (PCR) techniques have demonstrated the presence of acid-fast bacilli RNA, DNA and even whole bacteria in patients with sarcoidosis [15–17]. The majority of studies have, however, failed to find mycobacterial DNA in cells or tissue from disease sites. Other suggested potential trigger factors have included Epstein–Barr, adeno- and herpes virus [18,19]. Table 2 lists some of the many infectious agents that have been proposed as the cause

Table 2 Transmissible agents proposed as triggers for sarcoidosis

Mycobacteria

- Mycobacterium tuberculosis*
- Atypical mycobacteria
- Cell wall-deficient mycobacteria
- Phage transformed mycobacteria

Other bacteria

- Mycoplasma pneumoniae*
- Nocardia* spp.
- Corynebacterium* spp.
- Propionibacter* spp.

Viruses

- Epstein–Barr
- Adenovirus
- Herpes simplex

of sarcoidosis, although none of these has been linked unequivocally with the disease.

The failure to identify a single antigen responsible for sarcoidosis has led many to believe that in genetically predisposed individuals, a variety of environmental antigens can trigger the disease process. There is, however, a caucus of opinion that implicates mycobacterial infection and points to the considerable evidence of mycobacterial exposure in patients with sarcoidosis to support this [15–17]. To accept this suggested association between mycobacterial infection and sarcoidosis, a number of important clinical observations would have to be ignored. First, despite the routine use of corticosteroids in sarcoidosis, the development of a disease more classical of mycobacterial infection is not common. In addition, uveitis, salivary gland and cardiac involvement are common features of sarcoidosis, not seen in mycobacterial infection. Finally, despite the dramatic reduction in mycobacterial infection over the last 100 years, there has not been a corresponding reduction in the prevalence of sarcoidosis.

Kveim reagent

Following the observation that intradermal inoculation with pretreated sarcoid tissue resulted in a local granulomatous reaction in patients with suspected sarcoidosis [20], the Kveim reagent was developed as a diagnostic aid in sarcoidosis [21]. Despite extensive investigation, isolation of the 'sarcoid antigen' in the Kveim reagent has proved elusive [22]. When it was established that T cells failed to proliferate when exposed to Kveim reagent and monoclonal antibodies raised against the reagent recognized macrophages from sarcoid (and also non-sarcoid) immune granulomas [23], it was assumed that the antigen would be identified on the surface of the latter cell type. Work done with granuloma-inducing autologous BAL cells supported this concept when the activity of the preparation was found to lie within membrane fragments of alveolar macrophages [4].

Granulomas

Immune effector cells

Sarcoidosis is characterized by the accumulation, proliferation and spontaneous activity of macrophages and T cells at sites of inflammation. The evidence to date suggests that both cell populations are activated via normal physiological pathways and a primary defect in cell-mediated immunity does not exist. Much of our current knowledge regarding the function of these cells in sarcoidosis has been gained from the direct immunohistochemical analysis of granulomatous tissue and the analysis of BAL cells and fluid, that

is a reflection of the cellular milieu in which granulomas develop.

Alveolar macrophages Alveolar macrophages play a central role in sarcoidosis, through the secretion of pro- (and possibly anti-) inflammatory cytokines and by stimulating the lymphocyte population. In sarcoidosis there can be a five-fold increase in BAL cellularity compared with normal controls [24]. Although a BAL lymphocytosis is characteristic, there is also an increase in the absolute number of alveolar macrophages, which is probably due to the recruitment of peripheral blood monocytes but may also be in part due to local proliferation. The excess of immunophenotypically immature alveolar macrophages suggests that these cells have recently migrated from the periphery, but newly divided cells also bear an immature phenotype [25]. Consistent with this, there is a small increase in percentage of alveolar macrophages undergoing proliferation in sarcoidosis, contributing to the expansion of this cell population [24]. Alveolar macrophages are differentiated cells, but will proliferate in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF), a cytokine known to be present in increased concentration in sarcoidosis [26], which may explain this finding. Mice that over-express GM-CSF also show excess local proliferation of peritoneal macrophages [27]. Although expansion of the alveolar macrophage population is likely to represent a combination of peripheral recruitment and local proliferation, the evidence is compelling that the former mechanism is of most importance.

The high levels of tumour necrosis factor- α (TNF- α) [28] and IL-1 [29] spontaneously released by cultured alveolar macrophages indicates that these cells are activated *in vivo*. Studies looking at the kinetics of TNF- α gene expression support these findings, demonstrating that TNF- α mRNA is maximally expressed immediately after retrieval of cells from the lung [28]. Importantly, in the same study there was no corresponding spontaneous release of TNF- α from cultured peripheral blood monocytes. *In situ* hybridization techniques looking at cytokine mRNA transcript levels in cells from sarcoid lymph nodes [30] showed that macrophage cytokine up-regulation is 'compartmentalized' to sites of inflammatory activity. Alveolar macrophages from lung but not blood express increased IL-12 mRNA and increased concentrations of the p40 protein product of the IL-12 gene are found in BAL fluid. Importantly, the Th2 cytokines IL-10 and IL-4 are not up-regulated. IL-12 is known to up-regulate interferon- γ (IFN- γ), arguably the most pivotal cytokine in granuloma formation.

T cells Sarcoidosis is also characterized by the accumulation, proliferation and activation of T cells at sites of

disease activity. A lymphocyte percentage approximately three times normal is often seen in a sarcoid BAL, but is not correlated with progressive pulmonary disease [31]. The predominance of CD4⁺ T-cells is a feature of sarcoidosis and can be of diagnostic value. In one published series a CD4/CD8 ratio > 3.5 was 94% specific and 52% sensitive for the diagnosis of sarcoidosis [32]. The T cells, like the macrophages, are activated, spontaneously secreting a number of cytokines, including IL-2 [33], IFN- γ [34] and lymphotoxin *in vitro*, as well as expressing increased numbers of cell surface activation markers such as HLA-DR and very late activation antigen-1 (VLA-1) [35]. Restriction of variable region gene usage on peripheral blood TcRs indicates that these cells are responding to an antigenic stimulus [6,7], but spontaneous secretion of cytokines is only seen at sites of inflammation [36], suggesting that there is also 'compartmentalization' of the T cell response.

In a situation analogous to alveolar macrophages, the mechanisms governing the accumulation and activation of T cells at sites of inflammation is due to a combination of recruitment and local proliferation. It would appear that a number of different cell types participate in the intra-alveolar expansion of CD4⁺ T cells. Alveolar macrophages spontaneously secrete IL-8 [37], IL-12, IL-15 [38] and 'regulated on activation, normal T-cell expressed and secreted' (RANTES) [39] which are chemoattractive for lymphocytes. In addition, up-regulation of endothelial cell surface adhesion molecules (Table 3), by macrophage cytokines TNF- α , IFN- γ , IL-1 and IL-15, is seen in sarcoidosis and is likely to be an important factor in the tissue-specific accumulation of T cells [40]. Airway epithelial cells are also involved in the recruitment of lymphocytes, secreting IL-16, which is a cytokine specific for T cell recruitment [41]. Balancing this evidence for peripheral recruitment are studies demonstrating local proliferation of CD4⁺ T cells. Using immunohistochemical analysis, Chilosi *et al.* found that an increased proportion of BAL T cells express the Ki67 antigen, indicating active nuclear replication [42]. In addition, high levels of the lymphocyte growth factor IL-2 are secreted in active pulmonary sarcoidosis [36].

CD4⁺ T cells in the BAL of patients with sarcoidosis

usually secrete IL-2 and IFN- γ [33,34], cytokines traditionally associated with the Th1 cells, although Th2-type cytokines can be detected in some patients [43]. There is evidence that the ratio of Th1/Th2 T cells plays an important role in the clinical pattern of sarcoidosis, with a Th1 profile favouring granuloma formation and a switch to Th2-type T cells leading to resolution with or without fibrosis [44]. The ratio of Th1/Th2 T cells is the result of local cytokine concentrations, especially IFN- γ and IL-12, which inhibit Th2 proliferation. Culture of Th1 T cell clones in the absence of IFN- γ results in the expression of Th2-type cytokines, including IL-4 which is a potent chemotactic factor for fibroblasts [45]. As both the Th1 and Th2 T cell subsets are derived from the same Th0 progenitor T cell, it is likely that factors influencing Th0 differentiation determine the outlook of patients with sarcoidosis.

Polymorphonuclear cells (PMN) Increased numbers of PMNs are found in the BAL of patients with sarcoidosis when there is radiological evidence of established fibrosis, with the degree of neutrophil alveolitis correlating with disease severity [46]. Intrapulmonary accumulation of PMNs also requires recognition of endothelial adhesion molecules with their appropriate ligand on the PMN surface [47] (Table 3). Once in the lung, PMNs degrade the collagen matrix and damage lipid membranes through the release of proteases and oxidant species, which are found in raised concentrations in BAL fluid [48]

Granuloma formation

Up to this stage we have focused on the contribution of individual cell types to the immunopathogenesis of sarcoidosis. In this section we will outline the important relationships that exist between these immunocompetent cells and how they result in granuloma formation. Sarcoid granulomas are tightly formed aggregations of activated lymphocytes and cells derived from the mononuclear phagocyte cell line, i.e. tissue macrophages, epithelioid and multinucleate giant cells organized into a specific spatial arrangement. It appears that sarcoidosis shares common immunological pathways with other granulomatous diseases.

Table 3 Up-regulated endothelial cell surface adhesion molecules in sarcoidosis

Endothelial cell adhesion molecule	Cluster	Ligand	Adhesion of:
LFA-3 (lymphocyte function-associated antigen)	CD58	CD2	T cell
ICAM-1 (intercellular adhesion molecule)	CD54	CD11a/CD18	T cell and PMN
CD44 variants	CD44	hyaluronate	T cell
P-selectin (previously GMP-140)	CD62	Sialyl Lewis X	T cell and PMN
E-selectin (previously ELAM-1)	Nil	CD15	PMN

Table 4 Chemokine superfamily in sarcoidosis

C-C	Monocyte chemotactic protein-1 (MCP-1)
	'Regulated on Activation, Normal T-cell Expressed and Secreted' (RANTES)
	Eotaxin
	Macrophage inflammatory protein-1 α (MIP-1 α)
C-X-C	IL-8
	Interferon-gamma inducible protein (IP-10)
	Macrophage inflammatory protein-2 (MIP-2)
	Platelet factor-4 (PF-4)
C	Lymphotactin

In sarcoidosis, as in other granulomatous diseases where the causative antigen is known (e.g. *Mycobacterium tuberculosis* or avian proteins in 'bird fancier's lung'), tissue macrophages process the foreign protein and incite a specific T cell response. The subsequent spontaneous release of TNF- α and IL-1 [28,29] by activated macrophages is pivotal to the formation of immune granulomas. IL-1 acts as an accessory T cell growth factor and exists in two major forms: the secretory form, IL-1 β and membrane-bound IL-1 α . TNF- α is the most ubiquitous member of the TNF-ligand superfamily, inducing tissue fibrosis through IL-6-mediated fibroblast growth and promoting the release of other proinflammatory cytokines such as IL-1 β , GM-CSF and platelet-activating factor (PAF).

Animal models exploring the kinetics of immune granulomas induced by *Schistosoma mansoni* eggs showed evidence of sequential monokine production, with IL-1 produced in greatest amounts in the first 4 days and TNF- α

from day 8 to 16 [49]. The findings suggest that IL-1 is important in the early recruitment stages of granuloma formation and TNF- α may take part in later maintenance or effector functions. This theory is supported by more recent work by James *et al.*, who showed by *in situ* hybridization techniques that macrophages and Th1 lymphocytes centrally located in granulomas produced IL-1 and IFN- γ , respectively, while macrophages expressing TNF- α and cell surface markers of more recent activation were located more peripherally [50].

Other important cytokines secreted by locally active macrophages are IL-2, IL-6, IL-12 and IL-15. Of these, IL-12 [51] and IL-15 [38] are of particular importance because they promote Th1 proliferation. IL-12 specifically up-regulates IFN- γ secretion from Th1 lymphocytes, which in turn enhances accessory macrophage function. IL-6 is a ubiquitous cytokine found in many inflammatory conditions including sarcoidosis. IL-6 up-regulates the expression of acute-phase reactants, increases cortisol release and induces fever.

Macrophages also secrete a number of chemotactic chemokines that play important accessory roles in regulating immune traffic. Chemokines expressed in sarcoidosis (Table 4) are produced from many different cell types, including epithelial cells, which are not traditionally associated with immunocompetence. Chemokines form a superfamily of cell products divided into three subgroups based on variations in cysteine residues [52].

T cells in sarcoidosis secrete IL-2 [33], IFN- γ [34] and GM-CSF, which enhance the antigen-presenting capabilities of macrophages [53]. Of these cytokines it would appear that IFN- γ is most crucial in granuloma formation. This is highlighted by the absence of granulomas following exposure to thermophilic bacteria in IFN- γ gene knockout mice [54]. In separate animal studies of IFN- γ knockout mice, using mycobacterial antigens, a Th1-mediated

Table 5 Summary of key events in the immunopathogenesis of sarcoidosis

- Uptake and processing of a trigger antigen by antigen-presenting cells in the lower respiratory tract.
- Recognition of MHC class II bound conventional antigen on macrophages by T cells and subsequent normal physiological activation of the CD4⁺ lymphocyte subset via the TcR with appropriate down-regulation of TcR number.
- Release of macrophage-derived cytokines—TNF- α ; IL-1, IL-6, IL-12, IL-15; chemokines IL-8, 'regulated on activation, normal T-cell expressed and secreted' (RANTES), monocyte chemotactic protein (MCP-1), macrophage inflammatory protein (MIP-1) and the colony-stimulating factor GM-CSF.
- Oligoclonal proliferation of CD4⁺ T cells, with expression of GM-CSF and a Th1 cytokine profile, IL-2 and IFN- γ .
- Macrophage/lymphocyte interaction via intercellular signalling and cell contact leading to proliferation, activation and spontaneous cytokine release by both cell lines at sites of inflammation.
- Proliferation of the Th1 T cell subset and reciprocal suppression of the Th2 subset by IFN- γ , IL-12 and IL-15, leads to granulomatous inflammation.
- Fibrosis associated with shift to Th2 T cell profile, up-regulation of macrophage-derived fibrogenic cytokines (TGF- β , PDGF and IGF-1) and increased production of neutrophil protease products.
- Disturbance of normal programmed cell death (apoptosis) by dysregulation of the TNF-L/TNF-R superfamily, abnormal expression of oncogene products and change in Th1/Th2 ratio.

granulomatous response is converted to an IL-4 and IL-5 (Th2) eosinophilic inflammatory response [55]. It is likely that as well as being a promoter for accessory macrophage function, IFN- γ plays an additional role in suppressing the Th2 lymphocyte response.

Granuloma control

The key events in saroid granuloma initiation and control are outlined in Table 5.

Cell apoptosis

Programmed cell death (apoptosis) is an important component of normal tissue turnover and occurs by two separate mechanisms. The first mechanism is 'passive cell death' and occurs at the conclusion of the immune response when cytokine stimulation is withdrawn. The second mechanism is 'active' and involves the expression of factors that induce cell death. Dysregulation of the TNF-receptor superfamily, expression of oncogene products and the ratio of T helper cell populations, are important factors that drive apoptosis in sarcoidosis. The balance of proinflammatory cytokines promoting granuloma growth and resolution of tissue inflammation via cell apoptosis in conjunction with immunosuppressive cytokines, may be the reason why spontaneous remission and exacerbation occur in sarcoidosis.

Dysregulation of TNF-receptor/TNF-ligand superfamily The TNF-receptor (TNF-R) and TNF-ligand (TNF-L) superfamilies are involved in T cell apoptosis, which occurs in a number of fibrotic lung diseases, including chronic pulmonary sarcoidosis. In recently published data from sarcoid patients, Agostini *et al.* described up-regulation of CD95 and CD70 and down-regulation of CD27, which are all cell surface molecules belonging to the TNF-R superfamily [56]. This observation along with others noting similar dysregulation of the TNF-R superfamily in other forms of fibrotic lung diseases raises the possibility that the TNF-R profile expressed in sarcoidosis influences the development of chronic fibrotic disease. Important members of the TNF-L superfamily are TNF- α , produced by macrophages and lymphotoxin- β (LT- β) produced by Th1 cells. Investigating the role of TNF- α in cell apoptosis has yielded conflicting data. There is evidence that over-expression of TNF- α (and IFN- γ from Th1 lymphocytes) sets the stage for the persistence of inflammation and the development of fibrotic lung disease [57], but other studies have shown that TNF- α is increased in the resolution phase of granulomatous infection. Almost certainly the role that TNF- α plays in cell apoptosis depends on accessory factors such as the genetic profile of the patient. Fas is another

member of the apoptosis-regulatory TNF-L superfamily and is up-regulated in patients with active pulmonary sarcoidosis [56]. Binding of Fas to CD95/Fas-R results in the transduction of apoptotic cell signals, increasing clearance and limiting T cell proliferation at sites of activation [58].

Oncogene products Oncogene products also regulate apoptosis in sarcoidosis. The Bcl-2 oncogene was originally identified as the breakpoint in the translocation between chromosome 14 and 18 occurring in follicular lymphoma. Bcl-2 and Bcl-x_L are the protein products of the Bcl-2 oncogene and protect lymphoid cells from programmed cell death when IL-2 is withdrawn [59]. Bcl-2 and Bcl-x_L bind to sites on the mitochondrial membrane, where they regulate the cytochrome c system and inhibit calcium release, which is the final step in the apoptotic process [60]. Bcl-2 has been shown to be increased within T cells located in sarcoid granulomas [61]. One mechanism leading to Bcl-2 oncogene over-expression appears to be the release of an as yet undefined chemokine by fibroblasts that maintain high Bcl-x_L [62].

Th1/Th2 ratio The ratio of Th1/Th2 T cells is an important factor in regulating apoptosis and may explain why this ratio can be used as a predictor of disease prognosis. It would appear that Th cells influence apoptosis through cytokine release. For instance, IL-2 and IL-15, two Th1 cytokines, inhibit macrophage apoptosis, while the Th2 cytokines IL-4 and IL-10 enhance macrophage cell death [63]. In sarcoidosis IL-15 is also produced by macrophages [38] and prevents T cell apoptosis by up-regulating Bcl-2 and Bcl-x_L [64]. As well as preventing apoptosis, IL-15 has other important effects on macrophages. IL-15 and its receptor share homology with the IL-2 receptor/ligand family and have similar biological actions, in expanding the T cell and macrophage populations through the release of GM-CSF and TNF- α . In some circumstances the role IL-15 plays in apoptosis seems to serve a protective function, but in sarcoidosis there is evidence that it contributes to disease persistence. Higher levels of IL-15 mRNA are found in epidermal lymphocytes of patients with localized tuberculoid leprosy, compared with the poorly controlled lepromatous form of the disease [65]. It is postulated that in leprosy IL-15 contributes to better disease control by preventing T cell death and improving intercellular co-operation, while in sarcoidosis it is detrimental, causing a persistent T cell response.

Fibrosis

The clinical presentation of sarcoidosis has important implications for prognosis. It has long been speculated that differing immunological pathways are responsible for

the various disease phenotypes and dichotomy in prognosis. For instance, patients presenting with the clinical picture of fever, bilateral lymphadenopathy, uveitis and erythema nodosum originally described by Löfgren in 1946 [66] have an excellent outlook, with 80% having clinical and radiological resolution at 2 years [67]. Unfortunately, this favourable prognosis is not shared by patients who present with fibrotic chest radiological changes, neurological or granulomatous skin involvement, the majority of whom go on to develop chronic disease [68]. The spectrum of disease caused by sarcoidosis is best documented in the lung, where pathological changes can range from predominantly granulomatous inflammation with minimal chronic changes, through to extensive fibrosis with few granulomas [69]. Histologically it would appear that granulomas act as a focus for fibrosis that may then become more diffuse to eventually cause organ impairment [70]. It is likely that acute granulomatous and chronic fibrotic pulmonary sarcoidosis represent separate immunopathogenic processes. For instance, the neutrophils, eosinophils and mast cells seen in the BAL of patients with fibrotic disease are not seen in the acute presentation and are recruited by T cells releasing Th2-type cytokines.

Immunomodulatory cytokines

An imbalance between proinflammatory and immunomodulatory cytokines may play a role in the expression of the acute vs. chronic phenotype, but seems to provide only part of the explanation. Unfortunately, to date investigation into this crucial area of disease pathogenesis has yielded conflicting results and led to confusion. The role of transforming growth factor-beta (TGF- β), a cytokine with documented anti-inflammatory activity [71], highlights the difficulties in defining the immunological differences that exist between acute remitting and chronic persisting sarcoidosis. On the one hand higher levels of TGF- β have been found in the BAL of patients who subsequently have a spontaneous remission [72]. Furthermore, a strong negative correlation has been found between IL-2 and TGF- β levels, suggesting that the positive prognostic value of TGF- β may lie in its ability to suppress the Th1 lymphocyte subset. Contrary to these findings are the results of an immunohistochemical analysis by Khalil *et al.*, who found evidence of increased TGF- β expression causing extracellular matrix deposition in a number of fibrotic lung diseases [73]. Animal models have also suggested that epithelial cells can contribute to fibroblast growth via release of TGF- β [74]. Another cytokine with recognized immunomodulatory activity is IL-10, which is produced by activated Th2 cells and some B cell subsets. IL-10 has been shown to inhibit the release of IL-2 and IFN- γ by Th1 cells and regulate the accessory function of antigen-presenting cells [40]. Two

investigators have been unable to find evidence of IL-10 expression in the BAL of patients with pulmonary sarcoidosis [51,72].

Fibroblasts

Macrophage and T cell products including TNF- α , IL-1, IFN- γ , IL-6, platelet-derived growth factor (PDGF), TGF- β , insulin-like growth factor (IGF-1), IL-3 and GM-CSF [75], products of the coagulation cascade and collagen matrix peptides themselves contribute to fibrosis. Macrophages promote fibroblast recruitment principally through TNF- α and other members of the TNF-L superfamily [56]. Alteration of the expression of adhesion molecules on the surface of epithelial cells also plays a role in fibroblast chemotaxis. Once attracted to sites of tissue inflammation, fibroblasts proliferate in response to a number of macrophage products, including IL-1, IL-6, PDGF, TGF- β , fibronectin, IGF-1 and IL-3 [75]. Many of these cell products cause a general proliferative response, leading to epithelial and mesenchymal cell growth that characterizes chronic sarcoid inflammation.

In normal tissues inflammation is generally short lived and involves 'cytokine binding proteins'. There is evidence that in sarcoidosis increased fibroblast growth relates to abnormal expression of these proteins [76]. Release by macrophages of PDGF, TGF- β and IGF-1, combined with a shift from a Th1 to a Th2 response [56], promotes deposition of fibronectin and other collagen matrix products from recruited fibroblasts. Support for the importance of a change in Th1/Th2 profile contributing to fibrosis is based on evidence that IL-4, a Th2-type cytokine, stimulates collagen production in fibrosing alveolitis [77], while IFN- γ inhibits fibrogenesis.

Chemokines also play a role in the induction of chronic tissue injury. Recent studies have correlated the BAL levels of monocyte chemotactic protein (MCP-1), macrophage inflammatory protein (MIP-1), IL-8 and RANTES, with risk of disease progression [78,79]. Further work is required to define further how these proinflammatory cell products induce tissue injury.

Use of immunological markers in clinical practice

Although consensus guidelines exist outlining which patients with sarcoidosis should receive corticosteroids, there are no clinical, radiological or physiological features that predict the group that will need treatment. As a result the clinical goal has been to identify genetic or immunological parameters at presentation that will identify patients likely to develop progressive disease.

BAL cytokines as markers of disease prognosis

BAL provides relatively non-invasive access to the lower respiratory tract and has the potential to be used in serial studies. As a result BAL studies, along with peripheral blood analysis, are the basis for most published studies examining immunological measurements as potential prognostic markers in sarcoidosis.

Attempts to correlate TNF- α levels with disease outcome have yielded conflicting results. In a detailed study, Ziegenhagen *et al.* looked at BAL levels of TNF- α along with a number of serum markers and found that patients with raised BAL TNF- α had a 44% chance of developing progressive pulmonary disease compared with 8% in the group with normal levels [57]. Based on known actions as a T cell growth factor, IL-2 has also been investigated as a possible predictor of the development of chronic disease. Correlation of BAL IL-2 level with clinical outcome has proven difficult. In one study Muller-Quernheim *et al.* divided a cohort of 89 patients into two groups, those with and those without a BAL lymphocytosis and then subdivided these two groups based on level of IL-2. Both subgroups with excess IL-2 secretion had a greater risk of developing progressive pulmonary sarcoidosis [31]. Patients with elevated IL-2 and a lymphocytic BAL were at the greatest risk of progression. The conclusion drawn from these results was that patients without a lymphocytosis but with an elevated IL-2 level were at risk of increasing their alveolitis by recruiting more lymphocytes and subsequently deteriorating. It should, however, be pointed out that having a lymphocytosis is not a prerequisite for progressive pulmonary sarcoidosis and the two subgroups described may not represent a continuum of the same process, but rather two separate pathways, resulting in a similar physiological deficit. Unfortunately the difficulties in interpreting BAL cytokine levels means that currently the application of this diagnostic tool is limited to clinical research.

Serum markers of disease prognosis

In a clinical setting serial BAL is often not practical and as a result a number of serum markers have been investigated as possible surrogate indices of disease activity or prognosis. Molecules secreted into the serum by activated sarcoid inflammatory cells are logical candidates for investigation. Angiotensin converting enzyme (ACE) is secreted by epithelioid cells and when raised has been recognized for over 20 years as being of some value in the diagnosis of sarcoidosis [80]. An elevated serum ACE has a diagnostic sensitivity of 57% and specificity of 91% [81]. Serial measurements of serum ACE may be used to track progress but this tool is only of use when the concentration

is high initially. Variations in ACE concentrations may be partly explained by the recent recognition that polymorphisms in the ACE gene affect serum levels [82].

Another possible target is neopterin, a small protein released by activated macrophages that can be elevated in sarcoidosis. From investigations to date it would appear that it is a marker of overall granuloma burden, rather than a reliable predictor of disease progression [57].

Soluble extramembranous portions of cytokine receptors, cleaved from the cell surface, can be found in the serum. Fragments of the 55-kDa TNF- α -receptor (TNF-RI) and 75-kDa TNF-RII have been identified in serum. Nakayama *et al.* found that plasma levels of soluble TNF-RI (sTNF-RI) and sTNF-RII were significantly higher in patients with active sarcoidosis and changes in sTNF-RII levels could be correlated with clinical course and radiological changes [83]. Similarly, soluble IL-2-receptor (sIL-2R) is found in the BAL fluid and serum of patients with sarcoidosis. The relative contribution of cells from the macrophage and lymphocyte cell lines to this observation is uncertain, as both cell types express this receptor. Muller-Quernheim *et al.* felt that the lack of correlation between BAL TNF- α and sIL-2R level favoured the latter as being a marker of Th1 lymphocyte activity [28]. More recent work from the same group found sIL-2R was of significant prognostic value [57]. In a cohort of untreated patients with pulmonary sarcoidosis, 41% of patients with an elevated serum sIL-2R level had subsequent deterioration in pulmonary function testing, compared with none of the patients with a normal sIL-2R level.

Cell surface markers as an index of disease activity

Patients with higher levels of cell surface activation markers might be expected to be more likely to have aggressive disease. Suzuki *et al.* found that an elevated level of the activation marker HLA-DR on BAL T cells was associated with a poor clinical outlook [84]. Similarly the dual expression of two forms of mononuclear phagocyte markers, RFD1 and RFD7, has been found on the cell surface of macrophages in chronic pulmonary sarcoidosis, but not on normal macrophages [85]. It has been shown that cells co-expressing RFD1 and RFD7 down-regulate the mixed lymphocyte reaction, suggesting that this abnormal subset of cells contributes to the prevention of disease resolution by interfering with lymphocyte immune response modulation.

Lymphocyte subset analysis as a marker of prognosis

Once it was determined that total lymphocyte percentage was not a reliable indicator of disease outcome, attention turned to lymphocyte subset analysis. Conflicting data have

been published regarding the value of a high CD4/CD8 ratio at presentation. A ratio > 3.5 has been associated with a positive response to therapy [86] and a high likelihood of spontaneous remission [87]. Balancing these data are two studies that found that a high CD4/CD8 ratio predicted progressive disease [88] and a fall in the CD4/CD8 ratio precedes clinical and radiological improvement in pulmonary sarcoidosis [89].

Despite a vast amount of research, there remains no BAL or serum marker that has been validated as a clinically useful predictor of disease outlook.

Future therapy based on immunological models

Corticosteroids are first-line therapy for patients with pulmonary, ocular, central nervous system and cardiac sarcoidosis. After over a quarter of a century, controversy still exists regarding the value of second-line immunosuppressive agents and which patients with pulmonary sarcoidosis should receive corticosteroids. It is beyond the scope of this article to review the debate concerning the efficacy of second-line agents and use of corticosteroids in pulmonary sarcoidosis, except to say that a number of studies have failed to show sustained improvement in pulmonary function following a fixed duration of therapy [90–92]. In an effort to determine if long-term corticosteroids are of benefit, the Sarcoidosis Subcommittee of the British Thoracic Society designed a trial that eliminated some of the recognized design faults of earlier studies. The conclusion reached at the end of the trial was that patients with pulmonary sarcoidosis who remained asymptomatic, but who failed to show radiological improvement after 6 months of observation, should receive prolonged corticosteroid treatment, as there was a small but significant physiological benefit in this subgroup of patients [93].

It has been long speculated that the use of cytokines or cytokine blocking strategies will form the basis of the next generation of drugs for inflammatory diseases and be the first truly disease-modifying agents. Encouraging results from phase three clinical trials describing the efficacy of 'etanercept', a recombinant soluble TNF receptor/fusion protein combination (TNF-R:Fc) in patients with poorly controlled rheumatoid arthritis [94,95], have encouraged researchers to consider the application of this form of therapy to other inflammatory conditions such as sarcoidosis. While systemically blocking TNF- α has theoretical advantages in sarcoidosis, from earlier discussions it is clear that the role of TNF- α is not fully understood and it may be that this cytokine possesses important disease modulation functions.

An important concept in understanding sarcoidosis is that, unlike other diseases, outlook is not a function of the degree of inflammation, but rather the emergence of

chronic disease: it is generally accepted that those patients who present with the most florid disease have the better outlook. It may therefore be more important to identify and modify the initiation of the immune process, than to attempt to dampen down the existing inflammatory process, some of which is regulatory. Before cytokine therapy in sarcoidosis becomes a reality we need to have a better understanding of the balance between disease progression and disease regression networks.

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