Immunology and pathophysiology

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Sarcoidosis is considered the archetype of immune granulomatous disorders, since immunoregulatory mechanisms that play a part in the development of the sarcoid granuloma may modulate pathogenetic events leading to granuloma development in other granulomatous diseases [1]. Remarkable advances have been made in understanding general immunological and molecular aspects of the mechanisms leading to granuloma formation and the development of fibrosis in sarcoidosis. In particular, the sarcoid granuloma is considered to be the consequence of a crippled immunological response against an unidentifed antigen that has persisted at sites of disease involvement, perhaps because of its low solubility and degradability. Indeed, although hypersensitivity reactions commonly resolve, the balance between events that mediate resolution or perpetuation of inflammatory responses may be altered in patients with chronic sarcoidosis. The persistence of the aetiological agents and/or an imbalance of mechanisms for the removal of inflammatory cells and their by-products ultimately lead to an ongoing inflammatory response. As a result, cytokines with pro-inflammatory destructive biological functions are locally produced; overall, these cytokines set the stage for the development of irreversible remodelling of lung tissue, progression toward pulmonary granuloma formation, and, in some individuals, irreversible development of pulmonary fibrosis.

The aim of the present chapter is to provide an overview of the available knowledge concerning the mechanisms leading to the inflammatory processes that occur at common sites of involvement in sarcoidosis. A detailed description of the cellular interactions that govern the dynamics of granuloma formation is also included.

The infiltration of inflammatory cells in the formation of sarcoid granulomas

Mechanisms leading to T-cell infiltration at sites of disease activity

The infiltration of CD4+ activated T-cells represents the immunological hallmark of sarcoidosis (table 1). Although lung parenchyma normally contains only a few lymphoid elements, lymphocyte populations are strikingly compartmentalised in sarcoidosis air spaces and interstitium. The equivalent of 25×10^6 T-cells can be recovered from the bronchoalveolar lavage fluid (BALF) of patients with active pulmonary sarcoidosis, most probably in response to unknown exogenous or endogenous antigen(s). Sarcoid T-cells are predominantly CD4+, CD45R0 T-cells, coexpressing the $\alpha\beta$ T-cell receptor, mainly producing interferon gamma (IFN)-γ or interleukin (IL)-2 and, thus, belonging

Table 1. – Immunological features that have been observed in patients with sarcoidosis

	Feature
T-cells	Infiltration of CD4+ cells showing a Th1 cytokine profile with a marked increase in CD4:CD8 ratio at sites of disease activity.
	Expansion of T-cells bearing a restricted Vβ and Vα TCR repertoire in involved tissues. This pattern is consistent with TCR oligoclonality.
	Increased expression of members of TNF receptor superfamilies and molecules which regulate cell survival and death of sarcoid T-cells. This suggests that dysregulation of T-cell apoptotic mechanisms may explain the absence of apoptosis in the granuloma and the persistence of inflammation.
	Increased expression of chemokine receptors involved in the recruitment of activated Th1 cells (CXCR3, CCR6, CXCR6).
Macrophages	Tissue accumulation of macrophages expressing increased levels of activation markers, adhesion molecules (CD49a, CD54, CD102) and costimulatory molecules (CD80 and CD86, CD40 and CD72).
	Pulmonary macrophages showing increased antigen-presenting capacity
	Production of macrophage-derived cytokines (IL-1, IL-6, IL-15, TNF-α, GM-CSF) that favour tissue damage and granuloma assembly.
	Release of macrophage-derived chemokines that favour granuloma assembly (CCL5, CCL20, CXCL9, CXCL10, CXCL16)
	Production of macrophage-derived fibrogenetic cytokines (TGF-β and related cytokines, PDGF, IGF-I) involved in progression towards fibrosis.

Th: T-helper cell; V: variable region; TCR: T-cell receptor; TNF: tumour necrosis factor; CXCR: CXC chemokine receptor; CCR: CC chemokine receptor; IL: interleukin; GM-CSF: granulocyte-macrophage colony-stimulating factor; CCL: CC chemokine ligand; CXCL: CXC chemokine ligand; TGF: transforming growth factor; PDGF: platelet-derived growth factor; IGF-I: insulin-like growth factor-I.

to the T-helper (Th) cell type-1 subset. The marked accumulation of CD4+ lymphocytes can be observed in all tissues affected by the sarcoid immuno-inflammatory process [2, 3], where the CD4:CD8 ratio is extremely high (usually >10).

T-cells expanding at sites of disease activity bear a restricted variable region (V)β and Vα T-cell receptor (TCR) repertoire, a pattern which is consistent with TCR oligoclonality. Different mechanisms could account for the limited usage of the TCR repertoire in sarcoidosis. One hypothesis is that the putative antigen(s) drives oligoclonal expansion of T-cells using particular Vα or Vβ regions. In addition, the *in situ* release of cytokines probably plays a role in this phenomenon. After *in vitro* growth in IL-2-supplemented media, T-cells from sarcoid patients show selective expansion of particular Vβ-expressing subsets. Junctional region sequencing indicates that the IL-2-stimulated T-cells are strikingly oligoclonal and derive from T-cell clones already selectively expanded *in vivo*. Thus, it is thought that the antigen(s) that triggers the development of the granulomatous lesions favours the progressive accumulation and activation of a limited number of Th1 clones. When a sufficient amount of tissue is involved by inflammatory Th1 cells, clinical signs of disease activity appear, and are sensed by the individual, for instance, as dyspnoea when the respiratory tract is involved.

T-cell homeostasis is strictly controlled by soluble factors and membrane receptors that activate proliferative and apoptotic processes (fig. 1). It has been known since the 1980s that the *in situ* proliferation of Th1 represents a putative mechanism responsible for CD4 accumulation in sarcoid inflamed tissues (see later). A number of data also suggest that the binding of death-signal-transmitting receptors or modulators of T-cell apoptosis may have undesirable pathogenetic effects in subjects with progressive sarcoidosis. As has recently been shown, sarcoid T-lymphocytes exhibit resistance to apoptosis [4], which might contribute to the accumulation of inflammatory cells in the lungs, persistence of inflammation, and the development and maintenance of granulomas. Interestingly, the percentage of T-regulatory cells (Tr), defined as CD4+,
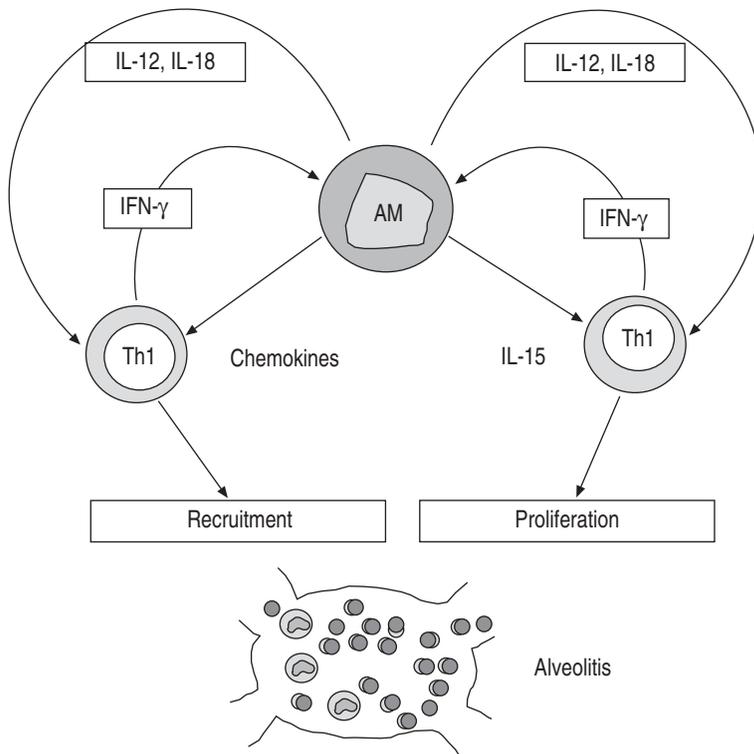
Fig. 1. – Following recognition of the unknown antigen(s) causing sarcoidosis, alveolar macrophages (AMs) are triggered to produce interleukin (IL)-12 and IL-18. Both cytokines enable the activation of macrophages. This favours the release of macrophage-derived chemokines (CXC chemokine 10/interferon (IFN)-γ-inducible protein 10) and cytokines (IL-15) involved in T-helper (Th) cell type-1 recruitment. In turn, newly recruited Th1 cells release IFN-γ which stimulates a further autocrine loop. The net effect is the development of T-cell alveolitis.

CD25+(bright) lymphocytes, is increased in the lungs of patients with active disease who show spontaneous clinical resolution, suggesting that an increased number of Tr in active sarcoidosis may favour the downregulation of cell-mediated immune responses [5].

Tumour necrosis factor (TNF)-α may also play conflicting roles in modulating the activities of sarcoid T-cells at sites of inflammation. There are data suggesting that the chronic overexpression of TNF-α and IFN-γ sets the stage for the persistence and progression of inflammatory events in patients with chronic sarcoidosis; in some circumstances, alteration of the TNF receptor/ligand balance leads to the chronic recruitment of inflammatory cells, which, once in the inflamed tissue, assemble new granulomatous structures. Conversely, there are data from animal models indicating that TNF-α is required for Th1 recruitment, granuloma assembly [6] and antigen clearance, and, thus, for recovery from granulomatous disorders. Both effects are likely to be possible. TNF-α may be essential or have little impact on the control of apoptotic mechanisms within the granulomatous structure depending on a combination of genetic factors, previous environmental exposure and local alterations in immunocompetence.

p21, a member of the Cip/Kip family, regulates cell survival and death, implying that it is a master regulator of cell fate. Specifically, activation of macrophages *in vitro* by IFN-γ or their adhesion to extracellular matrix decorin leads to suppression of apoptosis [7, 8]; this anti-apoptotic effect is mediated by the expression of p21 (Waf1). From recent studies, high levels of p21 have been demonstrated in sarcoid lung, and it has been

hypothesised that the hyperexpression of p21 could explain the absence of apoptosis in the granuloma and the persistence of inflammation [9].

Another system that is involved in the regulation of T-cell inflammatory processes is the Fas/Fas ligand (FasL) system. Fas, which is expressed at high levels by chronically stimulated T-cells, limits the expansion of antigen-reactive T-cells after ligation with a specific ligand belonging to the TNF ligand superfamily (FasL), thus preventing excessive accumulation of antigen-activated lymphocytes [10]. Both of these systems have been evaluated in sarcoidosis [11]. Fas molecules are expressed at higher levels on sarcoid T-lymphocytes than in normal T-cell subpopulations [12]. Furthermore, high concentrations of the soluble form of FasL, which is associated with downregulation of cytotoxicity, are detected in the BALF and serum of patients with sarcoidosis but not in normal subjects [13]. Programmed T-cell death is also inhibited by oncogene products. The oncogene encoding B-cell leukaemia/lymphoma 2 gene product (Bcl-2) (*BCL2*), in particular, belongs to a family of apoptosis-regulatory products that may be either death antagonists or agonists. Overexpression of some family members (*e.g. BCL2* and the gene encoding Bcl-2-like 1 (*BCLX*L)) protects lymphoid cells from programmed cell death when certain growth factors, such as IL-2, are withdrawn, whereas overexpression of others (*e.g.* the genes encoding the apoptotic protein BAD (*BAD*), Bcl-2-associated X-protein (*BAX*) and BH3-interacting domain death agonist (*BID*)) overrides the incoming signals from the cytokine receptor and induces apoptosis. Like Fas, *BCL2* is highly expressed by T-lymphocytes surrounding the granulomatous lesions of patients with sarcoidosis [14]. Applying high-density human gene chip probe arrays for RNA expression profiling, it has been shown that a number of apoptosis-related gene products, including growth factors and the *BCL2* family of genes, are upregulated in patients with sarcoidosis, consistent with a pro-survival profile [15]. Furthermore, patients with progressive disease show upregulation of nuclear factor (NF)-κB and a lack of downregulation of inhibitors of apoptosis [15].

It has also been shown that interaction with fibroblasts can inhibit the apoptosis of cytokine-deprived activated T-cells by a selective effect on *BCLXL*: this phenomenon is probably mediated by a soluble factor released by fibroblasts that upregulates glutathione synthesis and maintains high *BCLXL* levels, helping to maintain the granulomatous process. Therefore, it is possible that overexpression of this inhibitor of apoptosis may prevent the clearance of activated T-cells.

Th1 cell-derived molecules influence inflammatory events in sarcoidosis

Data on the pattern of lymphokine production during sarcoidosis may be summarised in the context of the Th1/Th2 paradigm. The idea is that alterations in Th1/Th2 balance play an important role in determining the organisation of the sarcoid granuloma and the progression of the disease. The cytokines released mainly by T-cells, and those released by macrophages that regulate Th1/Th2 subsets, are discussed in the following section.

Th1 cell-derived cytokines. IFN-γ is the key factor that triggers all inflammatory processes in sarcoidosis (fig. 1). IFN-γ is typically expressed by Th1 cells infiltrating the sarcoid tissue and favours the development of the typical hypersensitivity reaction, and, in general terms, inhibition of fibrogenetic processes. As a confirmation of the role of this cytokine, levels of IFN-γ are significantly high in the fluid component of the BALF of patients with active sarcoidosis [16].

Through its pleiotropic effects on cytokine production, IFN-γ upregulates the expression of the costimulatory molecules on accessory cells, including CD80 and CD86 [17]. IFN-γ also has crucial antifibrotic effects, since it inhibits the proliferation of

endothelial cells and the synthesis of collagens by fibroblasts. As previously reported, there is also a significant correlation between increased expression of IFN-γ and expression of the cdk inhibitor p21/Waf1 (an anti-apoptotic molecule) in sarcoid lung [9], suggesting that the high levels of IFN-γ-induced p21 may explain the absence of apoptosis in the granuloma and the persistence of inflammation.

However, by inducing non-ERL chemokines (monokine induced by IFN-γ (MIG)/CXC chemokine ligand (CXCL)9, IFN-γ-inducible protein (IP)-10/CXCL10, IFN-γ-inducible T-cell α chemoattractant (ITAC)/CXCL11), IFN-γ plays a major role in the recruitment and activation of sarcoid CXC chemokine receptor (CXCR)3+ T-cells in inflamed tissues (fig. 1). Indeed, there are a high number of CD4+ T-cells expressing CXCR3, CC chemokine receptor (CCR)5, IL-12 receptor (IL-12R) and IL-18R in the lung of patients with sarcoidosis, whereas the Th2-associated chemokine receptors CXCR4 and CCR4 are expressed by a low percentage of pulmonary sarcoid CD4+ T-cells [18].

IL-2 is also actively released at sites of disease activity during sarcoidosis, where it acts as a local growth factor for T-lymphocytes infiltrating involved tissues [19–21]. The presence of binding sites for IL-2 has also been demonstrated on human lung fibroblasts. Addition of IL-2 to fibroblasts leads to enhanced expression of the gene encoding monocyte chemoattractant protein (MCP)-1/CC chemokine ligand (CCL)2, a chemokine which is involved in fibrosis through the regulation of profibrotic cytokine generation and matrix. IL-2 may, thus, serve to integrate fibroblasts and sarcoid macrophages into a coordinated response of the connective tissue initiated by Th1 lymphocytes at sites of disease activity.

Th2 cell-derived cytokines. A switch to Th2 may occur in patients with progressive sarcoidosis. In these subjects, T-cells release Th2 cytokines, including IL-4, which is a cofactor for the proliferation of multiple cell lineages, including fibroblasts (see later) [22–24]. Activated Th2 cells also represent a source of IL-10, a molecule which has anti-inflammatory and immunoregulatory properties: it inhibits pro-inflammatory cytokine and chemokine production in addition to blocking T-cell responses to specific antigens. It has been proposed that the local secretion of IL-10 may represent a down-modulating mechanism involved in the spontaneous resolution of alveolitis in sarcoidosis [25]. In this regard, recent data suggest that the low macrophagic production of IL-22, a member of the human type-I IFN family, which includes IL-10, may play a role in the pathogenesis of sarcoidosis [26]. IL-22 has the potential to interact with IL-10 because it binds to the IL-10R2c chain with IL-22R1 in its receptor complex. Its role in the pathogenesis of sarcoidosis remains to be established. Finally, IL-13, a Th2 cytokine which is expressed by CD4+ T-cells and has been shown to suppress TNF-α in human blood monocytes is actively released in the lung of some patient with sarcoidosis [27]. However, alveolar macrophages rather than Th2 cells seem to be the cellular source of this pro-Th2 cytokine in sarcoid lung. In any case, the overproduction of IL-13 might have an anti-inflammatory effect on the surrounding microenvironment by acting on TNF-α release.

Monocyte/macrophage-derived molecules

The interaction between IFN-γ and its receptor triggers macrophages to become primed from the early phases of the sarcoid inflammatory process (fig. 1). This activation state of sarcoid macrophages is indicated by enhanced secretion of immunomodulatory molecules (table 1), as specified in the following section.

Pro-inflammatory cytokines. Sarcoid macrophages are capable of producing detectable amounts of IL-1, which regulates the development of alveolar inflammation in

sarcoidosis. Moreover, IL-1 *per se* may stimulate granuloma formation and fibrosis development by inducing fibroblast proliferation and increasing collagen production.

As previously discussed, TNF-α is another pro-inflammatory cytokine actively produced by sarcoid macrophages. It plays a critical role in pulmonary injury and the regulation of fibroblast growth *via* induction of IL-6. Furthermore, TNF-α stimulates and regulates the synthesis and release of other lymphokines (IL-1, granulocyte-macrophage colony-stimulating factor (GM-CSF), platelet-activating factor and IL-6) and increases prostaglandin (prostaglandin E_2) production. There are data suggesting that the chronic overexpression of TNF-α and IFN-γ sets the stage for the persistence and progression of inflammatory events and tissue damage during sarcoidosis [12, 28–30]. This suggests the importance of anti-TNF strategies in the treatment of sarcoidosis.

Cytokines involved in the regulation of Th1/Th2 cells. IL-12, the main macrophage-derived molecule involved in initiating Th1 immune responses, has been extensively evaluated in sarcoid lung. Its involvement in the development of lung granulomas, including sarcoidosis, has been clearly demonstrated [31–34]. IL-12 stimulates the proliferation of activated sarcoid T-cells. In synergy with IL-15, IL-12 favours contact between activated T-cells and sarcoid macrophages and induces the expression of chemokine receptors by Th1 cells (fig. 1). This cytokine acts by interacting with specific receptors (IL-12Rβ) expressed by lymphocytes accumulating at sites of disease activity during sarcoidosis [35].

IL-27, a newly described member of the IL-12 family, has been recently implicated in the pathogenesis of granulomas [36]. This cytokine synergises with IL-12 and is composed of two subunits, p28 and Epstein–Barr virus-induced gene 3 (EBI3). Immunohistochemical studies in granulomatous tissues have shown that EBI3 and p28 coexpression can be detected in epithelioid and multinucleate giant cells of sarcoid granulomas. In addition, sinus or tissue macrophages, endothelial cells and plasma cells coexpress EBI3 and p28. Taken together, these data suggest that IL-27 may play some role in granuloma pathogenesis, although the molecular mechanisms through which IL-27 participates in the assembly of the central macrophagic core of the granuloma remains to be defined.

Another macrophage-derived pro-Th1 cytokine which cooperates with IL-12 is IL-18 (fig. 1). Mainly produced by monocytes and macrophages, IL-18 induces expression of IFN-γ and GM-CSF, whereas it inhibits the production of IL-10. IL-18 and IL-12 act synergistically on sarcoid Th1 cells in the development and organisation of the Th1-type immune response [37–40]. However, it has been shown that IL-18, *via* activation of activation protein 1 and NF-κB, leads to enhanced IL-2 gene expression and production in sarcoid lung [37].

In confirmation of the local effects of IL-18, it has been shown that patients with active disease show upregulation of the NF-κB family of transcription factors [41]. Recent findings suggest that the local hyperexpression of these critical regulators of immediate transcriptional responses may be, to some extent, genetically regulated in sarcoidosis [42, 43]. Some molecular mechanisms that favour the upregulation of NF-κB on sarcoid macrophages begin to be clarified. The ligand-activated transcription factor peroxisome proliferator-activated receptor (PPAR)-γ has crucial effects in modulating the Th1 immune response, partly by down-regulating the production of inflammatory cytokines. Sarcoid macrophages exhibit activation of NF-κB and deficiency of PPAR-γ, suggesting that insufficient PPAR-γ activity contributes to ongoing inflammation in pulmonary sarcoidosis by failing to suppress pro-inflammatory transcription factors, such as NF-κB [44].

Sarcoid macrophages also release IL-15, a cytokine which supports the growth, survival and chemotaxis of sarcoid T-cells, favouring the development of Th1 infiltration

[45, 46]. It also behaves as a costimulatory factor for the production of other cytokines and chemokines (IL-17, CXCL8/IL-8, CCL2/MCP-1, GM-CSF, IFN-γ and TNF-α), and for the expression of molecules involved in the antigen-presenting capability of resident accessory cells (CD80/CD86). Furthermore, the finding that IL-15 down-modulates the apoptotic rate of lung T-cells indicates IL-15 as a possible inhibitor of physiological apoptotic stimuli that favour the persistence of inflammatory processes at sites of disease activity.

Colony-stimulating factors. Colony-stimulating factors, and in particular GM-CSF, are actively produced by sarcoid macrophages. In particular, GM-CSF is able to induce the growth and differentiation of sarcoid macrophages, facilitating the development of the macrophage-derived core of the sarcoid granuloma [47, 48]. Furthermore, GM-CSF modulates cytokine production and enhances the antigen-presenting capacity of sarcoid macrophages [49].

Chemokines

The trafficking and accumulation of immunocompetent cells are essential components in the pathophysiology of the inflammatory processes taking place in the lung of patients with sarcoidosis. A number of data suggest that most of these events are regulated by chemokines, which are highly expressed in the lung of patients with sarcoidosis (table 2). Monocyte inflammatory protein (MIP)-3β/CCL19 has been implicated in T-lymphocyte recruitment in sarcoidosis, is associated with disease progression and is down-regulated by drugs used for sarcoidosis treatment [50]. However, production of high levels of MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4 and regulated on activation, normal T-cell expressed and secreted (RANTES)/CCL5 cooperate to immobilise several leukocyte subpopulations in perivascular foci of inflammation. MCP-1/CCL2 and RANTES/CCL5, interacting with CCR1/CCR2 or CCR1/CCR3/CCR5, respectively, may be

Table 2. – Chemokines and chemokine receptors that have been implicated in the pathogenesis of sarcoidosis

Chemokine	Human ligand	Receptor
CC Chemokines		
CCL2	MCP-1, MCAF	CCR2
CCL3	MIP-1α, LD78a, LD78b, AT464.1, AT464.2, GOS19-1, GOS19-2	CCR1, CCR5
CCL4	MIP-1β, AT744.1, AT744.2, Act-2, G-26, HC21, H400, LAG-1	CCR5
CCL5	RANTES	CCR1, CCR3, CCR5
CCL19	MIP-3β/exodus-3	CCR7
CCL20	MIP-3α/exodus-1	CCR6
CXC chemokines		
CXCL8	IL-8, MDNCF, NAP-1, NCF	CXCR1, CXCR2
CXCL9	MIG, HuMIG	CXCR3
CXCL10	IP-10	CXCR3
CXCL11	ITAC, H174, β-R1	CXCR3
CXCL16	SR-PSOX	CXCR6

CCL: CC chemokine ligand; CXCL: CXC chemokine ligand; MCP-1: monocyte chemoattractant protein-1; MCAF: monocyte chemotactic and activating factor; CCR: CC chemokine receptor; MIP: monocyte inflammatory protein; RANTES: regulated on activation, normal T-cell expressed and secreted; IL: interleukin; MDNCF: monocyte-derived neutrophil chemotactic factor; NAP-1: neutrophil-activating protein 1; NCF: neutrophil chemotactic factor; CXCR: CXC chemokine receptor; MIG: monokine induced by IFN-γ; HuMIG: human MIG; IP: interferon-gamma (IFN-γ)-inducible protein; ITAC: IFN-γ-inducible T-cell α chemoattractant; SR-PSOX: scavenger receptor that binds phosphatidylserine and oxidised lipoprotein.

chemoattractant for different cell targets that characterise this different phase of the sarcoid inflammatory process, including macrophages and T-lymphocytes.

Three lymphocyte-specific CXC chemokines, which are produced in response to IFN-γ (*i.e.* CXCL10, MIG/CXCL9 and ITAC/CXCL11) [51], play an important role in the recruitment of activated T-cells into the pulmonary microenvironment during sarcoidoisis. Sarcoid macrophages are the main cell source for these molecules; they release large amounts of CXCL10 and CXCL9 that, by interacting with specific receptors expressed by Th1 cells (CXCR3), allow for the accumulation of pulmonary T-lymphocytes and contribute to granuloma formation [52]. Activated bronchial epithelium is another important source of CXCL9, CXCL10 and CXCL11.

The present authors have recently evaluated whether or not CXCR3 is coexpressed with other Th1-associated chemokine receptors. The preliminary data indicate that two other typical Th1 chemokine receptors, CCR6 and Bonzo/CXCR6, are highly expressed by sarcoid Th1 cells. In particular, it has been shown that, whereas CXCR3 is expressed early by sarcoid T-cells, CXCR6 expression is the result of prolonged exposure in the pulmonary microenvironment to IFN-γ and macrophage-derived cytokines, primarily IL-15.

IL-8/CXCL8, a chemokine that favours T-cell and neutrophil recruitment, is actively released in the airways during sarcoidosis, and its release is associated with lung damage. Immunolocalisation of IL-8 has demonstrated that the pulmonary fibroblast is the predominant cellular source of IL-8, even if there are data suggesting that macrophages may release this chemokine. Interestingly, pulmonary fibrosis may be associated with increased release of IL-8/CXCL8 and dysregulation of CXCL10 production, suggesting that the balance of chemokine production is an important factor in the regulation of local angiogenesis and fibrogenesis.

Collectively, these data emphasise the role of chemokines and chemotactic molecules in the development of sarcoid inflammation. It is also likely that cell-to-cell and cell-to-matrix interactions modulate local chemokine expression, contributing to the pathological progression toward fibrosis of inflammatory lesions. As recently reviewed [29], a number of antagonists of chemokine receptors are being developed by different pharmaceutical companies. Specific chemokine antagonists are now approaching their first clinical trials. The therapeutic use of molecules selective for chemokine receptors appears to have great potential for sarcoidosis and other T-cell mediated diffuse lung diseases.

Macrophage/T-cell interactions are key factors in granuloma formation

The central core of the typical sarcoid granuloma is made up of a number of monocytes/macrophages at various states of activation and differentiation, as well as epithelioid cells and multinucleate giant cells. Chemotactic and activating factors for leukocytes, which are actively secreted in tissues involved by sarcoidosis, are then capable of recruiting blood monocytes to the local milieu, favouring the development of the central structure of the granuloma.

Histopathological data have demonstrated the presence of macrophage and macrophage-derived interdigitating professional antigen-presenting cells (APCs) in the T-cell areas [53]. These cells, even when assembled into the mature components of the granuloma, maintain APC features as well as the ability to synthesise an array of cytokines. T-cell/macrophage interaction depends on the presence of a number of costimulatory molecules on lung macrophages, including members of the B7 family (CD80 and CD86), some molecules of the TNF receptor superfamily (CD40 and CD27)

and the CD5 co-ligand CD72. The pattern of CD80 and CD86 expression shown by pulmonary macrophages of patients with sarcoidosis is consistent with that of conventional APCs [17, 54, 55]. Indeed, sarcoid macrophages increase their expression of molecules which endow macrophages with accessory functions for T-cell activation and proliferation, including CD80 and CD86, and CD40 and CD72. Furthermore, it has been shown that mature dendritic cells can be found in the central core of sarcoid granulomas of patients with sarcoidosis, suggesting that blood dendritic cell subsets may migrate into the affected tissues, contributing to the formation of the granuloma [56].

The expression of cytokine genes, which ultimately accounts for the accumulation of immunocompetent cells inside granulomas, was recently investigated in sarcoid lymph nodes using *in situ* hybridisation techniques and immunohistological studies. IL-1β, IFN-γ, CXCL16 and CXCL10, and CCL20 and CCL5 are preferentially expressed by cells inside the granuloma [29, 57, 58], whereas cells containing TNF-α, IL-1α, IL-6 and IL-2 mRNA are scattered and randomly distributed. These findings suggest that the development of the new granuloma is due to molecules with chemotactic properties which cooperate to attract monocytes and lymphocytes in the perivascular foci of inflammation (fig. 2). It is probable that IFN-γ release by CD4 cells is essential to the assembly of the granuloma since it is not possible to induce the formation of granulomas in mice in which the IFN-γ gene has been disrupted [59]. IFN-γ-induced chemokines have also been implicated in the assembly of the granuloma structure. Indeed, immunohistological analysis of tissues displaying abundant sarcoid granulomas has revealed that cells bearing CXCL10 and CXCL16 are mainly epithelioid cells and CD68+ macrophages located inside the granulomatous areas (fig. 2). Both chemokines are functionally active. Indeed, by interacting with specific receptors expressed on Th1 cells (CXCR6 and CXCR3), the two chemokines are able to favour the migration and accumulation of the sarcoid T-lymphocytes that surround the central macrophagic core.

Fibrosis in sarcoidosis

Although the granuloma structure is aimed at containing the dissemination of inciting agents in hypersensitivity reactions, it is to be expected that the inflammatory response will spontaneously clear once the aetiological factors are isolated. This paradigm is not supported in the case of refractory sarcoidosis. In ∼60% of patients with sarcoidosis, the course of the disease is self-limiting with spontaneous resolution of the granuloma, whereas patients with progressive sarcoidosis show massive development of granulomas and do not recover even if strong immunosuppressive therapy is used. The uncontrolled development of granulomas results in fibrosis.

Although the reversible phases of initial alveolar injury in the sarcoid process are mediated by Th1 lymphocytes, the fibrotic changes that follow the sarcoid Th1 immune response are modulated by macrophages, neutrophils, eosinophils and mast cells [60, 61], which, *via* overproduction of the superoxide anion, oxygen radicals and proteases, can cause local injury, disruption of the epithelial basement membrane, alteration of epithelial permeability and consequent derangement of the normal architecture of lung parenchyma [62]. In confirmation of the role of polymorphonuclear cells in lung damage, an increased number of BALF neutrophils has been related to a more severe course of the sarcoid process [63].

By releasing a number of molecules, including transforming growth factor (TGF)-β and the family of TGF-related cytokines, platelet-derived growth factor and insulin-like growth factor I, sarcoid macrophages may, in theory, mediate fibrosis. These growth factors for fibroblasts and epithelial cells and their receptors are abundantly expressed in
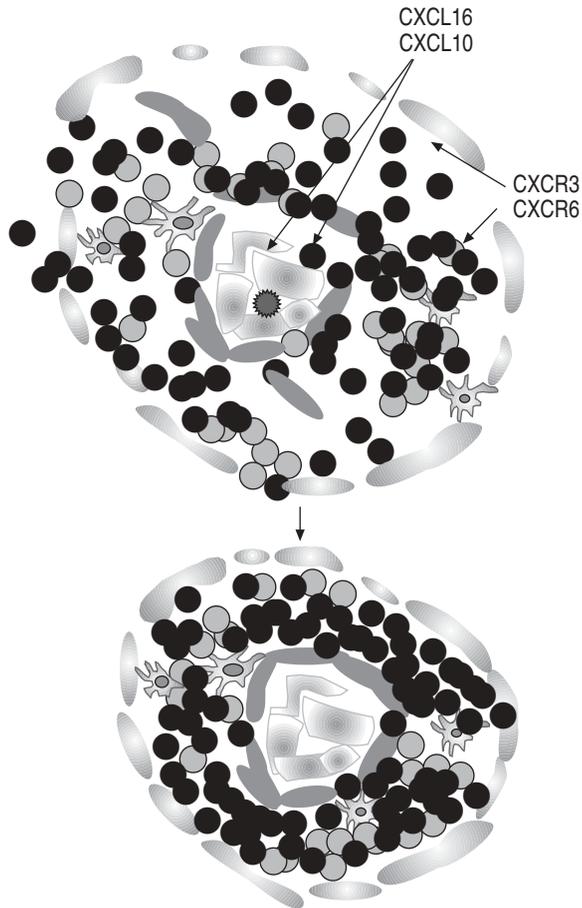
Fig. 2. – Molecules with chemotactic properties cooperate to attract monocytes in perivascular foci of sarcoid inflammation (see section on Chemokines). However, cells bearing CXC chemokine ligand (CXCL)10 and CXCL16 are located mainly inside granulomas. Indeed, epithelioid cells and CD68+ macrophages of the central core of the granuloma are CXCL10+/CXCL16+, whereas lymphocytes surrounding the granuloma core are CXC chemokine receptor (CXCR)3+/CXCR6+ T-cells, suggesting that interaction between chemokine receptors and their ligands are crucial to the mechanisms leading to granuloma assembly.

fibrotic lung. They cooperate with the TGF family in promoting fibroblast growth and deposition of collagen fibrils. Furthermore, macrophage-derived cytokines which are overexpressed at sites of granuloma formation (including IL-1, IL-6, IFN-γ, TNF-α and GM-CSF) [64–66] and immunoglobulin G immune complexes may upregulate the expression of the inducible form of nitric oxide synthase and nitric oxide production in granuloma cells [67], thus contributing to the injury and consequent reparative processes.

In general terms, the recruitment of fibroblasts and the subsequent increased production of matrix macromolecules are crucial to the fibrotic process. In particular, migration of fibroblasts and epithelial cells from the interstitium to the alveolar spaces and adhesive interactions of fibroblasts with the surrounding interstitial matrix are the major factors contributing to the development of fibrosis. The migratory process of fibroblasts reflects the local release of a variety of molecules which can act as chemoattractant factors for fibroblasts, such as chemokines, products of coagulation and

the fibrinolytic cascade, as well as matrix proteins (collagen peptides, laminin, fibronectin and elastin-derived peptides) [68–71]. Most of these are actively produced in sarcoid lung.

Molecules secreted by sarcoid inflammatory cells are also able to prime fibroblasts to enter the G1 phase of the growth cycle, and thus to proliferate. Indeed, an increased proportion of fibroblasts isolated from patients with pulmonary fibrosis demonstrate unexpected growth capability and a higher rate of cell division than fibroblasts isolated from normal lung. Furthermore, they show increased migratory capability [72].

Conclusions

Since 2000, much has been learnt regarding the pathogenesis of sarcoidosis, and improved therapies are being developed based on these findings. Currently, most interest in soluble cytokine and chemokine inhibitors, genetically engineered antagonists, and single or combinations of anti-inflammatory cytokines has focused on the possibility that they may become standard pharmacological agents for controlling and modulating the sequelae of the immunological events that lead to granuloma formation and fibrosis development. For example, molecules capable of neutralising TNF-α have been used in the clinical setting of patients with sarcoidosis [29, 73]. Not unexpectedly, these data suggest the real possibility of using anti-TNF strategies to treat refractory sarcoidosis. These data are preliminary and clinical trials are in progress to confirm both the efficacy and tolerability of anti-TNF agents when used in patients with sarcoidosis. Blockades of other inflammatory cytokines are also expected to be therapeutic in sarcoidosis and other T-cell-mediated diffuse lung diseases. In particular, therapies directed at neutralising chemokines and other molecules which control the trafficking and accumulation of immunocompetent cells are potentially more selective and attractive but require *a priori* knowledge of the precise pathways regulating the inflammation state involving the alveolar and interstitial structures.

Summary

By influencing many of the physiological properties of immunocompetent cells, including proliferation, differentiation, activation and chemotaxis, chemokines and cytokines act as critical mediators of cell function and cell-to-cell communication in sarcoidosis. In fact, it is well established that the release of a cascade of interacting extracellular signalling proteins orchestrates the trafficking of immune cells during sarcoid inflammatory process. Cytokines regulate the expression of adhesion molecules on the lung vascular endothelium within and around the site of inflammation by combining with relevant receptors on neighbouring cells. This in turn favours the entrance and activation of type-1 T-helper (Th) cells, and modulates the local survival and proliferation of different types of immune cells, including macrophages, which in turn release pro-inflammatory cytokines. As a result, cytokines with pro-inflammatory destructive biological functions set the stage for the development of irreversible remodelling of the lung tissue, the evolution toward pulmonary granuloma formation, and, in some sarcoid individuals, the development of fibrosis.

Keywords: Cytokines, immunopathogenesis, sarcoidosis, T-cell alveolitis.

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