Immune aspects of sarcoidosis

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Summary: Although the initiating factor(s) is unknown, it is now accepted that pulmonary sarcoidosis develops as a result of an over-stimulated local cellular immune response. Starting as a lymphocytic alveolitis, there is a progression to granuloma formation within the interstitium as stimulated T lymphocytes release mediators capable of attracting and activating monocytes to differentiate into macrophages and epithelioid cells. We are also aware that macrophage-like cells must act as antigen presenters to initiate T cell stimulation within the immune response. To date, interest in the alveolar macrophages of patients with sarcoidosis has focused more on their passive role as responders to the soluble T cell products released as the disease progresses. This paper explores the active role of mononuclear non-lymphoid cells as inducers of immune responses, by taking advantage of monoclonal antibodies capable of discriminating between phenotypically distinct subsets of macrophages. Recent results are presented that support a central role for these cells in controlling the course of this disease, focusing specifically on the mechanisms underlying the failure in some patients to resolve the interstitial inflammation and subsequently progressing to fibrosis. A new hypothesis proposes that aberrations in the functional capacity of macrophages may prohibit the emergence of a granuloma-resolving mechanism in some sarcoid patients.

Introduction

This article reviews the way in which investigation of immune mechanisms in pulmonary sarcoidosis has helped reveal the pathogenesis of the disease. Some comment and speculation is included which is intended to be somewhat provocative. Such speculation should not be taken as ‘state of the art’; rather, it should be seen as an attempt to raise relevant questions, answers to which will further our understanding of this disease.

Although the aetiology of sarcoidosis remains unknown, there is no doubt that the pathogenesis of this disease is critically influenced if not totally controlled by mechanisms within the immune response network of the individual.1–3 For the purposes of this article I shall concentrate on pulmonary sarcoidosis, making reference to extrapulmonary disease where relevant to the discussion at hand.

Accepting that the basis of the disease is an aberrant immune response, we must assume that this is triggered by antigenic insult. Various candidates for this role have been proposed, including viruses,4 mycobacteria,5 mycoplasma,6 lipopolysaccharides7 and auto-antigens (for review see reference 8). None has so far been consistently linked with the disease and it is possible, of course, that different sources of antigen may promote sarcoidosis in different patients, providing there exists an underlying immune abnormality perhaps associated to genetic influences.9,10

As well as the absence of an identifiable aetiological agent, another complication facing the immunological investigation of this disease is the considerable variability in clinical presentation of sarcoidosis. To understand the role of the immune response in pathogenesis it is necessary to relate immunological observations to clinical status. Even restricting the study group to patients with pulmonary sarcoid leaves you with a highly heterogeneous population that all present with disease of unknown duration and then proceed along differing routes either to resolution or progressive morbidity, some with, and some without therapeutic intervention.

Despite these problems our understanding of the role of the immune response system in the pathogenesis of sarcoidosis has now grown to the point where many of the pathological consequences of this disease can be explained, and for those that cannot, working hypotheses are proposed that can be tested in the future. The three main pathological features of this disease are alveolitis, granuloma formation and (in a proportion of patients), fibrosis.
in the lung interstitium. The role of the immune response must be considered in the light of these basic features. When aberrant immune responses are identified, it needs to be determined whether they represent cause or effect in relation to the disease process.

The immune basis of sarcoidosis

Early disease is characterized by an accumulation of inflammatory cells (predominantly lymphocytes) within the alveolar structures. The development of a lymphocytic alveolitis distinguishes sarcoidosis from other interstitial lung disease where polymorphonuclear cells predominate. Furthermore, the predominance of mononuclear cells in the interstitial infiltrates of sarcoidosis is an important clue that immune mechanisms are involved. The most prominent cell types are macrophages and T lymphocytes. Thus the histological picture of the alveolitis reflects that of a Type IV (delayed) hypersensitivity reaction. As early pulmonary sarcoidosis is also characterized by bilateral hilar lymphadenopathy (seen in Types I, II, chest X-ray) immune hyper-reactivity is strongly implicated. It is thought that immunological mechanisms promoted by the interaction of the T cells and macrophages infiltrating the alveolar areas are a prerequisite for the formation of granulomata within the interstitium. Such an association has been demonstrated in animal models of granulomatous lung disease. Furthermore, there appears on biopsy to be an inverse relationship between the extent of 'diffuse' mononuclear cell infiltration, and the number and maturity of the granuloma. These observations can be interpreted as reflecting a progressive pathogenesis with T cells and macrophages first infiltrating the interstitium as the result of an (as yet unknown) antigenic stimulus; and later the mediators released by the infiltrating cells of the immune response orchestrating the macrophages into granuloma formation.

Such suggestions are impossible to investigate in situ as tissue sections of biopsies give only a 'still life' plucked from a moving picture. However, the alveolitis and subsequent granuloma formation within the interstitium are accompanied by changes within the cell populations of the air spaces. These cells are available for investigation through the technique of bronchoalveolar lavage (BAL). Direct comparisons of the cellular constituents in the BAL and tissue biopsy from the same patients have revealed that a qualitative but not quantitative relationship exists. As quantitation of proportions of different cell types within tissues is, to say the least, rather imprecise, such differences may be apparent rather than real. Indeed, some authors have suggested that BAL does offer a cell suspension that reflects the proportions of cells present within the interstitium, at least in patients without high intensity alveolitis. There is no doubt, however, that BAL offers a valuable sample of the immunocompetent cells involved in the lung pathology.

The lymphocytic alveolitis in the lung tissue is reflected by a lymphocytosis in the lavage. The proportion of 4–10% lymphocytes found in normal volunteers rises to 15–50% in patients with sarcoidosis. This change is made even more significant by up to a 10-fold overall increase in lavage cells recovered from patients with sarcoidosis. This overall increase also means a significant rise in macrophage numbers, although their proportion falls. It is from studies of these lavage cells that most evidence of the immune mechanisms involved in sarcoidosis has come. More than 90% of the lymphocytes in sarcoid BAL are T cells. Of these, the CD4+ helper/inducer type subset outnumbers the CD8+ suppressor/cytotoxic type from 5.1 to 10.1 (normal 1.6:1). The fact that no significant numbers of B lymphocytes appear either in lavage or in the tissues adds further weight to the concept that cell-mediated rather than antibody-mediated mechanisms are at play, at least in the lung. Reports of raised circulating antibody levels and immune complexes in patients with sarcoidosis may thus reflect secondary B cell stimulation, possibly of a polyclonal nature. The raised ratio of CD4+ T cells to CD8+ cells in sarcoid lavage is also seen in other chronic inflammatory disease such as rheumatoid arthritis. Here the low number of CD8+ cells is taken as indicating dysfunction in immunoregulatory pathways.

The same situation may also exist in sarcoidosis as the BAL CD4+ T cells have been shown to be activated. Functional studies of these cells in vitro have shown them to express helper activity, and respond vigorously to mitogenic and antigenic stimulation. They have also been shown to express phenotypic signs of activation such as the presence of interleukin 2 (IL2) receptors and Class II MHC antigens on their surface. Separate studies have demonstrated that sarcoid T cells spontaneously release IL2 and raised levels of this mediator have been found in the lavage fluid. IL2 release is an essential step in T cell activation required to induce the proliferation of these cells. Raised IL2 levels in lavage therefore signify an active if not overactive immune response. More recent studies using techniques of molecular biology have demonstrated a genetic basis for the increased
production of IL2 by these cells in patients with sarcoidosis.\textsuperscript{39}

Interestingly, it is known that the T cells within the inflamed tissues replicate faster than normal in sarcoidosis.\textsuperscript{40} It is likely, therefore, that increased IL2 production detected in the lavage is also occurring within the tissues and that this results in increased local proliferation of T cells in the interstitium. These T cells accumulating in the interstitium can release monocyte chemotactic factor\textsuperscript{41,42} and interferon $\gamma$ (IF$\gamma$).\textsuperscript{43} These mediators could be active in promoting macrophage activation and granuloma formation within the lung tissue.

Together, these observations give a picture of pulmonary sarcoidosis as a local cell-mediated immune response induced by unknown antigen(s), resulting in the activation of CD4+ T cells in an environment lacking significant numbers of regulatory CD8+ cells. These circumstances would lead initially to an alveolitis and subsequently to the formation of granulomata as lymphokines released by the activated lymphocytes react with the macrophage populations.\textsuperscript{18}

The role of the macrophages

The role of the macrophage in pulmonary sarcoidosis has been described in terms of the formation of granulomata. With current knowledge of the heterogeneity of macrophage-like cells\textsuperscript{44,45} and their involvement in the induction,\textsuperscript{46} as well as expression of immune responses, this is clearly too simplistic a view.

There is little doubt that the presence of a population in the lung of 'activated' CD4+ T cells implicates the involvement of a macrophage-like cell as an 'antigen presenter', notwithstanding the fact that the triggering antigen has not been identified. The induction of acquired T cell-mediated immune responses requires the presence of an antigen-presenting cell.\textsuperscript{46} Although classical macrophages have been shown to be capable of antigen presentation, it is now established that so-called dendritic cells are uniquely designed for this purpose.\textsuperscript{47-49} It is of some importance, however, to note that alveolar macrophages in particular are relatively poor stimulators of T cell activity. This has been demonstrated in man\textsuperscript{50,51} and in animals.\textsuperscript{52} This is true despite the fact that even in normal lavage, cells with the phenotype of dendritic cells, and cells with the phenotype of macrophages can both be identified.\textsuperscript{53}

It is perhaps not surprising that both dendritic cells and macrophages are present within normal lavage fluid, as both the induction and expression of immune defence systems are naturally required for local protection. What happens, however, to these cells in pulmonary sarcoidosis? It is known that the absolute number of macrophages is increased in sarcoïd BAL,\textsuperscript{22,53} that the alveolar macrophages become larger,\textsuperscript{54} have a higher mitotic activity,\textsuperscript{55} have increased antigen presenting capacity,\textsuperscript{56} exhibit increased production of interleukin 1,\textsuperscript{57} and release super-oxide anions.\textsuperscript{58} One might say they were activated! However, alveolar macrophages in sarcoïd BAL have also been shown to express reduced phagocytic capacity,\textsuperscript{59} reduced expression of C3b receptors,\textsuperscript{60} and reduced levels of lysosomal enzymes.\textsuperscript{60} So they are not 'activated'! The explanation for this apparent paradox is simple and, arguably, it might be the key to our understanding of the pathogenesis of this disease. The fact of the matter is that the alveolar macrophage population (as mentioned above) is not homogenous, but contains sub-populations of cells that although morphologically similar differ phenotypically and almost certainly functionally. This has been demonstrated in animals,\textsuperscript{61} where it has been suggested that separate sub-populations of macrophages in the lung may influence the function of each other. To further complicate the issue, evidence is available that the phenotype of these subsets (and thus presumably their function) is altering during the course of the disease.\textsuperscript{36} By using monoclonal antibodies that distinguish dendritic cells and macrophages in normal tissues (RFD1 and RFD7, respectively)\textsuperscript{62} it has been possible in our laboratory to begin to monitor these changes, thus shedding new light on the pathogenic process.\textsuperscript{36} Using immunocytological methods it has been shown that in normal BAL 29% of 'macrophages' are RFD1+ , RFD7− (dendritic cell phenotype) and 32% are RFD7+, RFD1− (mature macrophage phenotype). The remainder do not express either of these specific marks, but do react with monoclonal antibodies Leu M3 and UCHM1 (a monocyte marker\textsuperscript{63}). Some 5% of cells in normal BAL express both RFD1 and RFD7 antigens.\textsuperscript{36,62} In sarcoidosis the proportions of RFD1+ cells rise to 63±21% and RFD7 cells rise to 43±15%.\textsuperscript{53} Interestingly, when sarcoid patients were grouped by chest X-ray type, those with a Type III X-ray gave even higher proportions of both RFD1+ and RFD7+ cells.\textsuperscript{36} The increase in the expansion of both RFD1+ and RFD7+ cells rested with the fact that a significant increase in macrophage-like cells expressing both markers occurred.\textsuperscript{39} Indeed, up to 40–50% of sarcoïd BAL 'macrophages' became RFD1+ , RFD7+.\textsuperscript{36}

What might this mean in terms of the immune response and the interaction of these cells with the T cells present? Let us imagine that initially only
two significant populations of non-lymphoid accessory cells exist in the lung, dendritic antigen-presenting cells and phagocytic macrophages. With the introduction of antigen, the dendritic antigen-presenting cells (RFD1+ cells) present the antigen to T cells that subsequently respond. In the individual destined to develop sarcoidosis the T cells appear to over-produce interleukin 238,39 and IFγ.64 both of which are to be found in high concentration in lavage, IFγ is known to activate macrophages64 and together with other lymphokines promotes the formation of granulomata within the lung interstitium. IFγ has also been shown to induce increased expression of HLA-DR molecules on cells,66 a phenomenon also observed on sarcoid lavage macrophages.67 Perhaps more significantly, when peripheral blood monocytes are cultured with IFγ, this has the effect of increasing the proportion of RFD1+ cells developing, while suppressing RFD7 expression.68 Were this to occur in the lung, it would result in a rise in the proportions of RFD1+ dendritic cells. Such cells do not have C3b receptors or lysosomal enzymes, nor are they active phagocytes.47 Thus the observed changes in the 'alveolar macrophages' of sarcoid patients of reduced phagocytosis49 and loss of C3b receptors and lysosomal enzymes60 could reflect a situation where 'macrophages' are switching phenotype and function under the influence of IFγ to become dendritic cells. An increased incidence of RFD1+ dendritic cells with antigen-presenting capacity could then further stimulate, or over-stimulate the T cell pool.

I have already mentioned a third population of 'macrophages', those that phenotypically express positivity with both monoclonal antibodies RFD1 and RFD7. Such cells could represent transitional forms between RFD7+ and RFD1+ cells. This would seem unlikely, however, as small but identifiable numbers of these cells appear in normal lavage fluid where presumably no stimulus is promoting such switching. They could be immature forms that have as yet to decide their mature phenotype; yet in vitro monocyte differentiation produces separate populations of cells being either RFD1+ or RFD7+ but not both.62 Preliminary work in this laboratory has shown these doubly labelling cells to be adherent to glass and to express strong lysosomal enzyme activity (Pouler et al. in preparation). When co-cultured with allogeneic lymphocytes they are found to suppress mixed lymphocyte reactivity. In studies of autologous mixed lymphocyte reactions using unfractionated lavage 'macrophages' as stimulators it has been found that sarcoid BAL cells suppress the autologous reactivity of peripheral blood lymphocytes (Spiteri et al. submitted). Furthermore, this suppression is most marked when samples from patients with Type III chest X-ray are used. As these are the patients with the highest proportions of the D1+/D7+ cells, it seems possible that these cells are in some way exerting a suppressive influence on immune reactivity, and represent the human equivalent of the suppressor macrophages reported previously in studies of rat lavage cells.81

One may therefore have a situation in sarcoidosis where initial overstimulation eventually leads to a build-up of a suppressive population of non-lymphoid accessory cells that neither function properly as antigen presenters not phagocytes. To speculate on the significance of this one must turn one's attention to the granulomata within the lung interstitium.

**Lymphocyte/macrophage interaction and the granulomata**

It is generally accepted that the formation of granulomata is a consequence of cell-mediated immunological reactivity (see above and reference 18). However, studies in sarcoid patients of granuloma formation induced by Kveim reagent do suggest that this may not be the whole story.69 Immunopathological evidence of cell-mediated immune reactivity was lacking in sarcoid patients developing cutaneous Kveim granulomas but present in normal volunteers injected with Kveim suspension. It is also well recognized that Kveim positivity is often associated with PPD anergy70,71 implying a suppression of specific cell-mediated reactivity at the periphery. Although this phenomenon has been explained by a compartmentalization of lymphocytes in the tissues and an associated lymphopenia,15 acceptance of this suggestion means acceptance that Kveim-induced granulomata can develop during a state of depressed immune responsiveness; and that sarcoid granulomas can also perhaps form and persist under such circumstances!

When considering the relationship between changing peripheral immune reactivity (as demonstrated by PPD anergy) and local immune reactivity in the lung, it is important to reflect on the time scale within the course of the disease. PPD anergy is generally observed sometime after the patient has developed frank pulmonary sarcoidosis with well established interstitial granulomata. This being the case, one can accept that the granulomata of pulmonary sarcoidosis are induced by activated cellular immune mechanisms,18 but subsequent to this, in some patients, a suppressive influence is imposed on the immune system (leading perhaps to

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PPD anergy at the periphery). Whether such suppression is induced by the progressive emergence of the aberrant RFD1+/RFD7+ macrophages mentioned above is not known, nor is it clear whether the changes in the phenotype of these macrophage subsets are related to immune suppression, or if such a phenomenon can be manifest systemically. What is clear, however, is that it is crucial to define the stage in the disease process that patients have reached when samples for immunological investigations are taken. Furthermore, it must be accepted that functional studies performed on isolated and manipulated BAL lymphocytes and macrophages demonstrate what these cells could do and not necessarily what they are doing (functionally) in the lung.

One crucial question that remains unanswered is why a proportion of patients progress to fibrosis, while the majority resolve their interstitial granulomatous inflammation at least to a point where lung function returns to normal. This is a difficult question to answer as most patients are treated with corticosteroids, which will clearly influence local immune reactivity. However, the fact that the persistence of granulomata and progressive fibrosis in some patients occurs despite aggressive steroid therapy implies in itself that these phenomena are not dependent on active immunological mechanisms. Indeed, one could speculate that quite the reverse might be true! That is, that persistent granulomata and fibrosis are the result of suppressed immunological activity. Before even considering this rather provocative suggestion it is necessary to accept at least the possibility that the resolution as well as the induction of sarcoid granulomas is under immunological control. There is no direct evidence for this suggestion, but by observing the development of sarcoid granuloma, and the disposition of immunocompetent cells in mature lesions, clues can be found that lead in that direction.

Kveim-induced granulomata begin as clusters of macrophages in the tissues with only scant CD4+ T cells, and not as perivascular accumulations of lymphocytes.59 The picture of a mature sarcoid granuloma is of a central core of macrophages and epithelioid cells interspersed with CD4+ T cells, surrounded by a mantle of CD8+ and CD4+ T cells and macrophages.7273 Particular attention has been focused by some workers on the restricted distribution of CD8+ T cells to the outer mantle of the sarcoid granuloma,7475 implying a unique clustering of these cells to this location. Similar observations have been made in other granulomatus disease.747677 If, however, the macrophage/epithelioid cell centre develops first, perhaps under the influence of activated CD4+ T cells, the mantle of lymphocytes may accumulate from the outside and not migrate out from the centre. If this occurred, both CD8+ and CD4+ lymphocytes accumulating from the outside could be seen as a response to the macrophage/epithelioid cell clusters rather than an integral part of original granuloma development. Indeed, the cellular constituents of the mantle zone represent the cell types in the right proportions characteristic of a cell-mediated delayed type hypersensitivity reaction. In other words, it could be argued that this mantle of T lymphocytes and macrophages may represent a distinct immune response mechanism directed against the granuloma.

If the inducing cells (i.e. macrophages/dendritic cells) in this reaction were functioning normally one could see this 'second reaction' resolving the lesion. But what if the inducer cells were aberrant RFD1+, RFD7+ cells? Would this reaction then fail, leading to the persistence of the granuloma and fibrosis? It is interesting to note that in the case of persistent foreign body granulomas the local injection of interferon results in their resolution.78 As interferons are recognized as stimulating effector function in immune responses, this observation implies that active immune mechanisms may be necessary for granuloma removal. One wonders, therefore, whether a suppression of immune reactivity might be occurring in those few sarcoid patients who reach the point where persistence of granulomata leads to fibrosis? From our studies to date it seems possible that such a situation could result from the presence of 'suppressive' (RFD1+ RFD7+) macrophages within the granuloma mantle zone reaction.

To answer this question biopsy material from patients with steroid-resistant progressive disease needs to be examined immunohistologically so that the phenotypes expressed by the macrophage-like cells within the mantle zones can be revealed. Such studies would determine the relationship, if any, between granuloma persistence and macrophage subsets present within the lymphocyte mantle. In the meantime, studies of the functional capacity of cells with the RFD1+/RFD7+ phenotype obtained from lavage are ongoing. Initial results (M. Spiteri et al. in preparation) do indicate that while D1+ D7− cells in lavage promote T cell reactivity, the D1+ D7+ cells are functionally suppressive.

Conclusions

A review of the immune aspects of sarcoidosis leaves no doubt that this is a disease promoted by local aberrations in immunological reactivity. CD4+ T cells are over-stimulated and promote the
formation of granulomata. The persistence of these lesions and a progression to fibrosis in some patients represents a more difficult puzzle to unravel. However, the emergence of phenotypically abnormal populations of macrophage-like cells may shift immune status to one where suppression of appropriate protective mechanisms occurs. If such a situation develops in some patients, the body is left with only the mechanism of fibrosis as a last resort to 'heal' the tissue damage created.

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