Dendritic Cells in the Pathogenesis of Sarcoidosis

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Sarcoidosis is a noncaseating granulomatous disease, likely of autoimmune etiology, that causes inflammation and tissue damage in multiple organs, most commonly the lung, but also skin, and lymph nodes. Reduced dendritic cell (DC) function in sarcoidosis peripheral blood compared with peripheral blood from control subjects suggests that blunted end organ cellular immunity may contribute to sarcoidosis pathogenesis. Successful treatment of sarcoidosis with tumor necrosis factor (TNF) inhibitors, which modulate DC maturation and migration, has also been reported. Together, these observations suggest that DCs may be important mediators of sarcoidosis immunology. This review focuses on the phenotype and function of DCs in the lung, skin, blood, and lymph node of patients with sarcoidosis. We conclude that DCs in end organs are phenotypically and functionally immature (anergic), while DCs in the lymph node are mature and polarize pathogenic Th1 T cells. The success of TNF inhibitors is thus likely secondary to inhibition of DC-mediated Th1 polarization in the lymph node.

Keywords: sarcoidosis; granuloma; dendritic cell; macrophage; inflammation

Sarcoidosis was first described in 1877 as a skin disease by British physician Sir Jonathan Hutchinson (1). It was later discovered that only 20% of patients with sarcoidosis have skin lesions, whereas over 90% have interstitial lung disease (2). In the United States, the incidence rate of sarcoidosis is 10.9 and 35.5 per 100,000 in persons of European and African descent, respectively (3).

Although the pathogenesis of sarcoidosis remains undetermined, many observations, including the presence of oligoclonal T cells in sarcoidosis bronchoalveolar lavage (BAL) fluid, blood, and skin granulomas, are suggestive of an antigen-driven autoimmune disease (4, 5). However, despite multiple proposed causative agents, a single agent has not yet been identified.

T cell subsetting studies have identified IFN- γ -producing Th1 cells as the dominant subtype in both sarcoidosis BAL fluid and skin lesions (6–8), although studies have not yet been done to rule out involvement of the recently described Th17 cell subset. The working model for antigen-driven inflammation involves peripheral organ dendritic cells (DCs) digesting antigenic material, migrating to the lymph node, presenting that antigen to naïve resting T cells and activating a clonal T cell response. T cells then migrate back to the end organ, where they produce a disease-specific profile of inflammatory cytokines and chemokines. Despite the necessity of DCs for induction of naïve T cell clonal expansion (9), to date sarcoidosis research has focused on alveolar macrophages as the causative antigen-

CLINICAL RELEVANCE

This review focuses on dendritic cells as integral players in sarcoidosis pathogenesis. As such, these cells become potential therapeutic targets.

presenting cell (APC). This review focuses on evidence supporting an important role for DCs in directing sarcoidosis Th1 polarization and immunopathogenesis.

DENDRITIC CELL DEFINITION

In 1973, Steinman and Cohn first described the dendritic cell as having three essential characteristics: (1) Ability to potently induce T cell proliferation (immunostimulatory capacity); (2) high expression of MHC class II peptides; and (3) ability to migrate in and out of tissue, blood, and lymph nodes (migratory capacity) (10). Subsequent work has shown that there are three major lineages of DCs—myeloid DCs (mDC), plasmacytoid DCs (pDC), and Langerhans cells (LC).

Myeloid DCs are descendants of blood myeloid hematopoietic precursor cells (11–13). They respond to innate bacterial stimuli (14) through ligation of Toll-like receptors (TLR) 2 and TLR4 that recognize components of the bacterial cell wall peptidoglycan/lipoteichoic acid and lipopolysaccharide (LPS), respectively (15, 16). One proposed causative agent for sarcoidosis is *Mycobacterium tuberculosis*, which contains ligands for both TLR2 and TLR4 (17–20). TLR2/4 ligation induces up-regulation of MHC-II and co-stimulatory molecules that promote T cell activation (21), and increases expression of inflammatory cytokines TNF and iNOS (14).

Plasmacytoid DCs are phylogenetically most similar to lymphocytes (22) and produce large quantities of interferon- α (IFN- α) in response to viral invasion (23). Specifically, pDCs express TLR9, which recognizes viral DNA and CpG oligodeoxynucleotides, and TLR7, which recognizes single-stranded viral RNA (24, 25).

Langerhans cells are derived from multipotent bone marrow stem cells and monocytes (26–30) and are primarily located in the suprabasal layer of the epidermis and other stratified epithelium, where they are thought to both present foreign antigen (31) and tolerize to self-peptides (32, 33). Although LCs and pDCs have the capacity to induce T cell proliferation, only mDCs are found in abundance in sarcoidosis BAL fluid (34).

Like mDCs, macrophages are also descendants of a myeloid pluripotent precursor (35), but differ phenotypically and functionally. Despite similar stellate “dendritic” shapes and a common monocyte precursor, macrophages are much less immunostimulatory, express lower (mid) levels of MHC-II, and are less migratory than DCs (36, 37). As their name suggests, macrophages engulf large particles, such as a bacteria, virus, or necrotic tissue and “digest” these antigens in large lysosomal compartments (38). In the lung, macrophages also digest extra surfactant and dirt. Much of sarcoidosis research focuses on macrophages (39, 40), but in this article, we present data suggest-

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ing that the pathogenic cells are in fact immunostimulatory, inflammatory myeloid DCs that drive the adaptive Th1 immune response (41).

MYELOID DENDRITIC CELLS IN SARCOIDOSIS

DCs in BAL Fluid Are Representative of a Large Interstitial Lung DC Population

The lung is the most commonly affected organ in sarcoidosis (95% of patients), compared with 20% with cutaneous disease (2). Lung biopsy and BAL are frequently performed during the diagnosis of sarcoidosis to rule out respiratory infection and malignancy (2). Thus immunology studies on sarcoidosis consist of mostly *in situ* immunohistochemistry on lung biopsies, and functional studies using BAL fluid as the source of cellular material.

BAL fluid contains 85% alveolar macrophages as defined by high forward (FSC) and side scatter (SSC) flow cytometry, indicating large cell size and high cellular complexity, respectively (42). DCs only constitute approximately 1% of BAL fluid, as defined by lower FSC and SSC, and high expression of both MHC class II receptor HLA-DR and myeloid lineage integrin CD11c (34, 43). This relative paucity of DCs in the BAL does not, however, reflect a lack of DCs in the airway. An electron micrograph of a rat alveolus (Figure 1) shows a large alveolar macrophage filled with characteristic electron-dense cytoplasmic inclusions (lysosomes, phagosomes) nestled within the alveolar space, and a smaller interstitial DC (44). Quantitative analysis of the interstitial lung DC population density and distribution in rats using *in situ* immunohistochemistry demonstrates an approximate 1:1 ratio of interstitial DCs to alveolar macrophages (45). Interstitial DCs are functionally active and increase in number by 50% after inhalation of LPS antigen (46). Therefore, although relatively few DCs extravasate into the alveolar space, the BAL DC population is a reflection of a much

more abundant interstitial DC population that has the capacity to expand during inflammation.

Phenotypically Immature Myeloid DCs Are Enriched in Sarcoidosis BAL Fluid and Cutaneous Lesions, while Mature DCs Are Located in the Lymph Node

In sarcoidosis BAL fluid, there is a 2-fold enrichment of myeloid DCs compared with normal, a finding that was specific to sarcoidosis but not to other inflammatory lung diseases, including idiopathic pulmonary fibrosis and pneumonia. These inflammatory DCs have decreased expression of DC maturation marker CD83 and co-stimulatory molecule CD86 compared with normal, indicating a predominance of immature DCs in sarcoidosis BAL fluid (34).

As in the lung, immature DCs accumulate in cutaneous sarcoidosis granulomas (47). Subcutaneous injection of sarcoidosis granuloma distillate (Kveim reagent) into patients with sarcoidosis induces granulomas containing 2-fold more DCs than in foreign body reactions (48), suggesting that sarcoidosis granulomas are more DC trophic than other granulomatous reactions. These infiltrating DCs are immature, as evidenced by their lack of DC maturation markers CD83 and DC-lysosomal-associated membrane protein (DC-LAMP). However, after treatment with thalidomide, the number of DCs expressing CD83 and DC-LAMP increases, suggesting an inverse relationship between DCs maturity in the skin and disease activity (49). This seemingly paradoxical observation may result from a thalidomide-dependent interruption of mature DC migration to the lymph node, where they would otherwise present antigen to T cells and promote disease.

In contrast to immature DCs found in the lung and skin, many mature DC-LAMP⁺ DCs surround granulomas in the lymph nodes. Double label immunohistochemistry shows mature DCs and T cells situated in close proximity, suggesting that the DCs are actively presenting antigen and stimulating T cell proliferation (8). Thus, although DCs in sarcoidosis do not mature in the lung and skin, they do mature in the lymph node. This pattern deviates from other inflammatory skin diseases, such as psoriasis, where DC-LAMP and CD83⁺ cells collect in the cutaneous lesions (50), and may explain why patients with sarcoidosis are anergic and patients with psoriasis are not. Alternative explanations include the observation that CD4⁺CD25⁺FoxP3⁺ cells proliferate in patients with sarcoidosis both in the peripheral blood and at the periphery of granulomas (51). These cells exert an antiproliferative effect on naïve T cells, which may also be used to explain the presence of anergy in patients with sarcoidosis.

Sarcoidosis Lung and Peripheral Blood DCs Are Less Immunostimulatory than Normal DCs

Consistent with their immature phenotype, sarcoidosis lung DCs are less able to induce T cell proliferation than normal lung DCs. Autologous mixed leukocyte reactions (MLR) using unfractionated BAL fluid indicates that sarcoidosis BAL cells are less immunostimulatory (52) than normal, reflecting the decreased immunostimulatory capacity of sarcoidosis alveolar DCs and macrophages (42, 53). Using histoincompatibility-insensitive Jurkat indicator cells, however, one study showed increased IL-2 secretion after co-culture with sarcoidosis compared with normal alveolar macrophages (54). The immunostimulatory capacity of sarcoidosis BAL fluid is inhibited with anti-CD86, suggesting that their ability to induce T cell proliferation is dependent on CD86 co-stimulatory molecule (42). Myeloid DCs from sarcoidosis peripheral blood are also less immunostimulatory than normal blood DCs, and immunostimulatory capacity is directly correlated with ability to mount a delayed-type hyper-

Figure 1. Interstitial dendritic cells (DCs) and alveolar macrophages in the alveolus. Electron micrograph of rat alveolar septal junction showing a large macrophage (M) containing many electron-dense vacuoles, spread upon the type I epithelial lining of an alveolus, and a smaller DC (dc) with an irregularly shaped indented nucleus, located in the interstitial space. c, capillary. Reprinted with permission from Reference 45.

sensitivity (DTH) response (55). Decreased DC function is a potential mechanism for sarcoidosis-induced anergy, and may suggest that reduced cellular immunity at the end organs is responsible for disease perpetuation rather than resolution.

Proposed Hypotheses for Lack of Sarcoidosis DC Maturation

Although no data are available to explain the lack of maturation in cutaneous sarcoidosis DCs, two theories exist to explain the lack of lung DC maturation. These include short lung DC half-life, and DC suppression from alveolar macrophage anti-inflammatory cytokines. Mouse models using adoptive precursor cell transfer and conditional cell ablation techniques have shown that blood monocytes can replenish lung DCs during inflammation (56, 57). Moreover, in sarcoidosis, morphologic studies showed monocytes migrating from capillaries of the alveolar interstitium toward lung granulomas (58). However, inflammatory lung DCs have a relatively short half-life of only 2 days (59). This rapid transit time for development from blood monocytes into inflammatory lung DCs and subsequent cell death may explain the relatively immature state of lung DCs in sarcoidosis. Alternatively, sarcoidosis alveolar macrophages secrete anti-inflammatory diffusible factors, such as IL-10, prostaglandin E₂ (PGE₂), and inducible nitric oxide synthase (iNOS) (45, 60, 61). These mediators inhibit DC maturation and production of IL-12, a Th1 T cell–polarizing cytokine involved in sarcoidosis immunopathogenesis (6, 62). Therefore, although myeloid DCs accumulate in sarcoidosis lung, the DCs are phenotypically and functionally immature secondary to either intrinsic sarcoidosis DC characteristics or macrophage-mediated inhibition of maturation.

DCs Are More Immunostimulatory than Macrophages

Despite the somewhat blunted immunostimulatory capacity of DCs in sarcoidosis lung and blood, DCs are still more effective inducers of T cell proliferation than macrophages. DCs purified from normal BAL induce 5 to 10 times more T cell proliferation than alveolar macrophages. This result has been replicated using multiple DC purification techniques including “high-tech” bead selection and cell sorting (63), and “low-tech” density gradient fractionation (43, 64, 65). While DCs have not yet been isolated from sarcoidosis BAL fluid for functional studies, it is likely that they are more immunostimulatory than sarcoidosis alveolar macrophages.

Although alveolar macrophages have a limited capacity to stimulate naïve T cells, they are moderately more effective at stimulating primed T cell clones with corresponding HLA-DR type (66). These findings may be explained by the less stringent requirements for clonal T cell activation compared with naïve T cell activation. Considering the high proportion of clonal T cells in sarcoidosis BAL fluid (67), alveolar macrophages may selectively induce these memory cells to proliferate, although DCs are still necessary to initiate clonal expansion of naïve T cells in the lymph node (68, 69).

The Role of Cytokines Released by DCs in T Cell Activation and Leukocyte Trafficking

Apart from the DC's ability to stimulate T cell proliferation through direct T cell receptor and co-stimulatory molecule engagement, DCs secrete potent inflammatory mediators that polarize Th1 T cells and potentiate T cell proliferation and leukocyte chemotactic factors that promote granuloma formation. Most of the studies assessing cytokines and chemokines involved in sarcoidosis pathogenesis have used unfractionated BAL fluid containing both DCs and macrophages, or use plastic adherence purification that isolates both pre-DCs and pre-macrophages (70). Therefore, we now describe inflammatory

mediators overexpressed in sarcoidosis BAL that may be produced by either DCs, macrophages, or both.

Th1 T Cell Polarizing Cytokines

IL-12 is a Th1 T cell–polarizing cytokine (71, 72) constitutively expressed by DCs (9) and found at high levels in sarcoidosis BAL supernatant and at sites of Kveim skin reactions (6). DCs and macrophages in sarcoidosis BAL fluid spontaneously produce IL-12 (73–75), augmented by co-culture stimulation with IFN- γ , *Staphylococcus aureus* extract, or LPS (6). IL-12 amplifies its own response through induction of more IL-12 release from APCs and up-regulation of IL-12 β expression on activated T cells, further polarizing them toward the Th1 lineage. Moreover, Th2 T cells do not develop in the presence of IFN- γ (76). A similar self-amplifying scenario exists for the cytokine IL-18, whereby IL-18 released by DCs/macrophages acts on T cells through the IL-18R to up-regulate IL-12R β 2 and IFN- γ expression. IL-12 also induces IL-18R expression on Th1 T cells, creating a synergistic interaction between IL-12 and IL-18 that is thought to promote sarcoidosis pathogenesis (74, 77).

The functional importance of IFN- γ –producing Th1 T cells for sarcoidosis pathology was first established in IFN- γ knockout mouse models of hypersensitivity pneumonitis that lack granulomas (78). IFN- γ is also the most highly expressed cytokine in sarcoidosis BAL fluid (6). One proposed mechanism for involvement of IFN- γ in sarcoidosis pathogenesis is through inhibition of macrophage peroxisome proliferators–activated receptor γ (PPAR γ) expression (79)—an important negative regulator of inflammation (80). During normal steady-state conditions, PPAR γ is constitutively expressed by alveolar macrophages, promotes phagocytosis and IL-10 production (81), and inhibits DCs from releasing TNF, IL-12, and matrix metalloproteases (MMPs) (82) (Figure 2A). In sarcoidosis, normally immunosuppressive alveolar macrophages may not be able to control inflammatory DCs, contributing to excessive cellular and adaptive immune system activation (Figure 2B). MMP-8 and MMP-9 produced by activated alveolar macrophages are increased in sarcoidosis BAL fluid without compensatory increase in levels of tissue inhibitor of metalloproteinase. These MMPs then induce lung damage and subsequent fibrosis (83, 84). IFN- γ is also a direct inducer of DC/macrophage chemokines CXCL9, CXCL10, and CXCL11 that induce T cell chemotaxis by ligating the T cell receptor CXCR3 (85, 86). As expected, these IFN- γ –induced chemokines are elevated in sarcoidosis BAL fluid (87, 88).

Cytokines Promoting T Cell Proliferation and Survival

TNF produced by inflammatory DCs and macrophages (89–91) promotes T cell proliferation and survival, both indirectly by inducing DC maturation into potent antigen-presenting cells (70) and directly through induction of IL-2R on T cells (92, 93). IL-15 is also a trophic factor for T cells and, like IL-2, binds to the IL-2R to promote T cell survival (94).

The importance of TNF to sarcoidosis pathology is shown by the effectiveness of TNF inhibitors, particularly the anti-TNF chimeric monoclonal antibody infliximab/Remicade (95). Other effective treatments whose primary mechanism of action may be through TNF inhibition include phosphodiesterase inhibitor pentoxifylline/Trental (96, 97), and thalidomide (98, 99). Patients with sarcoidosis who continue to have elevated TNF production from alveolar macrophages during steroid treatment are 1.8 times more likely to relapse into active disease during a steroid taper, also suggesting that TNF production may be a critical cytokine for disease pathogenesis (100).

A Normal Steady-State

lung/skin

B Sarcoidosis Inflammation

lung/skin

lymph node

Figure 2. Critical role of DCs in sarcoidosis pathogenesis. (A) During normal steady-state, macrophages constitutively express peroxisome proliferator-activated receptor (PPAR) γ , a transcription factor that induces macrophage IL-10 production and inhibition of myeloid DCs. (B) During sarcoidosis antigen-driven inflammation (1), DCs are activated to mature and migrate to the lymph node (2), where they induce T cell expansion (3). They also release Th1 polarizing inflammatory mediators TNF, IL-12, and IL-18 (4). Th1 T cells express IFN- γ , which directly inhibits macrophage PPAR γ (5), resulting in decreased expression of immunosuppressive cytokine IL-10 and increased expression of inflammatory cytokines TNF, MMPs, and CXCLs chemokines (6). Decreased IL-10 and increased TNF expression releases DCs from macrophage inhibition, completing the self-amplifying inflammatory loop of sarcoidosis. In addition, MMPs damage lung tissue and promote fibrosis, and chemokines attract more myeloid cells and T cells promoting granuloma formation. Purple lines indicate cell migration to and from the lymph node; black lines indicate cytokines released from one cell acting upon another; arrowheads indicate cell activation; and flat lines indicate cell inhibition.

In summary, during sarcoidosis inflammation, a self-amplifying cycle is created where antigen-driven DCs migrate to the lymph node where they mature and polarize Th1 T cells that release IFN- γ , which activates alveolar macrophages to secrete TNF and other mediators of fibrosis, DC maturation, T cell survival and proliferation, and leukocyte chemotaxis (Figure 2B).

LANGERHANS CELLS AND PLASMACYTOID DCs IN SARCOIDOSIS

Langerhans Cells Do Not Play a Large Role in Sarcoidosis Pathogenesis

Apart from myeloid DCs, other DC subsets potentially involved in sarcoidosis pathogenesis are Langerhans cells (LCs), and plasmacytoid DC. LCs are primarily located in the basal layer of human epidermis, and were the first DC subset identifiable both by ultrastructural features and using monoclonal antibody labeling techniques for immunohistochemistry (101, 102). Their presence in sarcoidosis granulomas was probed by Munro and coworkers in 1987 (103). Using the NA1-34/OKT6 antibody later known as CD1a, an MHC-I-like molecule that presents microbial lipids to T cells (104), Munro and colleagues identified peri-granuloma LC infiltrate in only 1/6 lung and 1/3 lymph node granuloma samples. Using a more specific LC antibody, Langerin/CD207 C-type lectin (105), Smetana and coworkers found almost no Langerin⁺ cells in sarcoidosis BAL fluid (106), similar to results from normal BAL fluid (63). The paucity of LCs in sarcoidosis lung granulomas suggests that LCs do not play an active role in sarcoidosis lung pathology.

In contrast, there is an enrichment in NA1-34/OKT6/CD1a⁺ cells in the epidermis overlaying dermal granulomas and within the granulomas themselves (103, 107). However, as NA1-34/OKT6/CD1a is also a marker for dermal myeloid DCs (108), without immunohistochemistry using Langerin/CD207 LC-specific antibody, it is impossible to definitively conclude that the dermal cells infiltrating sarcoidosis granulomas are LCs. It is particularly interesting that epidermal LCs accumulate in the epidermis above

the granuloma, but not in the draining lymph nodes, considering that the standard model of skin inflammation involves LCs migrating from the epidermis to the lymph node to present antigen (109, 110). This typically results in a sparseness of epidermal LCs at the sight of inflammation with an increased abundance in draining lymph nodes (111). The inverse pattern in cutaneous sarcoidosis may suggest that epidermal LCs are not acting as antigen presenters at the lymph node. As previously described, myeloid DCs have an immature phenotype in sarcoidosis granulomas, and are abundant and mature in draining lymph nodes, supporting the role for mDC, not LCs in lymph node antigen presentation.

Plasmacytoid DCs Do Not Play a Large Role in Sarcoidosis Pathogenesis

Like Langerhans cells, plasmacytoid DCs do not appear to play a crucial role in sarcoidosis pathogenesis. In sarcoidosis BAL fluid (34) and in blood (55), pDCs appear in similar frequency and absolute numbers as in normals. Stimulation of sarcoidosis and normal blood pDCs with TLR9 ligand CpG-A showed no difference in IFN- α protein expression.

Despite the lack of evidence for increased pDC frequency or activation in sarcoidosis, IFN- α therapy is a well documented risk factor for development of IFN-induced sarcoidosis (IIS) (112, 113), occurring in 5% of patients with chronic hepatitis C treated with IFN- α (114). Also, an IFN- α 17 polymorphism, that shows increased IFN- α secretion when whole blood is activated with Sendai virus, has been documented in a cohort of Japanese patients with sarcoidosis (115). However, exogenous IFN- α -induced autoimmunity is not specific to sarcoidosis and has been implicated in inducing systemic lupus erythematosus, thyroiditis, and psoriasis vulgaris (116, 117). The mechanism by which IFN- α induces autoimmunity is still unclear, although IFN- α induces both MHC class II and co-stimulatory molecules on blood monocytes (118) and may enhance DC and macrophage antigen presentation to Th1 T cells (119). Th1 T cells are important mediators of sarcoidosis (62, 120), lupus (121), and psoriasis

immunopathogenesis (36, 50). IFN- α also directly increases IFN- γ and IL-2 production from T cells, thus promoting granuloma formation (122). Therefore, although pDCs are likely not causal to most patients' sarcoidosis, the IFN- α they secrete may potentiate the disease.

Genomic Signature of Myeloid DCs in Sarcoidosis

While a comprehensive review of sarcoidosis genetics has been recently published (123), we now focus on genetic associations that may be linked to myeloid DCs. Two genome wide scans have identified candidate genes within genomic loci linked to sarcoidosis. One study in 63 white German families with sarcoidosis found the greatest linkage on the short arm of chromosome 6 (6p21 to 6p22), a 16cM region containing both the MHC genes and TNF. Other minor loci included 3p21, 1p22, 9q33, Xq22, 7q22, and 7q36 (124). As previously described in this review, TNF is both a product and an activator of myeloid DCs. The second study was conducted in 229 African-American families, and identified a major linkage on 5q11 with minor loci at 3p21-14, 1p22, 9q34, 2p25, 5p15-13, 5q35, 11p15, and 20q13 (125). Both the white German and the African-American groups shared allele 3p21 containing chemokine receptors CCR2 and CCR5, which bind macrophage chemotactic protein 1 (MCP-1), promoting monocyte, macrophage, and DC recruitment into the lungs (64). CCR2^{-/-} mice are unable to control tuberculosis infection in the lungs secondary to impaired early recruitment of macrophages and later DCs (126). CCR5^{-/-} mice, however, retain the capacity to control tuberculosis infection, form granulomas, and induce a Th1 T cell response, making CCR2 the more likely sarcoidosis candidate gene (127).

Smaller studies probing individual candidate genes have identified TNF polymorphisms conferring sarcoidosis disease risk in British and Dutch (128), Japanese (129), and Indian patients (130). However, there is also evidence that TNF promoter polymorphisms may be irrelevant, or may predispose patients to variant forms of sarcoidosis, such as Lofgren syndrome (131–134). Other DC-related candidate genes include innate bacterial antigen receptors TLR2 (135) and TLR4 (136), although these genes have not yet been confirmed using unbiased genome-wide scans.

DISCUSSION AND FUTURE DIRECTIONS

DCs in sarcoidosis have paradoxical function. Although they have the capacity to initiate an antigen-driven, inflammatory oligoclonal T cell response (4), they are also anergic and less immunostimulatory than normal DCs (55). In this review of the literature, we show that DCs in sarcoidosis lymph nodes are mature, while those in the peripheral blood, lung, and skin are immature, suggesting a spatial segregation of DC function in sarcoidosis and a proposed resolution of the anergy paradox (8, 34, 47, 55). It is still unclear, however, what immunosuppressive factors in these end-organ sites are preventing DC maturation.

TNF is clearly an important mediator of sarcoidosis pathogenesis, as evidenced by high TNF concentrations in sarcoidosis BAL fluid that correlate with disease prognosis, genetic linkage, and the newly discovered therapeutic effects of TNF inhibitors (100, 137, 138). While TNF is produced by many cells, including DCs, macrophages, and T cells, TNF receptors are found in high abundance on DCs, making them a likely target for this inflammatory cytokine (139). Recent studies using TNF inhibitors to treat psoriasis, another autoimmune disease of the skin, identified DCs as the primary target of these drugs (50).

Fundamentally, sarcoidosis causation can be organized into two schools of thought: (1) extrinsic antigen-driven immune activation, and (2) intrinsic genetically determined overactiva-

tion of inflammatory pathways. Recently TLR2/4 ligand *Mycobacterium tuberculosis* (MTB) has been suggested as a causative agent of antigen-driven immune activation in sarcoidosis based on MTB nucleic acid identification within granulomas (140) and enhancement of proinflammatory cytokine release from sarcoidosis peripheral blood monocytes when incubated with MTB ligands (18) and antigens (141). In this review, we suggest that myeloid DCs are involved in pathogenesis, regardless of the initiating factor(s).

Although there is much evidence supporting the importance of DCs for the pathogenesis of sarcoidosis, many experiments must still be performed. In the lung BAL fluid, DCs can be separated from macrophages and tested for their immunostimulatory capacity. Similarly, immunostimulatory capacity of DC emigrés from sarcoidosis skin lesions could be tested against DCs from both normal skin and from other granulomatous skin diseases to determine if their decreased phenotypic maturity translates into decreased function. While most research proposes that macrophages are the major antigen-presenting cells in sarcoidosis, the relative immunostimulatory capacity of DCs versus alveolar macrophages has not yet been tested. A clinical trial treating patients with sarcoidosis with TNF inhibitors could be performed to determine if DCs are indeed the target cell for these drugs in sarcoidosis. Indeed, DCs in sarcoidosis will be an interesting field to study in the future.

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