Molecular profiling and gene expression analysis in cutaneous sarcoidosis: The role of interleukin-12, interleukin-23, and the T-helper 17 pathway

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Background: Cutaneous sarcoidosis (CS) skin provides relatively noninvasive access to granulomatous sarcoidosis tissue.

Objective: We sought to explore the role of the T-helper (Th)1 and Th17 pathways in sarcoidosis.

Methods: We used molecular profiling and gene expression analysis to analyze the Th1 and Th17 pathways and other immune-mediated pathways in CS. Molecular profiles were obtained from sarcoidosis skin lesions (lesional skin [LS]), unaffected skin from patients with CS (non-LS), and the skin of healthy control subjects. Whole blood was collected to compare the molecular profile of sarcoidosis skin lesions and whole blood.

Results: Twenty participants were enrolled: 15 with active CS and 5 healthy volunteers. Microarray analyses comparing non-LS and healthy volunteer skin with LS showed several thousand genes differentially expressed ($\geq$ 2-fold change false discovery rate, $P < .01$). Targeted selections of genes associated with Th1 and Th17 phenotypes showed a strong Th1 profile of sarcoidosis and expression of interleukin (IL)-23 and IL-23R with limited expression of other Th17 pathway genes. IL-21 and signal transducer and activator of transcription 3 (STAT3) were also dysregulated in skin and whole blood, providing additional evidence for involvement of the IL-12 pathway and potential activation of the Th17 pathway.

Limitations: Measurements were made at a single point in time and may not identify mechanisms that may be identified in patients followed up longitudinally.

Conclusion: These findings provide novel insight into the dysregulated pathways that may be involved in the pathogenesis of sarcoidosis. (J Am Acad Dermatol 2012;66:901-10.)

Key words: interferon-gamma; interleukin-12; interleukin-21; interleukin-23; lesional sarcoidosis; nonlesional sarcoidosis.

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Sarcoidosis is a multisystem granulomatous disease of unknown cause. As with known granulomatous diseases, sarcoidosis is thought to result from contact of an external antigen with antigen-presenting cells. The antigen-presenting cells process and present the antigen via HLA class II molecules to T-cell receptors attached to T lymphocytes.[1] Once this interaction occurs, the antigen-presenting cell and T lymphocytes secrete various cytokines of the T-helper (Th)1 class, resulting in the formation of granulomas. Cytokines such as tumor necrosis factor (TNF)-alpha and interleukin (IL)-12 are important Th1 cytokines that are released during the formation of sarcoid granulomas.[2-7] TNF antagonists have demonstrated some success in the treatment of sarcoidosis, including cutaneous forms.[8] Although IL-12 has been reported to be up-regulated in pulmonary sarcoidosis, its role in the development of skin sarcoidosis has not been elucidated.

CAPSULE SUMMARY

- Genes involved in the T-helper 1 and T-helper 17 pathways were up-regulated in the skin lesions of patients with sarcoidosis.
- These genes were differentially up-regulated in sarcoidosis skin lesions compared with nonlesional skin of these patients and the skin of healthy control subjects.
- Gene profiling in skin and blood suggests there is a potential for determining whether therapy that blocks particular pathways will be useful in assessing the sarcoidosis treatment response.

Evaluating the role of IL-12 has been complicated by the identification of IL-23, a heterodimeric cytokine comprising the common p40 subunit of IL-12 but binding a specific p19 subunit. IL-23 is a key cytokine in the Th17 pathway.[9] Although IL-12 has been linked to sarcoidosis, the role of IL-23 or the Th17 pathway, including IL-17, has not been examined in this disease.

Much of the information concerning cytokine networks involved in sarcoidosis is derived from samples obtained from the lung or periphery (ie, serum, plasma, peripheral blood).[10] Lung samples have predominantly been obtained by bronchoalveolar lavage. Because pulmonary sarcoidosis is a patchy disease, such samples contain material from involved and uninvolved lung segments.[11] In addition, the assessment of pulmonary activity in sarcoidosis is problematic.[12] Biopsy specimen of sarcoidosis skin lesions provides a sample with active granulomatous inflammation. In addition, the activity of sarcoidosis skin lesions is readily assessable. Furthermore, biopsy specimen of unaffected skin in a patient with cutaneous sarcoidosis (CS) allows each patient to serve as his/her own control. These facts suggest that analysis of granulomas from sarcoidosis skin lesions may reflect active mechanisms of disease more accurately than from sarcoidosis lung lesions. To this end, we assessed the expression of IL-12, IL-23, and the Th17 pathway in sarcoidosis skin lesions by comparing their expression in lesions versus areas of uninvolved skin of patients with sarcoidosis, the skin of healthy volunteers, and in whole blood from these patients.

METHODS

This study was approved by the Medical University of South Carolina Institutional Review Board. Patients aged 18 to 80 years were eligible for this study if they had biopsy-confirmed sarcoidosis; visual evidence of facial lupus pernio skin lesions or active sarcoidosis skin lesions over the remainder of the body; and a primary skin lesion for assessment (target lesion) that could be photographed. Control subjects were healthy volunteers aged 18 to 80 years. Patients with sarcoidosis and healthy volunteers were excluded if they were pregnant; had a history of keloid formation or psoriasis; had active tuberculosis or nonsarcoidosis active granulomatous disease; or had an underlying medical condition that, in the opinion of the investigators, would place them at undue risk or preclude the completion of follow-up measurements. Organ involvement in patients with sarcoidosis was determined using an established protocol.[13]

All patients with sarcoidosis had the skin lesions that were biopsied and assessed using a standardized visual evaluation system (the Sarcoidosis Activity and Severity Index scoring system).[14] In patients given the diagnosis of lupus pernio, a skin biopsy (two adjacent 3 mm) was performed on a facial lesion. Patients with sarcoidosis skin lesions on areas other than the face had a skin biopsy (two adjacent 3 mm) performed on lesional skin (LS) and two additional adjacent biopsies performed in an area of non-LS (NLS). Whenever possible, the NLS biopsy was performed at a site contralateral to that of the LS biopsy. Biopsies for healthy volunteer skin (two adjacent 3 mm) were performed in the axilla to minimize the cosmetic impact. Absorbable sutures were used for all biopsies. A total of 12 mL of blood was phlebotomized from each participant. Participants were contacted by telephone 7 to 10