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Original contribution

Granulomas are a source of interleukin-33 expression in pulmonary and extrapulmonary sarcoidosis☆

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Summary Sarcoidosis is a chronic inflammatory disease characterized by noncaseating epithelioid granulomas. These granulomas consist of highly differentiated mononuclear phagocytes—epithelioid cells and multinucleated giant cells (MNGCs)—surrounded by a proinflammatory infiltrate. Interleukin-33 (IL-33) is an inflammatory cytokine that is constitutively expressed in barrier tissues such as skin and lung and up-regulated in inflammation. Because sarcoidosis occurs most frequently in lung and skin, we studied the expression of this cytokine by immunohistochemistry in these tissues from patients with sarcoidosis, with foreign body granulomas, with other granulomatous diseases, and in corresponding normal tissues. We identified nuclear IL-33 staining of epithelioid cells and MNGCs in biopsies of skin (18/25 patients, 72%) and lung (10/19 patients, 53%) sarcoidosis. In contrast, sarcoidal granulomas in lymph nodes did not show IL-33 expression. Other granulomatous diseases showed only occasional and weak IL-33 expression. In sarcoidosis, we found a strong correlation between IL-33 expression and systemic disease, presence of MNGCs, and an M2-like macrophage phenotype as assessed by CD163 staining. Therefore, we propose that IL-33 plays a critical role in pathogenesis and disease progression of sarcoidosis. Because IL-33 is less commonly and only weakly expressed in other granulomatous diseases, the detection of IL-33 might serve as an adjunctive diagnostic marker. IL-33 expression in sarcoidosis seems to be dependent on the specific tissue microenvironment of sarcoidal granulomas and represents a novel biomarker for systemic involvement.

1. Introduction

Granulomas represent compact aggregations of mononuclear phagocytes and their derivatives including epitheloid cells and multinucleated giant cells (MNGCs). Granuloma formation in chronic immune responses is thought to occur as foreign particles or microorganisms get encapsulated to prevent their systemic spread [1,2]. Most granulomas are associated with proinflammatory M1-type cytokines such as tumor necrosis factor (TNF) α [1,3]. Macrophage fusion and fibrosis are instead associated with M2-type cytokines such

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as IL-13 [4]. Because granulomatous diseases contain both M1- and M2-type cytokines, it is important to understand the local tissue milieu in these diseases and to elucidate whether it favors M1 or M2 responses.

Sarcoidosis is a granulomatous inflammatory disorder of unknown etiology. It most commonly affects the lungs, skin, intrathoracic lymph nodes and eyes [5]. Research of sarcoidosis is hindered by striking heterogeneity in its clinical manifestations [1]. The disease either resolves spontaneously or develops into a more chronic disease, where the sarcoidal granulomas are accompanied by fibrotic changes that eventually result in loss of organ function. Thus, a key priority in sarcoidosis research is to understand the molecular mechanisms of fibrogenesis and to design antifibrotic therapies [6].

Interleukin-33 (IL-33) is a member of the IL-1 family. It binds to a heterodimeric receptor consisting of ST2 and IL-1 receptor accessory protein (IL-1RAcP) and leads to NF-κB and mitogen-activated protein kinase activation [7,8]. IL-33 is released upon tissue damage and may function as a nuclear alarmin [9]. Unlike the other IL-1 family members IL-1α, IL-1β, and IL-18, it is involved in T-helper (T_H) 2 immune responses that are characterized by a cytokine profile similar to M2 responses. It can induce chemotaxis of T_H2 lymphocytes and drive production of T_H2-associated cytokines in mast cells, basophils, and T_H2 cells themselves [10,11]. In addition, IL-33 promotes the activation of other cells that are involved in a T_H2-type inflammatory response, such as mast cells and eosinophils, and it acts as an amplifier of M2 macrophage polarization [8,12,13].

IL-33 is constitutively expressed in nuclei of epithelial or endothelial barrier cells and up-regulated in some granulomatous diseases of these tissues such as giant cell arteritis and Crohn disease [9,13-17]. Therefore, we hypothesized that IL-33 might be generally highly expressed in granulomas as a marker of granuloma reactivation, particularly in barrier tissue, and could be used as an indicator for the activity of granulomas.

In the context of giant cell arteritis, IL-33 immunoreactivity has been associated with an M2 phenotype [13]. We extended this finding to other granulomatous diseases of skin, lung, and lymph nodes. In sarcoidosis and foreign body granulomas, we found a correlation between IL-33 expression and CD163 but not CD206 immunostaining, consistent with local M2 macrophage polarization. Therefore, IL-33 expression may be involved in an M2-polarizing cytokine milieu. Because IL-33 was most consistently detected in sarcoidal granulomas of various tissues, our findings implicate a potential role of IL-33 in pathogenesis and progression of sarcoidosis specifically. In addition, because the heterogeneous staining for IL-33 in sarcoidal granulomas pointed to a stage-specific involvement, we found that its expression was most strongly correlated with systemic disease.

Table 1 Clinical and pathologic features of 49 patients with sarcoidosis with a total of 54 biopsy samples representing different tissues

Parameter	No. of patients/biopsies
Systemic disease	
Yes	26
No	12
Unknown	11
Localization of biopsy material	
Skin	25
Lung	19
Lymph nodes	7
Liver	2
Stomach	1
MNGCs	
Present	32
Absent	22
IL-33 expression in MNGCs	
Yes	14
No	18

Abbreviation: *MNGC*, multinucleated giant cells.

2. Materials and methods

2.1. Patient selection

After approval by the Ethics Review Board Basel, archived formalin-fixed and paraffin-embedded tissues representing sarcoidosis (n = 54), foreign body granulomatous reaction (FBGR, n = 22), and other granulomatous diseases (n = 9) as well as corresponding normal tissues (skin, lung, lymph node; n = 15) were reviewed and selected for our study by 1 board-certified pathologist (K.D.M.) and 1 board-certified dermatopathologist (W.K.). Hematoxylin and eosin staining was performed under standard conditions. Clinicopathological patient characteristics are summarized in Tables 1 and 2.

We examined biopsy and surgical samples of skin (n = 25), lung (n = 19), lymph nodes (n = 7), liver (n = 2), and stomach (n = 1) from 49 patients (21 males, 28 females) with sarcoidosis (Table 1). The median age of these patients was 48 years (range, 5-73 years). The diagnosis of sarcoidosis was based on the clinical picture, no evidence of concomitant infection by *Mycobacterium tuberculosis* or other organisms known to produce granulomatous diseases, and the presence of noncaseating granulomas in biopsy or surgical specimens of involved tissues, according to the criteria published in 1999 [18]. If skin or lungs/mediastinal lymph nodes were the only affected organs and there was no evidence of involvement of other organs, sarcoidosis was classified as localized disease. If more than 1 organ system was involved as assessed by imaging and/or histopathologic examination, the patient was diagnosed with systemic sarcoidosis. In both sarcoidosis and all other granulomatous diseases, granuloma-associated MNGCs were independently documented as present or absent by W.K. and K.D.M.

We also evaluated biopsy and surgical samples of skin (n = 18), peritoneum (n = 2), and joint (n = 2) from 22 patients with FBGR; skin biopsies (n = 3) from 3 patients