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Alveolar and intraparenchymal proteasome in sarcoidosis

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Summary
Background: In sarcoidosis, an antigen specific immune response is a putative mechanism, resulting in granulomatous inflammation. Since the proteasome is involved in antigen presentation, alterations in the alveolar and parenchymal proteasomal system may be a feature of sarcoidosis.
Objectives: To explore the role of proteasomes and immunoproteasomes in sarcoidosis.
Methods: Total proteasome concentration and activity was assessed in bronchoalveolar lavage (BAL) supernatant obtained from sarcoidosis patients ($n = 67$) and healthy controls ($n = 18$) using ELISA and cleavage of specific fluorogenic substrates (±epoxomicin), respectively. Immunohistochemistry of lung tissue sections and immunocytochemistry of BAL macrophages for immunoproteasome was performed in sarcoidosis patients and controls.
Results: Proteasome was present in BAL supernatants of all sarcoidosis patients. In sarcoidosis, abundant immunoproteasome staining was seen in pneumocytes type II and granulomas. Total proteasome concentration was greater in active sarcoidosis, stages II (101 ng/ml ± 79; $p = 0.009$) and III (119 ng/ml ± 66; $p = 0.012$), than in inactive sarcoidosis or in healthy

Abbreviations: TNFα, tumor necrosis factor α; IL, interleukin; IFNγ, interferon γ; ARDS, Adult Respiratory Distress Syndrome; SD, standard deviation; FVC, forced vital capacity; DCLO, diffusing capacity of the lung for carbon monoxide; BAL, bronchoalveolar lavage; LDH, lactate dehydrogenase.

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controls (35 ng/ml ± 34). In the absence of epoxomicin, all fluorogenic substrates were hydrolyzed by BAL supernatant of sarcoidosis patients and controls.
Conclusions: Patients with active sarcoidosis but not healthy controls demonstrate immunoproteasome in the lung tissue and in granulomas. Thus, the putative immune response in sarcoidosis may be mediated or sustained by the proteasomal system.

Introduction

Sarcoidosis is a systemic disorder of unknown etiology characterized by noncaseating epithelioid cell granuloma formation in affected organs including the lung [1]. Sarcoidosis is believed to result from an antigen-specific immune and inflammatory response to a yet unidentified antigen [2,3]. Regardless of the specific etiologic basis of the granulomatous inflammation, the pathologic changes observed are suggestive of an exaggerated immune response, and a marked increase in the CD4/CD8 T lymphocyte ratio is observed in affected organs including the lung. Effector cells produce pro-inflammatory cytokines, such as Tumor Necrosis Factor α (TNF-α), interleukin-2 (IL-2), IL-15, IL-18, and interferon-γ (IFN-γ) resulting in a Th1 pattern of cytokine production [4—6]. INF-γ has also been noted at sites of disease activity and is produced by CD4-positive Th1 cells. Production of INF-γ along with other lymphokines induces uncommitted CD4-positive T cells to differentiate into Th1 effector cells and evokes greater production of interferon [5]. This inflammatory feed-back loop may eventually result in the granulomatous response.

It is unknown whether the antigen presumed to evoke sarcoidosis is processed by the proteasomal system, a system involved in many basic cellular processes [7—10] and in antigen presentation [11]. However, since normal proteasomal composition is altered by exposure to TNF-α and IFN-y [12], resulting in the synthesis of immunoproteasomes [11], and both cytokines are believed to be important mediators in sarcoidosis, there might be a link between sarcoidosis and the immunoproteasome.

The standard 20S proteasome is a multicatalytic enzyme complex responsible for the degradation of most intracellular proteins and is involved in immune, inflammatory responses and antigen presentation [7—10]. The 20S proteasome is a 660—700 kDa multicatalytic proteinase complex with a cylinder shaped structure composed of seven α subunits (outer rings) and seven β subunits (inner rings), respectively [13]. Its proteolytic activities are characterized by trypsin-, chymotrypsin-, and caspase-like properties and are exclusively associated with proteasome subunits β_1, β_2 and β_5.

In cells exposed to IFN-γ and TNF-α, catalytic proteasomal subunits β_{1i} (LMP2), β_{2i} (MECL-1), and β_{5i} (LMP7) are expressed that replace the constitutive β subunits of the standard proteasome during proteasome biogenesis thus forming immunoproteasome [14]. This immunoproteasome efficiently generates peptides that are loaded on major histocompatibility complex class-I molecules and presented to cytotoxic T-lymphocytes [15].

Although it was a prior paradigm that the proteasome is present only intracellularly, it is now accepted that the proteasome also exists in the extracellular space [16] such as in plasma or sperm. We reported the extracellular presence of biologically active 20S proteasome in the alveolar space of healthy subjects [17] and, in much greater concentrations, in patients with Adult Respiratory Distress Syndrome (ARDS) [18,19].

Since sarcoidosis is characterized by an alveolitis with secretion of proinflammatory mediators [20], we tested the hypothesis that 1) proteasome is detectable extracellularly in the alveolar space in pulmonary sarcoidosis but not in healthy controls; and 2) immunoproteasome is present in lung tissues affected by sarcoidosis.

Methods

Patients and clinical procedures

67 adult patients with sarcoidosis (36 males, 31 females, mean age: 50years ± 14.5 standard deviation; SD) were studied prospectively. The diagnosis was established by one of the authors (U.C.) on the basis of clinical and radiographic features, histological evidence of noncaseating granulomas in transbronchial biopsy specimen, and the exclusion of other granulomatous lung diseases [20]. 27 patients were considered to have active disease and 40 to have inactive sarcoidosis at the time studied. The criteria for active disease considering the preceding 3 months were: 1) recently developed or worsening clinical features and/or 2) worsening of lung function tests (decrease of the forced vital capacity/FVC by $\geq$10% and/or decrease of diffusing capacity of carbon monoxide/DLCO by $\geq$15%); and/or 3) progression of chest radiographic findings, i.e., increase in densities or new densities (no quantification done). According to these criteria, 27 patients had worsening clinical features and active disease; 10 of these had worsening of lung function, and 22 patients had progression shown by chest X-ray, no patient received corticosteroids. 33% of sarcoidosis patients were ex-smokers, 55% were non-smokers, and 12% were smokers.

Eighteen adult, non-smoking subjects (8 males, 10 females, mean age: 48years ± 15), free of lung, cardiac, infectious, and allergic disease, and without a history of chemotherapy or radiation therapy served as controls. Characteristics of sarcoidosis patients and healthy controls are depicted in Table 1. The study was approved by the Ethical Committee of the Medical Faculty of the University of Duisburg-Essen.