SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2013; 30; 194-200

Oncostatin M in bronchoalveolar lavage correlates with the severity of sarcoidosis

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Abstract. *Background:* Recent studies have shown that oncostatin M (OSM) might have a role in T cell-mediated inflammatory processes in which mast cells are also involved. Patients with severe sarcoidosis might develop fibrotic changes in the lung. We assessed whether there was a correlation between mast cells expressing OSM in bronchoalveolar lavage (BAL) and the severity of sarcoidosis. *Patients and methods:* Twelve consecutive patients with new diagnosis of sarcoidosis were eligible for the study. All underwent complete lung function tests, angiotensin converting enzyme (ACE), and bronchoscopy that included BAL and biopsies. Cytospins of BAL were prepared. All samples were incubated with the primary antibody rabbit anti-human c-kit, CD117 and stained for total mast cell count. The mouse anti-human Oncostatin M was applied and activated mast cells were counted. Clinical sarcoidosis parameters including ACE and lung functions were correlated with mast cells in BAL, as well as with OSM positive mast cells. *Results:* FEV1 % was correlated with the percentage of activated mast cells, as well as with the percentage of OSM positive mast cells (r=0.61, p=0.033, 95% CI: 0.06-0.87; r=0.58, p=0.04, 95% CI: 0.015-0.86, respectively). FVC and FEV1/FVC correlated with activated mast cells (r=0.58, p=0.05; r=0.63, p=0.028, respectively). *Conclusions:* Direct correlation was found between clinical parameters including lung function tests (FEV1 and FVC) and OSM secretion from mast cells in patients with sarcoidosis. These findings suggest that mast cells and OSM have a role in sarcoidosis. Further studies to confirm these preliminary results are suggested. *(Sarcoidosis Vasc Diffuse Lung Dis 2013; 30: 194-200)*

Key words: sarcoidosis, oncostatin M, mast cell, fibrosis, lung function

Introduction

Many cells participate in fibrotic processes in the lungs; primarily fibroblasts, macrophages, and mast cells (1), orchestrated by T cells (activated CD8+ and T cells). They are cellular sources of profibrotic cytokines and chemokines in lung disease. These cytokines include transforming growth factor (TGF)-β, connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF) and oncostatin M (OSM) (2-5). Additional potential cellular sources of these profibrotic cytokines and chemokines in lung disease can be eosinophils, mast cells, and epithelial cells (6,7).

Most commonly known for their role in the elicitation of IgE-mediated allergic inflammation, mast cells have been implicated in a range of other non-allergic inflammatory processes (8,9). Observations such

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as the close physical proximity between mast cells and T cells in inflamed tissues, and the capability of the former to release a wide range of immunomodulatory mediators and to express surface molecules important in costimulation in both adaptive and innate immunity, have led investigators to propose a functional relationship between these two cell populations. Indeed, morphologic studies have documented an increase in the local density of mast cells and their activation during T cell-mediated inflammatory processes, as observed in cutaneous delayed-type hypersensitivity, graft-versus-host reactions, sarcoidosis, Crohn's disease, rheumatoid arthritis, and fibrosis (9-11).

We have previously reported on the effects of direct contact between mast cells and T lymphocytes on mast cell activation and mediator release (12). Mast cells were found to degranulate in response to direct contact with activated T cells or their membranes, and to produce cytokines, such as TNF-α and IL-8. Thus, direct contact between surface molecules on mast cells and on activated T cells was found to provide the stimulatory signal in mast cells necessary for degranulation and cytokine release, independent of T cell intracellular function and in the absence of demonstrable soluble mediators.

Oncostatin M (OSM) is a multifunctional cytokine that belongs to the IL-6 subfamily and is produced mainly by activated T cells, neutrophils, monocytes, and macrophages (13). More recently, it has also been demonstrated that OSM is produced by dendritic cells. However, and in contrast to other members of the IL-6 family, there is no evidence to suggest that OSM is produced by resident cells, such as mast cells or smooth muscle cells. Because of its pleiotropic nature, a vast array of biologic activities are exhibited by OSM. These activities include inhibition of tumor cell growth, induction of neurotrophic peptides, stimulation of fibroblast proliferation and collagen production, regulation of cholesterol metabolism, and induction of cartilage destruction (14-15).

Recent studies have shown that OSM might have a role in T cell-mediated inflammatory processes in which mast cells have also been found to be involved, including rheumatoid arthritis, multiple sclerosis, and pulmonary fibrosis (13-15). Sarcoidosis, in less than 15% of patients becomes a chronic process with devastating, fibrosing changes in the lung. Our assumption was that increased levels of mast cells expressing OSM in the bronchoalveolar lavage (BAL) of patients with sarcoidosis would correlate with continuing chronic inflammation and fibrosis that could be seen on pulmonary function testing.

Patients and Methods

Study population

The first 12 consecutive patients with a new diagnosis of sarcoidosis who presented at the Pulmonary Department between November 2010 and May 2011 were eligible for the study.

The eligibility included all patients with clinical and radiological findings of sarcoidosis that underwent transbronchial biopsy and found to be sarcoidosis The diagnosis of sarcoidosis was based on the ATS statement (16). Patients younger than 18 years were excluded. The study was approved by the Ethics Committee of Meir Medical Center and all patients signed an informed consent.

All patients underwent bronchoscopy for diagnosis with transbronchial biopsies (TBB) and BAL. During bronchoscopy, 6 biopsies were taken from the most involved side. The BAL was performed before the biopsies and on the same side, during bronchoscopy for cytological diagnosis. Differential count of cells harvested from lavage and CD4/CD8 lymphocytic pattern was done by flow cytometry. Serum was taken for angiotensin converting enzyme (ACE).

The laboratories results were assessed by two readers. Both were blinded to the pulmonary function test results.

Pulmonary function tests

The pulmonary function tests *(*PFTs) were performed according to the ATS/European Respiratory Society (ERS) guidelines (17-19) using the Medical Graphics Pulmonary Function System (1070-series 2, St. Paul, MN, USA). Lung volumes were obtained by body plethysmography (model 1085, Medical Graphics). Maximal voluntary ventilation (MVV) was assessed by asking the patient to breathe as fast and as deeply as possible for 12 sec, and the result was multiplied by 5 to obtain a 1-min measure. Carbon monoxide diffusion capacity (DLCO) was measured with a gas mixture containing air, 10% helium, and 0.3% carbon monoxide; each measurement was adjusted to

standard temperature and pressure. The predicted values of the pulmonary function parameters were obtained from the regression equations of the European Community for Coal and Steel.

Immunocytochemistry

Cytospins of BAL from patients with pulmonary sarcoidosis were prepared (5 min, 1000 rpm). Cells were fixed in cold methanol (BIO Lab Ltd., Jerusalem, Israel) for 5 min. Following PBS rinsing, endogenous peroxide activity was quenched in 1% H_2O_2 (diluted in PBS) and rinsed with PBS. The cells were then permeabilized with 0.2% Triton X-100 (Sigma Aldrich) for 5 min at room temperature and rinsed three times with PBS. Samples were covered with Axioskop microscope (Zeiss, Oberkochen, Germany). Images were taken using a digital normal blocker serum, immersed in PBS, and incubated with the primary mouse anti-human mast cell tryptase (CHEMICON International, Inc., Temecula, CA) overnight at 4°C. Then, the slides were rinsed and incubated with broad spectrum polyHRP conjugate for 30 min at room temperature, rinsed with PBS, and covered with AEC-chromogen (both materials are from Zymed laboratories, San Francisco, CA). For double staining procedure, following AEC development, slides were covered with denaturing solution (Biocare Medical, Concord, CA) and incubated with the second primary antibody mouse anti-human OSM (R&D Systems, Minneapolis, MN) for 2 h at room temperature. Next, the slides were incubated with biotinylated antibody (Biocare Medical), washed and covered with alkaline phosphatase streptavidin (Zymed), and developed with BCIP/NBT-chromogen (Chemicon). Irrelevant isotype-matched antibodies were used to exclude non-specific staining.

Slides were examined using a 40X objective under a Zeiss camera (C-5050ZOOM; Olympus Optical Co., Ltd., Tokyo, Japan). Brightness and contrast levels were achieved using the PhotoImpact Bundled Ed., Version 5.0 (Ulead Systems, Inc., Taipei, Taiwan) program.

Immunohistochemistry

Paraffin-embedded sections of lung biopsies from patients with sarcoid granuloma were deparaffinized in xylene, dehydrated in ethanol, rinsed with PBS, immersed in EDTA solution(pH 8), and heated in a microwave oven. Endogenous peroxide activity was quenched in 1% H_2O_2. Samples were covered with blocker serum, and incubated with the primary antibody rabbit anti-human c-kit, CD117 (DAKO, Glostrup, Denmark) overnight at 4°C. Then, the slides were rinsed and incubated with biotinylated antibody (Biocare Medical), washed and covered with alkaline phosphatase streptavidin (Zymed), and developed with BCIP/NBT-chromogen (Chemicon). For double staining procedure, following BCIP/NBT development slides were covered with denaturing solution (Biocare Medical) and incubated with the second primary antibody mouse anti-human OSM (R&D Systems) for 2 h at room temperature. Next, the slides were incubated with Broad Spectrum polyHRP conjugate for 30 min at room temperature, rinsed with PBS, and covered with AEC-chromogen (Zymed). Slides were examined using a 40X objective under a BX41 microscope (Figure 1). Images were taken using a DP70 digital camera and the image acquisition software DP Controller (all from Olympus). Brightness and contrast level were achieved using the PhotoImpact Bundled Ed. (Ulead Systems, Inc.) program.

Statistical analysis

The SPSS 14 statistical software package was used to evaluate the data.

A Mann-Whitney test and t test were used to compare all data from the same patient – (correlation

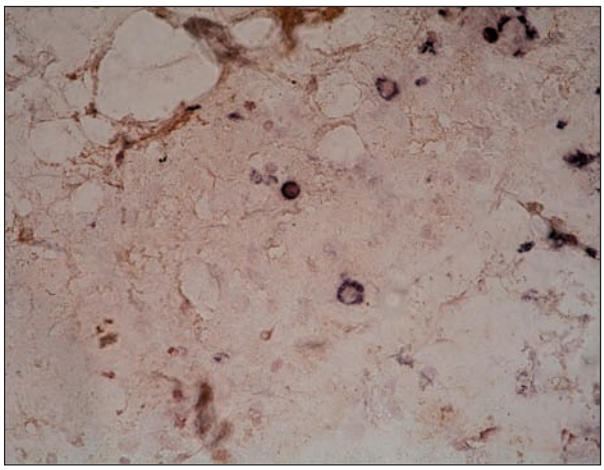

Fig. 1. Pulmonary activated mast cells from patients with sarcoidosis express oncostatin M.

between Oncostatin M expression and ACE values, FEV1, FVC, CD4/CD8). Results were given as mean ± standard deviation. P values less than or equal to 0.05 were considered statistically significant.

Results

The study group included 12 patients with sarcoidosis. Six (50%) were male. Table 1 summarizes the clinical characteristics of the study population. As noted, all patients had restrictive lung disease with decreased diffusion capacity. Four patients (33%) were smokers. Eight patients presented with unproductive cough and four patients developed gradual dyspnea. None had a history of B symptoms or weight loss. The mean BMI was 26.22 kg/m^2.

All had radiological findings of bilateral lymphadenopathy suggesting sarcoidosis.

All patients underwent a bronchoscopy before specific treatment was given.

BAL analysis results, including mean CD4, CD8, and CD3 were 50±0.15, 50±0.14, and 95±0.024, respectively. Differential analysis of BAL cell count revealed mean percentage of macrophages of 60±0.29%, neutrophils 28±0.31%, lymphocytes14±0.11% and eosinophiles, 1.0±0.5%. None correlated with clinical parameters.

Table 2 summarizes the functional parameters, as well as the mast cell positive OSM data of the patients with sarcoidosis.

Table 1. Clinical characteristics of patients with sarcoidosis (n=12)

Parameters	Value
(mean ± standard deviation)	
Age, years	45±13
Mean time from symptoms, months	4±2
Smokers, number	4 (33%)
Mean BMI, kg/m2	26.2
Angiotensin converting enzyme	62±22
Stage according to CXR	
Stage I	2
Stage II	7
Stage III	3
FEV1, liter	2.7±0.94
FEV1, % predicted	87±25
FVC, liter	3.48±1.0
DLCO, % predicted	73±11

FVC=Forced vital capacity; FEV1=Forced end expiratory volume at 1 second; DLCO=Diffusion capacity of CO

Table 2. Functional parameters and mast cell positive oncostatin M data of patients with sarcoidosis (n=12)

No.	Age	% of mast cells/slide	Number mast cells/slide	% Mast OSM+/slide	ACE	FVC (liter)	FEV1 % Predicted	DLCO % Predicted
1	35	4.9	295	78	50	4.6	90	81
2	26	7.7	80	94	40	3.5	101	70
3	27	1.5	101	24	83	4.2	88	78
4	59	0.9	54	33	84	3.0	67	71
5	64	4.5	410	80	29	3.5	138	57
6	49	1.6	51	10	52	2.7	43	81
7	38	1.3	15	13	76	3.3	98	70
8	55	1.7	45	47	84	2.6	95	92
9	56	1.8	99	36	81	1.3	91	65
10	57	0.7	6	17	77	3.4	79	86
11	41	1.3	68	50	76	4.9	99	51
12	34	0.5	19	74	82	4.7	80	74
Average	45	2.4	104	46	78	3.5	87	73

OSM=Oncostatin M; ACE=Angiotensin converting enzyme; FVC=Forced vital capacity; FEV1=Forced end expiratory volume at 1 second; DLCO=Diffusion capacity of CO

Correlations between lung function parameters and OSM cytokine findings

Figure 2 present the correlations between FEV% and the OSM. As presented, FEV1% was correlated with the percentage of activated mast cells, as well as with the percentage of mast cell OSM positive (r=0.61, p=0.033, 95% CI: 0.06-0.87; r=0.58, p=0.04, 95% CI: 0.015-0.86, respectively). FVC and FEV1/FVC were also correlated both with activated mast cells (p=0.05, r=0.58; and r=0.63, p=0.028) respectively. No correlation was noted between BM and smokng status and OSM or mast cells and OSM.

Discussion

In the last two decades, a large number of cytokines were found and classified into several families based on their structural properties, as well as their receptor components (20, 21). OSM is a multifunctional cytokine that belongs to the interleukin 6 subfamily (6). Since OSM is the most closely related to leukemia inhibitory factor (LIF) structurally, functionally, and genetically, among the family members, OSM had long been considered as another LIF. However, OSM exhibits unique activities that are not shared with LIF. Accumulating evidence indicates that OSM is a unique cytokine that plays an important role in various biological systems, such as inflammatory response and tissue remodeling and development (6,12).

In this study, we found direct correlations between clinical parameters including lung function tests (FEV1 and FVC) and OSM secretion from mast cell in patients with sarcoidosis. To the best of our knowledge, this is the first report of these findings.

Our observations were based on the role of mast cells in inflammatory disease and fibrotic processes (5-8, 10). In addition to being a major effector cell in the elicitation of allergic inflammation, mast cells have been found to be activated in various T cell-mediated inflammatory processes and to reside in close physical proximity to T cells (21). Such observations

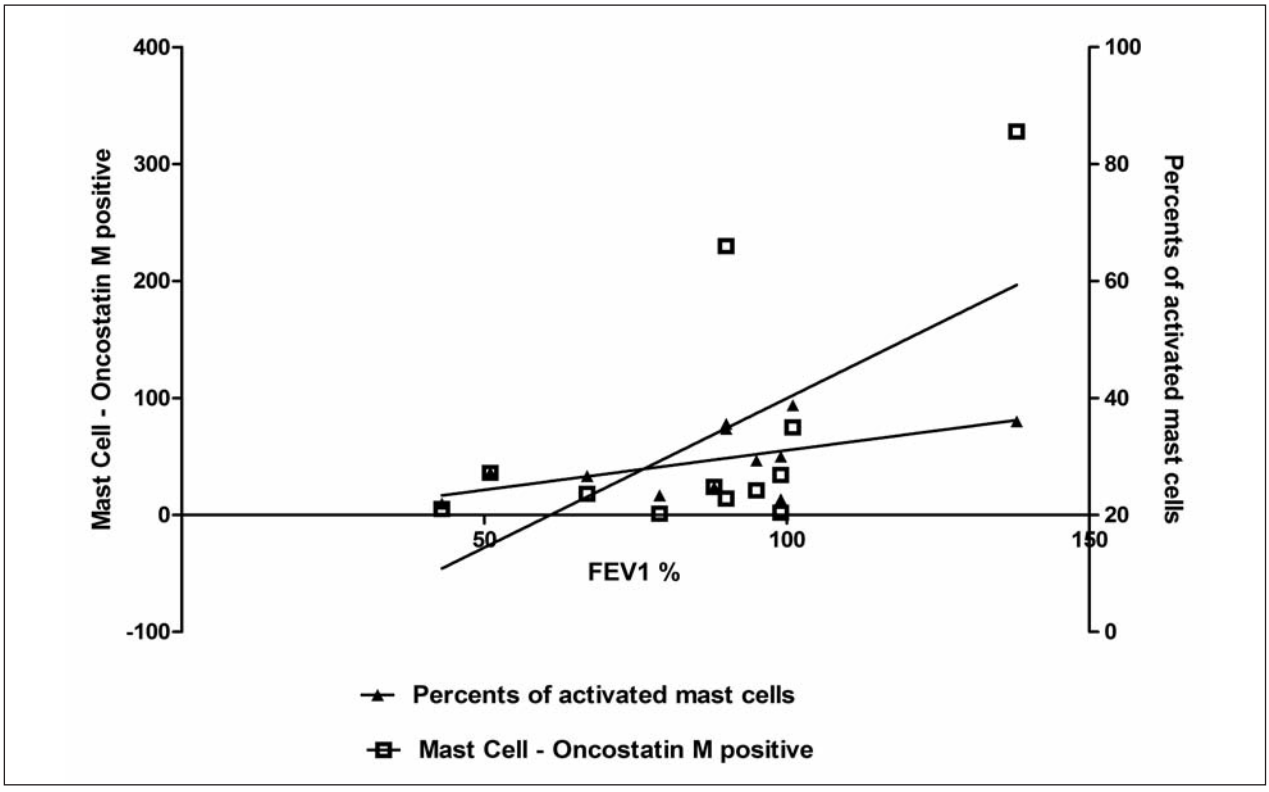

Fig. 2. Correlations between FEV1% and percentage of activated mast cells and percentage of mast cell OSM positive (n=12).

and the wide spectrum of mediators, in addition to OSM produced and secreted by mast cells have led investigators to propose a functional relationship between these two cell populations (22). Indeed, mast cell activation has been reported to induce T cell migration either directly by the release of chemotactic factors, such as lymphotactin or IL-16, or indirectly by the induction of adhesion molecule expression on endothelial cells (14, 15). Mast cells are also able to present antigens to T cells, resulting in their activation in either an MHC class I- or class II-restricted and co-stimulatory, molecule-dependent fashion. Adhesion molecule-dependent intercellular contact or MHC class II cognate interactions between T cells and mast cells results in the release of both granule-associated mediators and cytokines from the latter (9-11). Also, T cell-derived mediators, such as -chemokines, directly induce mast cell degranulation. On the other hand, mast cell-derived cytokines, such as IL-4, have been found to polarize T cells to differentiate preferentially into the TH2 subset (8-11). Thus, T cell-mast cell interactions are bidirectional, fulfilling regulatory and/or modulatory roles affecting various aspects of the immune response. Therefore, mast cells have a pivotal the role in inflammatory as well as fibrotic processes, which was the reason for our observation.

Recent studies have shown that OSM might have a role in T cell-mediated inflammatory processes in which mast cells have also been found to be involved, including rheumatoid arthritis, multiple sclerosis and pulmonary fibrosis (6). Sarcoidosis, although present in this spectrum of lung abnormalities, causes fibrotic changes in some patients, and a minority of patients will proceed to unresolving fibrotic changes, and might even require lung transplantation.

As was previously shown, OSM is specifically expressed on T cell-induced mast cell activation, but not on IgE cross-linking. Also, heterotropic adhesion to activated, but not resting, T cells resulted in OSM release from mast cells that was found to be biologically active, inducing human lung fibroblast proliferation (5, 10).

This study had several limitations. It included a small number of patients with sarcoidosis. There was no control group of healthy patients or patients with other types of pulmonary fibrosis. However, despite these limitations, our preliminary findings are unique and further studies to validate our findings are suggested.

Recently, we reported that cell-to-cell contact with activated T cells results in OSM release from mast cells (12). We assessed the role of OSM in the activation of human mast cells and found that OSM was expressed and released specifically on T cell-induced mast cell activation, but not on IgE cross-linking. OSM was localized to the cytoplasm and its expression was inhibited by dexamethazone (21, 22). OSM was also found to be biologically active in inducing lung fibroblast proliferation that was partially, but significantly inhibited by anti-OSM mAb. Therefore, we concluded that production of OSM by human mast cells might represent one link between T cell-induced mast cell activation and the development of a spectrum of structural changes in T cell mediated inflammatory processes in which mast cells have been found to be involved (18-20). OSM may serve as a noninvasive and simple test in BAL to predict the severity of patients with sarcoidosis

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Role of authors: Alexander Guber: Data collection, reviewed draft. Abdel Qader Jawad: Data collection, reviewed draft. Pazit Salamon: Study design. Yosef A. Mekori: Study design, wrote and reviewed draft. David Shitrit: Study design, data collection, wrote and reviewed draft.

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