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Serum alarm antiproteases in systemic sclerosis patients

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ABSTRACT

Alarm antiproteases, i.e. secretory leukocyte protease inhibitor ad elafin, are key mediators in innate immune response and integrate innate and adaptive immunity systems. The aim of the study was to assess clinical significance of serum levels of alarm antiproteases, elafin and secretory leukocyte protease inhibitor (SLPI) in patients with systemic sclerosis (SSc). Twenty-eight patients with SSc, 25 patients with rheumatoid arthritis (RA) and 22 healthy controls were recruited. Serum elafin and SLPI levels were examined using enzyme-linked immunosorbent assay (ELISA). The patients with SSc had significantly increased serum levels of SLPI in comparison with the RA patients and the healthy controls ($p < 0.01$), and the RA patients presented significantly higher serum levels of elafin in comparison with the controls ($p = 0.003$). In the SSc subgroup serum SLPI level negatively correlated with diffusing capacity of the lung for carbon monoxide (DLCO) ($r = -0.41$, $p = 0.03$) and total lung capacity ($r = -0.42$, $p = 0.03$). Both alarm antiproteases, elafin and SLPI could be potentially implicated in the pathogenesis of SSc and SLPI may be considered a candidate for serum biomarker of lung involvement in SSc.

1. Introduction

Despite the development of so called "danger hypothesis" model explaining key phenomena of innate immune system [1], studies on innate immunity effectors engaged in these "dangerous"/"safe" decisions in the area of autoimmune rheumatic diseases are still lacking. After an inflammatory reaction has been initiated, rapid innate immunity effector mechanisms have notable potential to cause damage to host tissues. Proteases, enzymes produced mainly by inflammatory phagocytes, provide a perfect example of such action. Recent evidence highlights how innate immune defense can promote autoimmunity and cause damage to host tissues by excessive release of proteases being its effectors. In response to these enzymes, antiproteases belonging to a group of either "alarm" or "systemic" inhibitors, are secreted [2]. They neutralize excessive protease load and protect host tissues. Alarm antiproteases include secretory leukocyte protease inhibitor (SLPI) and elafin, two members of the four-disulphide core family, which are synthesized and secreted locally at the site of inflammation and are produced in response to pro-inflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF) [2]. It soon turned out that alarm antiproteases are key mediators in innate host defense as they are pleiotropic molecules modulated in multiple pathological conditions [3]. Elafin and SLPI have not only potent antimicrobial effect, but also exert influence on the adaptive immune response and therefore can be responsible for the integration of innate and adaptive immunity systems [4,5].

Human SLPI was first identified as 11.7 kDa cationic protein. It was isolated from parotid secretions, its amino acid sequence was determined and its properties characterized with a noted high affinity for leukocyte elastase, which resulted in its name – leukocyte protease inhibitor [6]. SLPI expression has also been identified

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in lungs of chronically infected patients [7]. The elafin precursor protein trappin-2 (pre-elafin) is cleaved to form the mature 6 kDa protein elafin. Elafin expression has been identified in bronchial secretions and in the skin [8,9]. Both proteins are regarded as potent protease inhibitors and are important neutrophil elastase inhibitors. Moreover, SLPI has also been shown to inhibit cathepsin G, but not proteinase 3. On the contrary, elafin is a known proteinase 3 inhibitor, but cannot inhibit cathepsin G [10].

Systemic sclerosis is an incurable, autoimmune disease characterized by microvascular endothelial cell damage, excessive collagen deposition and perivascular infiltration of mononuclear cells in skin and affected organs. Known association of certain environmental factors with scleroderma-like illnesses supports the observation that non-antigen specific immune response to inflammatory stimuli can trigger the development of SSc. Newly published data revealed that SLPI serves as a useful serum marker for evaluating interstitial lung disease in SSc patients (SSc-ILD) [11]. There is also evidence that elafin has significant diagnostic and prognostic value as a biomarker of skin graft versus host disease (GVHD) [12], and that there exist many similarities between skin sclerodermatous subtype of GVHD and SSc [13]. In addition, anti-cathepsin G and anti-proteinase 3 antibodies have been found in SSc patients [14,15], which further supports the potential role of imbalance in protease/antiprotease system in SSc pathogenesis.

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease characterized by synovial hyperplasia caused by leukocyte inflammatory infiltrates and high expression of proinflammatory cytokines, which may result in development of bone erosions and joint damage. The observation by Mourão et al. reinforces a critical initial role of neutrophils in RA onset, which is later surpassed by the activation of adaptive immune system [16]. Neutrophil elastase, proteinase 3, and cathepsin G, three serine proteases stored in large quantities in neutrophil cytoplasmic primary granules [17], beside their antimicrobial activity are also important as specific regulators of the immune response by controlling cellular signaling through processing of chemokines, modulating the cytokine network, and activating specific cell surface receptors [18].

Given the role of serine proteases in immunopathogenesis of rheumatic diseases, the aim of our pilot study was to evaluate serum concentrations of the alarm antiproteases in SSc patients and to assess the relationship of their levels with clinical symptoms. The results obtained in SSc were compared with those determined in RA individuals and healthy controls.

2. Materials and methods

All patients with SSc and RA were Caucasian and were recruited consecutively from the Department of Rheumatology and Clinical Immunology, Poznan University of Medical Sciences, Poland. All of the 28 patients with SSc fulfilled American College of Rheumatology (ACR) classification criteria [19] and 2013 classification criteria for SSc [20]. All of the 25 RA patients fulfilled 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria for RA [21].

The patients with SSc were grouped according to the 2-cutaneous subset classification [22]as having diffuse cutaneous (dcSSc, n = 11) or limited cutaneous (lcSSc, n = 17) form of the disease on the basis of the extent of their skin involvement. The SSc group was also divided according to the disease duration into early SSc (n = 14) and late SSc (n = 14), defining early SSc as patients having disease duration of less than 2 years and late SSc as patients having disease duration longer than 2 years, measured from the onset of the first symptom other than Raynaud's phenomenon consistent with SSc.

The twenty-five patients with RA, who formed the reference group to the SSc cohort, were grouped as having early RA (n = 6) or established RA (n = 19), based upon the duration of the disease, defining early RA as patients having disease duration of less than 2 years and established RA as patients having disease duration longer than 2 years.

Patients with malignancy, other connective tissue disease and on biological therapy were excluded.

Healthy controls (n = 22) were volunteers and were matched according to gender and age (18–29 years, 30–44 years, 45–54 years, 55–70 years).

Informed consent was obtained from all study participants and the project of this study has been approved by the Institutional Review Board at Poznan University of Medical Sciences. The protocol of the conducted research conforms to the principles of the - World Medical Association's Declaration of Helsinki.

The workup encompassed patients' history and clinical examination. In SSc patients it included ulcer assessment, assessment of skin involvement using modified Rodnan skin thickness score, evaluation of the presence of gastrointestinal, renal, and/or joint involvement and calculation of the European Scleroderma Study Group (EScSG) disease activity index (DAI) for SSc [23–25]. Disease onset was defined as the first clinical event other than Raynaud's phenomenon, which was a clear symptom of SSc. Lung involvement was assessed functionally (diffusing capacity of the lung for carbon monoxide (DLCO), body plethysmography) and radiologically (high resolution computed tomography of the lungs, HRCT). To characterize SSc patients as "without pulmonary function impairment" three functional parameters: DLCO, total lung capacity (TLC) and vital capacity (VC) were defined as >80% predicted.

In RA patients the workup included patients' status/disease activity assessment using Disease Activity Score (DAS 28) [26]. In addition, the 66/68-joint count was performed (68 peripheral joints were evaluated for tenderness or pain on motion and 66 peripheral joints were evaluated for swelling – the hip joint was excluded). The patients were also asked to assess pain using visual analog scales (VAS) [27].

Blood samples from all patients were collected at the time of clinical examination on fasting conditions. Aliquots of sera were frozen at −70 °C until assayed. The degree of inflammatory activity was determined by erythrocyte sedimentation rate (ESR, Westergren), C-reactive protein concentration (CRP, enzyme-linked immunosorbent assay (ELISA), BioCheck, USA) and concentration of complement components C3 and C4 (radial immunoelectrophoresis). Antinuclear antibodies (ANAs) were determined by indirect immunofluorescence on mosaic containing as substrates HEp2 cells/monkey liver tissue (Euroimmun, Germany), and antibodies to extractable nuclear antigens (ENA) were estimated using blot type test (Euroimmun, Germany). The presence of IgM rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (aCCP) was defined using commercially available ELISA kits (Euroimmun, Germany). Serum levels of elafin and SLPI were measured using commercial ELISA kits (Uscn Life Science Inc., China) and calculated using standard curves generated with specific standards, according to the manufacturer's instructions.

Patients' demographic data were analyzed using descriptive statistics. All continuous data were tested for normal distribution using Kolmogorov-Smirnov test. In case of normally distributed data, results were presented as mean ± standard deviation (SD), whereas non-normally distributed data were expressed as median (interquartile range, IQR). A p value of less than 0.05 was considered statistically significant. Comparisons between the investigated groups were tested for significance using Mann-Whitney *U* test and Kruskal–Wallis one-way analysis of variance (ANOVA) by ranks and *post hoc* multiple comparisons of mean ranks. Correlations between variables within the group were analyzed using