

Investigation of basophil cell proteinase release in progressive systemic sclerosis

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The aim of this study was to evaluate the function of basophil granulocytes in 37 patients with progressive systemic sclerosis (PSS). The *in vitro* proteinase release test using Chromozyme TH as a chromogenic substrate was performed to measure the basophil cell releasability. Anti-IgE, calcium-ionophore (A 23187) and N-formyl-methionyl-leucyl-phenylalanine were used as activatory agents. Our results showed that the reactivity pattern to those agents did not differ in PSS compared to controls. Patients whose basophil cells release proteinases to all of the 3 activators tended to have more severe organ symptoms compared to the rest of the patients, while teleangiectasia was significantly less frequently ($p < 0.02$) found in cases with proteinase release. These findings suggest that there may be a certain relationship between basophil function and severity of the PSS.

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Progressive systemic sclerosis (PSS) is a multisystem disorder characterized by degenerative, inflammatory, vascular and fibrotic changes involving the skin and several internal organs. Mast cells are believed to participate in the development of certain fibrotic processes (2, 6, 7, 8, 11, 12). Many years ago an increased dermal mast cell population was found in PSS (1). Recently, new studies have highlighted the importance of mast cells in the pathogenesis of the cutaneous lesions in PSS (7, 8, 13, 18, 20, 23). Myocardial mast cells were described in fatal cases with PSS (16).

There are certain difficulties in testing the activation and functions of mastocytes. It has already been shown that several *in vitro* functions of basophils correlate with those of mastocytes (17). A correlation between dermal and lung mastocyte functions and the *in vitro* histamine release from leucocytes has been described in ragweed allergic asthma (5) and hay fever (19). Testing the *in vitro* activation of basophils may provide some information about the *in vivo* activation of mastocytes in PSS.

In our recent study, the *in vitro* basophil activation was investigated by proteinase release test (PRT) in patients with PSS.

Patients

Thirty-seven patients with progressive systemic sclerosis were investigated. The patients' mean age was 50.8 ± 11.3 years (21 to 76), female/male ratio 33/4 and the duration of disease was 10.8 ± 7.9 years. All

patients fulfilled the preliminary diagnostic criteria for scleroderma (24). The clinical symptoms of the patients (Table 1) were characterized by a standard protocol in all cases as previously described (9). Besides evaluation of the case history and the physical examination, chest roentgenogram, evaluation of vital capacity by spirometry, barium esophageal passage, assessment of the lacrimal and salivary excretion, and measurement of the serum creatinine levels were also performed. Cardiac involvement was evaluated by clinical symptoms, ECG and, occasionally, echocardiography. Atrioventricular and intraventricular conductory disturbances, signs of myocardial ischemia, congestive heart failure (without signs of atherosclerosis on the fundi of eyes), and pericarditis (without uremia) were taken as the cardiac manifestation of disease. Serum creatine kinase was also determined, and in cases with relevant complaints or symptoms electromyography and biopsy were also performed. All drug administrations were stopped 36 h before the investigation (Table 1).

Nineteen sex-matched healthy volunteers were used as controls.

Methods

The polymorphonuclear basophil cell reactivity to specific and non-specific agents was determined colorimetrically by proteinase release test (4, 27, 28) with minor modifications. The peripheral blood was separated by Ficoll-Uromiro (1.077 density) and the interface and mononuclear cell layer was used for

Table 1. Clinical profile of 37 patients with systemic sclerosis

Number of patients with:	
"Proximal" scleroderma	27
Lung involvement	31
Esophageal dysmotility	12
Sjögren's syndrome	11
Myositis	9
Cardiac involvement	8
Renal manifestation	1
Subcutaneous calcinosis	6
Truncal skin involvement	12

the test. These 2 layers contained almost the total amount of basophil cells which release the proteases detected by our test, as described by Williamson et al. (28). Cells were carefully washed and suspended in Tris buffer at pH 7.6 before stimulation. $2-5 \times 10^6$ cells/well were stimulated by anti-human IgE (dilution 1/4000, Behring, Germany), calcium ionophore A 23187 (0.15 $\mu\text{g/ml}$, Sigma, USA), and *N*-formyl-methionyl-leucyl-phenylalanine (f-met peptide) (10^{-5} M, Sigma, USA). After 1 h incubation with the agents described above, cells were eliminated by centrifugation and proteinase activity was measured colorimetrically at 405 nm in the supernatant of the samples, using Chromozyme TH (Boehringer, Germany) as the chromogenic substrate.

The absorbance changes during 1 h in the supernatant of the samples containing only buffer solution (B), anti-IgE and buffer (E), cell suspension in the buffer (C), and stimulated cells by anti-IgE (CE), Ca^{2+} -ionophore (CC), and f-met-peptide (CF) were counted. The basophil proteinase releasability (PR) was defined as follows:

PR by anti-IgE stimulation = CE-C-(E-B)

PR by Ca^{2+} -ionophore stimulation = CC-C

PR by f-met-peptide stimulation = CF-C.

Positive proteinase release was defined if the PR was found to be > 0.01 (4).

Results

When anti-IgE was used as a stimulatory agent, 20 patients showed positive proteinase release (positive PRT). Positive release was detected in 33 patients, with Ca^{2+} -ionophore, and 31 patients with f-met-peptide. Positive PRT reaction to all 3 agents was shown in 16 cases. The reactivity pattern of the patient group to these agents was found to be similar to that of the control group. Patients were divided into 2 groups based on the PRT reactions to anti-IgE, Ca^{2+} -ionophore, or f-met-peptide. The first group consisted of the patients with no response to the stimulatory agents (negative PRT), the second group consisted of the patients with measurable releasability (positive PRT). No significant differences were found between the proportion of organ mani-

Table 2. Reactivity pattern to basophil stimulatory agents in systemic sclerosis

Reactivity to agent	Anti-IgE	Caio	FMLP	All 3 agents	
pos. PRT	20	33	31	16	Cases with PSS (37) controls (19)
neg. PRT	17	4	6	21	
pos. PRT	11	15	14	10	
neg. PRT	8	4	5	9	

Abbreviations used: pos. PRT=positive proteinase release test; neg. PRT=negative proteinase release test; Caio= Ca^{2+} -ionophore, FMLP=*N*-formyl-methionyl-leucyl-phenylalanine.

festations of these subgroups. It is noteworthy that the patients with no release to Ca^{2+} -ionophore did not show esophageal dysfunction, myositis or, cardiac involvement. Similar differences in the other subgroups were not found (Table 2).

The correlation to certain clinical symptoms is shown in Table 3. Patients with teleangiectasia ($n = 21$) had less frequently positive PRT with anti-IgE compared to Ca^{2+} -ionophore ($p < 0.05$) or f-met-peptide ($p < 0.05$) stimulation. A similar tendency for other symptoms was not demonstrated (Table 3).

Patients with positive PRT to all 3 agents ($n = 16$) were compared to patients with other reaction patterns ($n = 21$). The frequency of teleangiectasia was significantly lower in the first group ($p < 0.02$). The internal organs' manifestations, such as esophageal involvement, myositis, cardiac, or pulmonary manifestations, were more frequently found in the first group (Fig. 1).

Table 3. Basophil proteinase release and clinical symptoms in 37 patients with systemic sclerosis

	algE	Caio	FMLP	All
T (21) ^a	9 ^{bc}	19 ^b	17 ^c	5
E (12)	8	12	10	7
M (9)	6	9	7	5
C (8)	5	8	6	4
P (31)	17	28	26	13

	B1	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3	B1	B2	B3
T	7	11	3	1	6	2	6	10	3	6	10	1	0	5	0
E	0	8	4	0	6	2	0	8	4	0	8	2	0	6	1
M	1	6	2	0	4	2	0	6	3	1	5	1	0	4	1
C	1	3	4	0	2	3	1	3	4	1	3	2	0	2	2
P	7	18	6	3	10	4	7	15	6	7	16	3	2	9	2

Abbreviations used: T: teleangiectasia, E: esophageal, M: myositis, C: cardiac, P: pulmonary involvement.

B1, B2, B3: Types I (11 cases), II (20), and III (6) of systemic sclerosis according to Barnett's classification (3); algE=positive proteinase release test (PRT) to anti-IgE; Caio=positive PRT to Ca^{2+} -ionophore, FMLP=positive PRT to *N*-formylmethionyl-leucyl-phenylalanine, all=positive PRT to all of the 3 agents.

^a Number of patients with symptoms is depicted in parentheses ^{b,c} $p < 0.05$ by chi-square test.

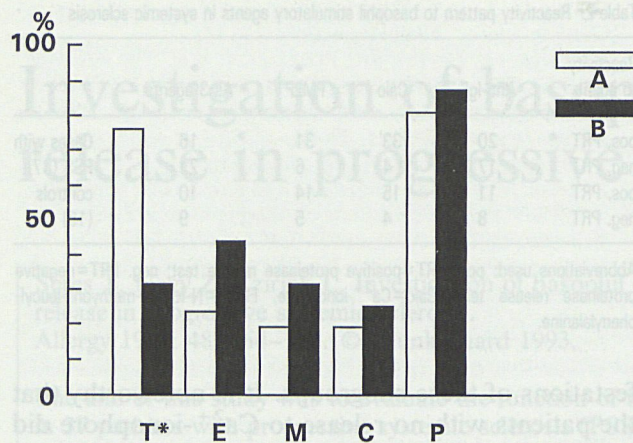


Fig. 1. Proteinase release and proportion of clinical symptoms in the patients with PSS.

Abbreviations used: T: teleangiectasia, E: esophageal, M: myositis, C: cardiac, P: pulmonary involvement.

A: patients with negative PRT to one or more of the 3 agents (anti-IgE, Ca^{2+} -ionophore, *N*-formyl-methionyl-leucyl-phenylalanine) ($n = 21$).

B: patients showing positive PRT to all 3 agents (anti-IgE, Ca^{2+} -ionophore, *N*-formyl-methionyl-leucyl-phenylalanine) ($n = 16$).

* $p < 0.02$ by chi-square test.

Discussion

Although basophils and mast cells are different cell types, they are functionally similar from several aspects, therefore *in vitro* investigation of basophil cell functions may be a good tool for the evaluation of mast cell activity in PSS. Evidence of mast cell activation has been shown in PSS (7, 13, 16) and in the tight skin mouse model of scleroderma (26). A possible aetiological role of factors deriving from activated mastocytes in PSS is attractive, because certain products influence the fibroblast functions, including proliferation. The *in vitro* mast cell degranulation is associated with fibroblast stimulation and collagen production, and mast cell granules can be internalized by fibroblasts (13).

The possible pathogenic role of mast cells has also been suggested in other fibrotic processes, such as toxic oil syndrome (11), chronic graft versus host disease (6), keloid formation, and wound healing (21). Basophil and mast cell histamine release can be initiated by a variety of immunologic and non-immunologic stimuli. The activation of these cells is classically associated with the interaction between antigen and antigen-specific IgE. Other factors, such as complement components, mononuclear cell products including interleukin 1, are also involved in the activation of basophils (15, 25).

Recently, an elevated plasma histamine level was described in PSS (10). Ketotifen was described as a new therapeutic tool for the treatment of PSS (12).

In our study, the proportion of positive PRT was

similar in PSS and the control group, indicating that the possible genetic background for all 3 activating mechanisms may not differ significantly between patients and controls. We have also shown that the presence of positive basophil protease release tends to be associated with a more severe disease state. The proportion of internal organ involvement was higher in the group showing positive protease release to all 3 agents (Fig. 1) than in the rest of the patients.

Teleangiectasia was more frequently associated with the milder subset (CREST variant) of PSS. Our findings also showed that cases with teleangiectasia less frequently had high releasability to anti-IgE compared to the other 2 ways of stimulation (Table 2). Recently, anti-IgE autoantibodies have been described in PSS (14); therefore, at least this type of stimulatory mechanism for basophils may even be present in the *in vivo* situation.

As a working hypothesis, it is tempting to speculate that a relationship may be present between the "activatory capability" of basophils and the severity of PSS. Our present findings show that many of the pathologic events leading to endothelial injury, fibrosis, inflammatory processes, and production of autoantibodies are a complex mechanism mediated by activated mast cells, platelets, and monocyte-macrophage-derived products in PSS.

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