The association of serum matrix metalloproteinases and their tissue inhibitor levels with scleroderma disease severity

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ABSTRACT

Objective

Matrix metalloproteinase 3 (MMP-3) is reported to play an important role in the pathogenesis of systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Studies have also investigated the association of different tissue inhibitors of MMPs (TIMPs) with fibrosis in scleroderma (SSc). The aim of this study was to evaluate the correlation of serum MMP-1, 3 and TIMP-1 with severity and disease specific markers of SSc and RA.

Methods

Serum MMP-1,3 and TIMP-1 were measured in 42 SSc patients (age range 28-68 yr, mean 47 yr) and compared to 29 RA and 30 healthy age- and sex-matched individuals. Elevated values of MMPs and TIMP-1 were defined as those greater than 2 SD above the normal mean. All SSc and RA patients were scored for disease severity.

Results

Serum MMP-1 was significantly elevated in 8/42 (19%) SSc patients ($p = 0.01$) but only in 2/29 (7%) RA patients ($p = 0.2$). Whereas MMP-3 levels were elevated in 10/29 (34%) RA patients ($p = 0.002$), it was elevated in only 5/42 (12%) SSc patients ($p = 0.03$). TIMP-1 was found elevated in 17/42 (40%) SSc patients ($p = 0.001$) and in only 4/29 RA patients (with a strong trend towards significance, $p = 0.052$).

We found a significant association between the elevation of both MMPs and TIMP-1 levels, with the severity of SSc. Those who had an increase of more than one MMP and/or TIMP, demonstrated life-threatening major organ involvement such as end stage lung fibrosis, GI aperislasis, and severe cardiac failure. Contrary to that in SSc, the severity of RA showed some trend of association with MMP-3 only.

Conclusion

We confirm previous observations that MMPs and TIMPs may play an important role in various rheumatic diseases. Whereas serum increase of MMP-3 correlated with RA severity, SSc severity was more characterized by the increase of both MMP-1 and TIMP-1. This suggests that the MMPs and TIMPs involved in SSc are different than those playing a role in RA, which may indicate that in SSc they are produced in different locations than in RA.

Introduction

Scleroderma (systemic sclerosis, SSc) is an autoimmune disorder in which excessive extracellular matrix is deposited in the skin and internal organs (1). Metalloproteinases (MMPs) are a group of enzymes, dependent on Zn^{2+} ions for activity, which degrade extracellular matrix (2). In rheumatic diseases such as rheumatoid arthritis (RA) and systemic lupus erythematousus (SLE), certain cells (*i.e.* synovial cells, fibroblasts, macrophages and neutrophils) are exposed to pro-inflammatory cytokines such as IL-1, IL-6 and TNF-a which in turn leads to the overproduction of MMPs (3-5).

An increase in serum concentrations of MMP-3 was recently reported in patients with RA and SLE and has been shown to correlate with various systemic markers of inflammation such as the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) (6-10). These reported studies concentrated mainly on the role of MMP-3 in the pathogenesis of RA and SLE without investigating the role of other MMPs and/or tissue inhibitors of metalloproteinases (TIMPs).

One of the suggested contributory factors to the development of dermal fibrosis in SSc is the increased production of extracellular matrix together with a decrease in collagenase activity, which may be related to elevated levels of serum TIMP-1 (1, 8). Cells from SSc patients simultaneously produce less stromelysin but substantially higher amounts of TIMP-1 than do normal dermal fibroblasts, suggesting that abnormalities in the regulation of the matrix enzymes and their inhibitors play an important part in the molecular pathology of SSc. Additionally, it has been noted that SSc fibroblasts produce increased amounts of TIMP-1 relative to that of normal fibroblasts, suggesting that TIMP-1 may serve as an autocrine growth factor in the fibrotic process of SSc (11, 12). Due to the differing activities of the various cyto-

kines in disparate autoimmune diseases it has been suggested that the balance between the production and activation of MMPs and their inhibition by TIMPs are the crucial determinants in inflammation and/or fibrosis, rather than the level of each one alone. In this regard it is well accepted that RA is a disease of Th1 activity dominance whereas SSc has been reported to be more of a Th2 disease (13, 14).

This study was designed to assess the possibly different roles of MMP-1, 3 and TIMP-1 in SSc and RA and to evaluate their individual association with disease severity as well as specific serological markers. We assumed that it is important in such studies to analyze different MMPs and TIMPs simultaneously and investigate their role in such diseases as SSc.

Patients and methods

Patients

We studied 42 SSc patients (31 women and 11 men, age 22 - 68 yr, mean 42 yr) and compared them with 29 RA patients (the disease comparison group), and 30 healthy individuals. Both the SSc and RA patients satisfied the American College of Rheumatology criteria (15, 16) and were followed in the outpatient rheumatology clinic. All studied individuals underwent the following laboratory studies: protein electrophoresis (PEP), ESR, CRP, complement levels, antibodies to snRNP, RO/SSA, La/SSB, centromere, Scl-70, Jo-1 and rheumatoid factor (RF).

A severity score for SSc was defined by two rheumatologists based on the proposed Medsger scale (17): severe SSc = patients with score of 4 (end stage) in any organ system or a score of 3 in two or more categories at the time of investigation; moderate SSc = patients with Medsger scores ≤ 2 in most systems, except for the possible presence of digital tip ulcerations (Medsger score 3) as an expression of severe Raynaud's; mild SSc = patients with Medsger grade 0 - 1 in all organ systems, with the exception of possible digital pitting scars (Medsger score 2), as a manifestation of their peripheral vascular disease.

RA severity was defined using a global physicians' score, namely articular swelling/deformity, radiographic erosions, subcutaneous nodules, and serological markers of activity such as CRP, PEP, RF and anti-keratin antibodies.

Detection of MMPs/TIMPs and autoantibodies

MMP-1, 3 and TIMP-1 were analyzed using commercial ELISA kits: the BINDAZYME Pro MMP-1 and Pro MMP-3 Enzyme Immunoassay Kit (The Binding Site, Birmingham,UK) and TIMP-1 ELISA (Oncogene Research Products, Cambridge, MA, USA). Normal values were adjusted by relating them to the range and mean for our 30 normal control individuals +2 SD. All of the above mentioned autoantibodies were measured in serum using commercial ELISA kits. The sera of all the individuals studied were stored at -20°C and analyzed together by the same technician. The results were reported positive when they were +2 SD of the mean cutoff; the detected values were the mean of triplicate runs for each sample.

Statistical analyses

The significance of the MMP and TIMP elevations in different groups was analyzed using the Chi square test or Fisher's exact test, as appropriate. The correlation between SSc disease severity and autoantibodies was analyzed using the Spearman's R coefficient of correlation. Two-tailed P values of 0.05 or less were considered to be statistically significant.

Results

The mean age and sex were similarly distributed within the three studied groups. Forty-two patients suffering from SSc of differing severity were studied. 9/42 had severe SSc (21%). In 2 of these Raynaud's resulted in gangrenous extremities together with end-stage lung disease (one died during the study). The other 7 suffered from severe organ failure: GI involvement (malabsorption syndrome in one), renal failure (creatinine > 3.0 in one), congestive heart failure in another, and skin involvement with a total skin thickness score (TSS) of 30 - 35 in 4 patients. 21/42 (50%) had moderate SSc; all suffered from moderate Raynaud's (digital pitting scars), together with either distal esophageal aperistasis, pulmonary hypertention, or TSS of 15 - 18. The other 12 (29%) were classified as having mild SSc (Raynaud's requiring vasodilatation, TSS of 10 or mild distal esophageal dysmotility).

Out of 29 RA patients, 7 (24%) were considered to have severe disease (multiple articular erosions, subcutaneous nodules, and positivity to both RF and anti-keratin antibodies). The remaining 22 patients were scored moderate to mild.

The normal mean values for MMPs and TIMP-1 were as follows: MMP-1 = 5.6 ± 3.7 ng/ml, MMP-3 = 15 ± 7.9 ng/ml, and TIMP-1 = 168.4 ± 33.2 ng/ml. Values were considered to be significantly elevated when + 2 SD greater than the normal mean. Accordingly, both dis-

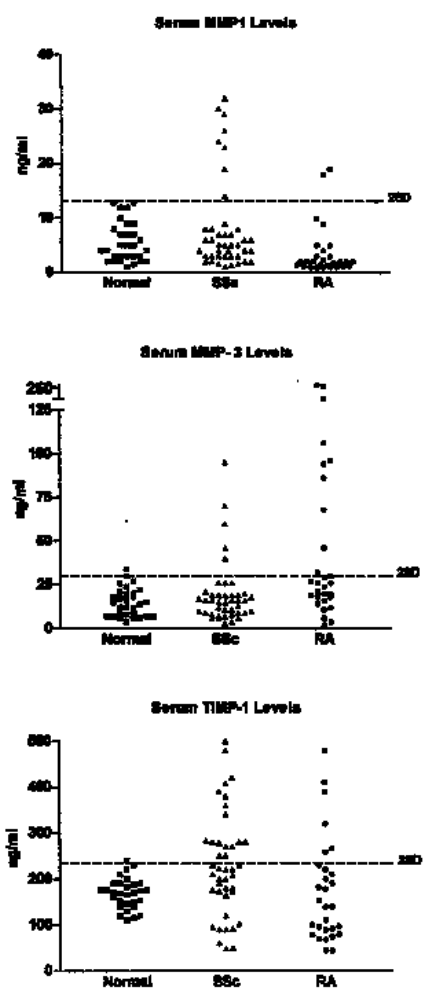

Fig. 1. The elevation of serum MMPs and TIMP-1 in normals, SSc and RA individuals.

ease groups and the normal controls were divided into two categories, those above and those below 2 SD (Fig. 1). The association between categories was statistically tested.

Serum MMP-1 levels were found to be significantly elevated in 8/42 (19%) of SSc patients ($p = 0.01$), whereas high serum MMP-3 was documented in only 5/42 (12%, $p = 0.03$) (Fig. 1). Serum TIMP-1 elevation was significant in 17/42 (40%) SSc patients ($p = 0.0003$) (Fig. 1). Only 2/29 (7%, $p = 0.2$) and 6/29 (21%, a strong trend towards significance $p = 0.052$) RA patients had high levels of MMP-1 and TIMP-1, respectively. However, serum MMP-3 levels were found to be elevated in 10/29 (34%) RA patients ($p = 0.0002$).

We found a significant positive association between SSc severity and the existence of MMPs/TIMP-1. Six out of 9 severe SSc patients were found to have simultaneous elevation of three markers, in contrast to the remaining 33 patients in whom only one marker was increased, and those presented a moderate to mild disease course ($p < 0.0001$). SSc severity was found to be most highly correlated with an elevated TIMP-1 ($p = 0.01$).

Similarly, 4 out of 7 severe RA patients demonstrated a simultaneous increase in two enzymes, whereas the remaining 22 patients had moderate to mild disease and presented only one marker ($p = 0.001$). Whereas MMP-1 and TIMP-1 did not correlate with RA severity, MMP-3 showed some trend of association ($p = 0.1$).

Whereas hypergammaglobulinemia was recorded in all SSc patients, with no relation to disease severity, it was found in 11/29 (38%) of RA patients and was correlated with disease severity ($p = 0.001$). The elevation of CRP was documented in 29/42 (69%) and 16/29 (55%) of SSc and RA patients respectively, and was correlated with disease severity ($p = 0.01$ and $p = 0.001$, respectively).

Anti-centromere or Scl-70 antibodies were found in 20/42 (48%) of the SSc patients and in none of the RA patients. RF was found in 9/42 (21%) and in 16/29 (55%) of the SSc and RA patients, respectively. Whereas anti-Ro/SSA antibodies were found in 8/42 (19%) of SSc, it was documented in 3/29 (10%) RA patients. Anti-keratin antibodies were found in 11/29 (38%) of the RA, and in none of the SSc patients. Anti-nRNP and Jo1 antibodies were found in 3/42 (7%) of SSc patients. We found a significant correlation between the intensity of SSc severity and the simultaneous detection of multiple autoantibodies ($R = 0.4$, $p = 0.008$). The presence of anti-centromere/Scl-70, RF, and anti-Ro or nRNP antibodies simultaneously in one sera was found in 4 severe and 7 moderate SSc patients, whereas in mild SSc patients this was found in only one. The elevation of TIMP-1 was found in good association with anti-Scl-70/centromere antibodies.

Whereas 14/17 (82%) TIMP-1 positive patients were also positive to anti-Scl-70/centromere antibodies, only 6/25 (24%) TIMP-1 negative patients were positive to anti-Scl-70/centromere antibodies ($p = 0.0002$).

As mentioned above, RA severity showed some trend of association with MMP-3 elevation. However, no association could be demonstrated between this elevation and any of the above analyzed autoantibodies.

Discussion

This study demonstrates that in SSc patients serum elevations of MMP-1/TIMP-1 and much less of MMP-3 are to be found, whereas in RA patients MMP-3 elevation was the more prominent of the studied enzymes. In addition, a direct correlation between the disease severity of SSc and the simultaneous elevation of both MMPs and TIMP-1 was shown.

It has been proposed that pro-inflammatory cytokines such as IL-1, IL-2, IL-6 and TNFa are involved in many aspects of the pathogenesis of autoimmune diseases. The exposure of neutrophils and macrophages to these cytokines is followed by the heightened production of MMPs and the degradation of extracellular matrix (3).

Systemic lupus erythemathosus, SSc and RA are known to be characterized by different type 1 (Th1) and type2 (Th2) cytokine profile shifts (18). Systemic sclerosis is a disease of predominant activation of Th2 type cytokines. Skin T cells showed high mRNA expression of IL-4 and little or no IFN . Such a cytokine shift may account for the major alterations (endothelial cell injury, fibrosis and multiple autoantibodies) occurring in this disease (19-21).

In RA in contrast to SSc, CD4+ cells, infiltrate the synovium and produce a pathogenic immune response. Also, positive rates of IFNg producing cells among CD4+T cells were significantly higher than those of IL-4 producing ones in both PBMCs and synovial fluid cells (3, 22, 23).

Whereas the main secretion of MMP-3 has been reported to originate from chondrocytes and synoviocytes of RA patients, its secretion in other organs such as the kidneys has only been hypothesized. The significantly higher elevation of MMP-1 and TIMP-1 than MMP-3 in SSc raises the further speculation that a specific cytokine imbalance might induce their local production, mainly in the skin and lungs of these patients.

MMPs and TIMPs have been shown to be secreted by activated mast cells, contributing to the extensive matrix lysis characteristic of diseases such as RA and SSc (24). In the light of our findings it may be speculated that active mast cells in the skin, GI and lung connective tissues of SSc patients may release more MMP-1 and TIMP-1 than MMP-3, thus encouraging the development of fibrosis as a dominant finding in SSc patients; on the other hand RA and SLE, diseases in which inflammation dominates, are more associated with MMP-3. All of this is supported by another study which has discussed the importance of mast cells in the development of pulmonary fibrosis, suggesting that the local concentration of heparin released by mast cells, increase MMP-1/ TIMP-1 activity and lung fibroblast proliferation (25).

This may explain why, similarly to what we assume in our study, various inflammatory diseases and their disease severity depend on the activation of different connective tissue cells followed by the different production or

imbalance of MMPs/TIMPs. Whereas MMP-1 elevation was found in a few, TIMP-1 was found in 40% of our SSc patients. This is in agreement with other reports that have shown the molecular pathology in SSc to be in part due to a decrease in the MMP-3/TIMP-1 ratio in SSc fibroblasts (26).

The present findings suggest that these enzymes play a more important role in the development of the upregulation of extracellular matrix biosynthesis, end organ damage and fibrosis than in the inductive inflammatory stage of SSc. Whereas hypergammaglobulinemia and CRP elevations were found in association with RA severity, the existence of the various mentioned autoantibodies, mainly RF and anti-Keratin antibodies, did not. In contrast to RA, the association of SSc severity with the simultaneous elevation of more than one enzyme, along with the simultaneous detection of multiple autoantibodies, is in agreement with the hypothesis that SSc is a disease of predominant Th2 activation. Further studies are needed in order to better understand the role of different MMPs/TIMPs in different autoimmune diseases. It is also important to investigate how these markers, and by which mechanism, are probably variably produced in different organs.

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