Von Willebrand factor cleaving protease (ADAMTS-13) in 123 patients with connective tissue diseases (systemic lupus erythematosus and systemic sclerosis)

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Background and Objectives. Autoantibodies inactivating the von Willebrand factor (VWF) cleaving protease, ADAMTS-13, are among the most frequent causes of thrombotic thrombocytopenic purpura (TTP). We evaluated whether or not ADAMTS-13 deficiency and autoantibodies inactivating the protease prevalent in patients with the prototypic autoimmune diseases systemic lupus erythematosus (SLE) and systemic sclerosis (SSc).

Design and Methods. We measured, in parallel, the protease and VWF antigen (VWF:Ag) in 123 patients, 36 of whom had SLE and 87 of whom had SSc. In 14 patients with either disease who had low plasma protease levels (below 40%) we also looked for anti-ADAMTS-13 inactivating antibodies.

Results. ADAMTS-13 levels were significantly lower in SLE (p=0.0013) and in SSc (p=0.0002) than in normal controls. No anti-ADAMTS activity was measurable in patients with low ADAMTS-13 levels. VWF:Ag was high in both SLE and SSc (p=0.001).

Interpretation and Conclusions. Systemic connective tissue diseases are other conditions besides TTP that are associated in some instances with low but detectable levels of ADAMTS-13. Autoantibodies inactivating protease activity are not the cause of the low plasma levels of ADAMTS-13.

Key words: systemic lupus erythematosus, systemic sclerosis, ADAMTS-13, von Willebrand factor V, thrombotic microangiopathies.

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Furlan *et al.*[1] and Tsai and Lian[2] independently demonstrated that in the majority of patients with the microangiopathy thrombotic thrombocytopenic purpura (TTP) have a deficiency of the plasma metalloprotease ADAMTS-13 (A Disintegrin-like And Metalloprotease with ThromboSpondin type 1 motif).[3,4] In TTP, protease deficiency impairs physiological cleavage, at the bond between tyrosine 1605 and methionine 1606, of the adhesive glycoprotein von Willebrand factor (VWF), resulting in the abnormal appearance in plasma of a set of ultralarge highly thrombogenic multimers.[5] These multimers, normally present only in endothelial cells and platelets, interact with platelet membrane glycoproteins and the subendothelial matrix more avidly than multimers of regular size, thereby triggering intravascular formation of aggregates and occlusive thrombi in terminal arterioles.[6] In turn, VWF-rich occlusive thrombi produce the ischemic symptoms typical of TTP, mainly in the central nervous system but also in other organs.[7]

The main causes of ADAMTS-13 deficiency are mutations in the gene encoding the protein or, more frequently, the development of IgG autoantibodies that inactivate protease activity.[1,2,7] The trigger for the development of autoantibodies is not always identifiable, even though drugs, cancer, infections and pregnancy are frequently associated.[7] It is plausible that the mechanisms of immune-mediated ADAMTS-13 deficiency are similar to those of other immune-mediated coagulopathies such as acquired hemophilia, acquired von Willebrand syndrome or antiphospholipid syndrome. However, TTP cases lack some of the features typical of these syndromes, such as the prevalent association with the post-partum period, aging and monoclonal gammopathies. On this background we decided to evaluate whether or not ADAMTS-13 deficiency and autoantibodies inactivating this protein are prevalent in patients with two prototypic connective tissue diseases: systemic lupus erythematosus (SLE), which has in common with TTP some pathogenetic triggers and clinical findings (fever, CNS involvement, renal impairment, thrombotic events);[8] and systemic sclerosis (SSc), in which microvascular occlusion is an early pathogenetic event and is probably mediated by immunologic mechanisms.[9]

Design and Methods

The case material consisted of 123 consecutively observed patients, 36 with SLE and 87 with SSc, fulfilling the American College of Rheumatology criteria for the classification of these diseases,[10,11] and followed at the out-patient Immunology Unit of the Milan Maggiore Hospital. No patient with SLE or SSc had a history, signs or symptoms of thrombotic microangiopathies.

SLE patients (30 women and 6 men) had a median age of 35 years (range 22–62), with a median disease duration from diagnosis of 9 years (range 1-36). Skin involvement was present in 72% of them, arthralgias/arthritis in 64%, SLE-related hematologic abnormalities in 11%, active renal involvement in 8%, and CNS involvement in 6%. Antinuclear or antiphospholipid (lupus anticoagulant and/or IgG and IgM anti-cardiolipin) antibodies were positive in 89% and 29%, respectively; no SLE patient had symptoms and signs of antiphospholipid antibody syndrome (APS) or other atherothrombotic or venous thromboembolic disease.

Of the 87 SSc patients, 82 were women and 5 men: 26 of them had diffuse cutaneous disease, 55 limited cutaneous disease and 6 did not manifest scleroderma. Their median age was 56 years (range 22–62), with a median disease duration from diagnosis of 6 years (range 1–31). Raynaud's phenomenon was observed in 98% of patients, esophageal involvement in 90%, teleangiectasia in 75%, calcinosis in 41%, interstitial lung involvement in 56%, and pulmonary hypertension in 34%. Anti-SCl-70 antibodies were positive in 40% of cases and anti-centromere antibodies were positive in 34% of the patients. Antiphospholipid antibodies were found in one SSc patient of the 54 tested; this woman did not have clinical signs of APS. Three SSc patients, negative for antiphospholipid antibodies, had a history of a thrombotic episode: two had had a deep vein thrombosis and pulmonary embolism; another, an alcohol abuser, had had thrombosis of the superior mesenteric and splenic veins, complicating an attack of acute pancreatitis.

Thirty-three patients (9 with SLE and 24 with SSc) were on treatment with high dose corticosteroids (pulse methylprednisolone i.v. or oral prednisone >1 mg/kg daily) and/or immunosuppressants such as cyclophosphamide, azathioprine or methotrexate. Other medications commonly administered, also to the remaining patients, were low dose steroids, non-steroidal antiinflammatory agents, hydroxychloroquine, antiplatelet agents, calcium channel blockers and inhibitors of the angiotensin-converting enzymes.

As controls two groups of healthy individuals matched for sex and age for each disease group (36 for SLE and 87 for SSc) were also investigated.

Blood samples

Nine volumes of venous blood for the measurement of VWF antigen and ADAMTS-13 were collected into one volume of 0.129 M sodium citrate. Platelet-poor plasma was obtained by centrifugation at 3000 × g for 20 min, snap frozen and stored at –80°C until tested. Pooled plasma used as reference plasma for all assays was prepared from 50 individuals different from those used as controls and with the following features: age- and sex-matched, healthy, not pregnant and not on treatment with oral contraceptives. Reference plasma was assigned an arbitrary value of 100% of average normal plasma.

ADAMTS-13 activity assay

ADAMTS-13 activity was measured with the collagen binding assay described by Gerritsen *et al.*[12] To evaluate the presence of antibodies inactivating ADAMTS-13, plasma samples were incubated at 56° C for 60 min to destroy any residual protease activity. Then serial dilutions of test samples in phosphate-buffered saline with 1% bovine serum albumin were mixed with equal volumes of normal pooled plasma and incubated at 37° C for 90 min; the levels of residual ADAMTS-13 activity were then measured. In the same assay normal plasma, normal plasma diluted one in four (taken as 100% and 25% activity) and normal plasma diluted one in two (taken as 50% activity) were also run. The dilution of patients' plasma that corresponded to 50% of residual ADAMTS-13 activity was arbitrarily defined as 1 U/mL of inhibitor.

VWF antigen assay

To assay plasma VWF antigen (VWF:Ag), we used an enzyme immunoassay involving rabbit anti-human VWF polyclonal antibodies as first and second antibodies (DAKO, Glostrup, Denmark).

Statistical analysis

The values of ADAMTS-13 and VWF:Ag are given as mean ± standard deviation; the Student's t test for unpaired samples was used to compare the values in patients with SLE and SSc with those in the corresponding control groups.

Results

Figure 1 shows the plasma levels of ADAMTS-13 activity (expressed in percent of average normal plasma) in each patient with SLE or SSc and in the corresponding control individuals. The levels of ADAMTS-13 were significantly lower in SLE patients than in normal controls (mean values ± standard deviation: 89±33 vs 107±27, p= 0.013), the lowest value being 22%. The levels of SSc ADAMTS-13 were also significantly reduced in patients with SSc (79±32 vs 97±28, p=0.0002), the

lowest values being 20%. In the 3 SSc patients who had a history of thrombotic events (*see above*) ADAMTS-13 values were normal (72, 82 and 98%, respectively). The presence of ADAMTS-13 inactivating antibodies was searched for in plasma from 2 patients with SLE and 12 with SSc who had ADAMTS-13 levels below 40%, the lower limit of the normal laboratory range. No inactivating antibody was found in these plasma samples. Figure 2 shows the plasma levels of VWF:Ag in the same patients and controls. For both diseases VWF:Ag was significantly higher than in controls (SLE: 171±69% vs 112±39, p=0.0001; SSc: 168±82 vs 121±45, p=0.0001).

Discussion

The connective tissue diseases SLE and SSc are considered prototypic expressions of multisystem autoimmunity. In SLE immunologic alterations cause polyclonal hypergammaglobulinemia, excessive autoantibody production and immune complex formation; the deposition of immune complexes leads to complement activation, leukocyte infiltration and release of mediators, which underlie microvascular inflammation. A minority of SLE patients suffer from APS, a thrombotic microangiopathy with minimal or no vascular or perivascular inflammation, secondary to the procoagulant effect of antiphospholipid antibodies. A thrombotic microangiopathy with no true vasculitis also characterizes SSc, in which endothelial cell damage or perturbation and immune system activation lead to fibrosis and microvascular occlusions. Hence, these diseases can have features — such as endothelial involvement and occlusions of the terminal circulation — similar to those observed in an autoimmune microangiopathy such as acquired TTP. Therefore, we hypothesized that the presence of inactivating autoantibodies might reduce ADAMTS-13 activity in patients with autoimmune diseases, and that this deficiency might contribute to the microangiopathy of SLE and SSc.

In no instance in patients with SLE or SSc were protease levels as low as those found in patients with typical TTP (less than 10% of normal).[13] Furthermore, anti-ADAMTS-13 autoantibodies were not found in a subgroup of patients chosen on the basis of low protease levels and on the assumption that autoantibodies of clinical significance were unlikely to occur in patients with normal protease levels. The majority of our cases (90 of 123) were receiving no treatment that significantly suppressed immune responses. In the remaining 33 cases, the possibility that the absence of anti-ADAMTS-13 was related to immunosuppressive treatment cannot be ruled out, because Gungor *et al.*[14] reported a case of SLE with TTP in whom immunosuppressive treatment caused the disappearance of the circulating protease inhibitor. However, anti-ADAMTS-13 autoantibodies were absent not only in 5 immunosuppressed patients (2 with SLE and 3 with SSc), but also in 9 untreated SSc patients, all with low protease levels, indirectly suggesting that therapy was not responsible for this negative finding.

On the whole, this study indicates that the occurrence of autoantibodies inactivating the VWF-cleaving protease is not frequent in the connective tissue diseases SLE and SSc. Nevertheless, mean plasma levels of the protease were somewhat lower than in age- and sex-matched controls. Having excluded in this study that the

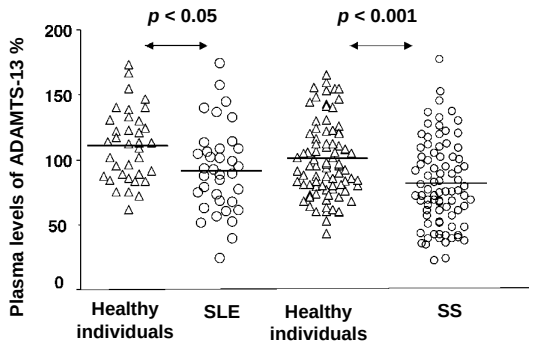

Figure 1. Values of ADAMTS-13 (expressed in percent of average normal plasma) in patients with systemic lupus erythematosus and systemic sclerosis and in two age-and sex-matched groups of healthy individuals. Horizontal solid lines indicate mean values for each group.

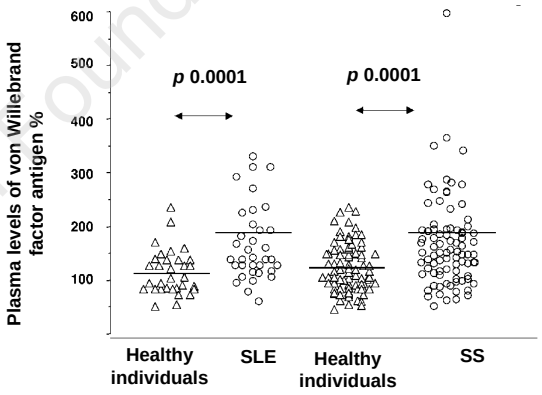

Figure 2. Values of von Willebrand factor antigen. Legend as for Figure 1.

ADAMTS-13 is inactivated by autoantibodies, it cannot be ruled out that autoantibodies bind to the protease at sites other than the enzymatic site and form complexes that retain enzymatic activity but are associated with an accelerated clearance of the protein from plasma. This hypothetical mechanism cannot be investigated at the moment by evaluating ADAMTS-13 plasma half-life, because the protease is present in small amounts in plasma (approximately 1 μg/mL) and cannot be purified in sufficiently large amounts for *ex vivo* clearance studies. Another possible mechanism explaining the low ADAMTS-13 level is decreased production. The synthesis of ADAMTS-13 occurs in the liver, and plasma levels are sometime very low in conditions of severe liver failure.[15] Although this mechanism cannot be formally ruled out, none of our patients had clinical or laboratory evidence of severe liver failure. Approximately one quarter of them received potentially hepatotoxic immunosuppressive drugs. However, low protease levels were observed in both untreated and treated patients, supporting the views that this therapy was not responsible for the reduced plasma levels of ADAMTS-13.

SLE and SSc are other examples of the several clinical and experimental conditions characterized by changes in opposite directions of VWF (high) and ADAMTS-13 (low).[15] Whether this inverse relation is due to consumption of the protease that had to cleave a larger VWF burden or to the fact that ADAMTS-13 is a negative phase reactant while VWF is a positive phase reactant, remains speculative at the moment.

Do low levels of ADAMTS-13 play a role in the thrombotic tendency that often accompanies SLE and SSc? None of our SLE patients had thrombotic symptoms, even in the presence of antiphospholipid antibodies, which are a well known cause of a thrombophilic state in patients with connective tissue disorders. Among the SSc subjects, those who reported past thrombotic events were negative for antiphospholipid antibodies and had fully normal values of ADAMTS-13 activity. ADAMTS-13 levels of 10% or more are thought to be sufficient to dispose ultralarge thrombogenic forms of VWF and to avoid platelet thrombi.[16] Even though multimeric analysis could not be carried out in this study, in all instances patients who were free of thrombotic symptoms had protease levels of at least 20%. On the other hand it has not been established whether low levels, albeit higher than the allegedly critical limit of 10%, interact with other thrombogenic abnormalities present in patients with autoimmune disease to cause a thrombotic tendency. For instance, most patient had high plasma levels of VWF, which is a risk factor for thrombosis.[17]

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Pre-publication Report & Outcomes of Peer Review

Contributions

MV and RS were responsible for the enrollment of the patients, MTC and IF for the laboratory tests and for statistical analysis of the data. PMM wrote the article with contributions from MV and was responsible for the conception and planning of the study.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Vicente Vicente, Deputy Editor. The final decision to accept this paper for publication was taken jointly by Professor Vicente and the Editors. Manuscript received June 4, 2003; accepted June 24, 2003.

In the following paragraphs, Professor Vicente summarizes the peer-review process and its outcomes.

What is already known on this topic

The majority of patients with microangiopathy thrombotic thrombocytopenic purpura (TTP) have a deficiency of the plasma metalloprotease ADAMTS-13. Autoantibodies inactivating this protease are the most frequent causes of TTP. Systemic connective tissue diseases are clinical conditions in which low levels of ADAMTS-13 have been detected.

What this study adds

Patients with autoimmune diseases such as systemic lupus erythematosus and systemic sclerosis presented low but detectable ADAMTS-13 levels. However, this study reveals that autoantibodies inactivating protease activity are not the cause of this biological fact. Moreover, none of the patients investigated had experienced TTP symptoms.