Evidence for a role of anti-ADAMTS13 autoantibodies despite normal ADAMTS13 activity in recurrent thrombotic thrombocytopenic purpura

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ABSTRACT

Background

Severe ADAMTS13 deficiency is a critical component of the pathogenesis of idiopathic thrombotic thrombocytopenic purpura but is found only in about 60% of patients clinically diagnosed with this disease.

Design and Methods

Over a period of 8 years and six episodes of thrombotic thrombocytopenic purpura we studied the evolution of the anti-ADAMTS13 antibody response in a patient using different ADAMTS13 assays and epitope mapping.

Results

Anti-ADAMTS13 autoantibodies were found in all episodes but were inhibitory only in the last two episodes. In a flow-based assay, normal ADAMTS13 activity was found only during the first disease episode, while ADAMTS13 activity was normal using a static assay in episodes 1 and 3, and severely deficient in the last two episodes. Fluorescence evolution in a modified fluorescence resonance energy transfer assay using a von Willebrand factor A2 domain peptide substrate was linear in episodes 1, 5 and 6, but increased exponentially in episodes 3 and 4. Despite the variable functional characteristics of the anti-ADAMTS13 autoantibodies, their principal epitope was the ADAMTS13 spacer domain in all episodes.

Conclusions

The patient is unique as he displayed features of maturation or shaping of the anti-ADAMTS13 autoantibody response during the course of multiple episodes of thrombotic thrombocytopenic purpura. Anti-ADAMTS13 autoantibodies may be important *in vivo* despite normal ADAMTS13 activity in routine assays. Consequently, treatment decisions should not be based solely on activity assay results.

Key words: anti-ADAMTS13 autoantibodies, ADAMTS13 activity, thrombotic thrombocytopenic purpura, ADAMTS13 assays, HIV infection

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Introduction

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disorder characterized by microangiopathic hemolytic anemia, thrombocytopenia and often organ dysfunction as a result of microvascular thrombosis.[1,2] Severe ADAMTS13 deficiency (<5% of the normal) due to circulating anti-ADAMTS13 autoantibodies, inhibiting ADAMTS13 enzymatic activity or increasing ADAMTS13 clearance, defines a subset of patients with idiopathic TTP. These patients have less renal dysfunction, may require more plasma exchange treatments to achieve remission and have an increased risk of relapse compared to clinically indistinguishable patients without severe ADAMTS13 deficiency.[3]

Although severe ADAMTS13 deficiency is a specific finding of TTP, only about two-thirds (range 33-100%) of patients clinically diagnosed with acute idiopathic TTP have severe ADAMTS13 deficiency at disease presentation.[4] The pathophysiology of TTP without severe ADAMTS13 deficiency remains unknown.

Here, we report on a patient who had normal ADAMTS13 activity at presentation with a first acute episode of TTP and experienced five relapses over 8 years during which he subsequently developed a severe autoantibody-mediated ADAMTS13 deficiency.

Design and Methods

Patient

A native American/African-American male[3,5] was admitted to hospital for his first episode of TTP in July 1998. At that time he had no past history of illness and was not taking any regular medication. Three days prior to admission he had developed abdominal pain, nausea, vomiting and diarrhea. He saw a physician because of weakness and a syncopal episode. Laboratory investigations revealed thrombocytopenia and a profound anemia with many schistocytes on the peripheral blood smear. The direct Coombs' test was negative in this and all subsequent episodes. Further laboratory investigations showed mild proteinuria and hematuria, while coagulation studies were normal (Table 1). An infectious illness was considered, although he had neither fever, nor physical signs of an infection and blood cultures were negative. On his 3rd day in hospital he had a transient episode of expressive aphasia and left-sided numbness. TTP was diagnosed and plasma exchange was begun. On the 5th day of plasma exchange the patient presented with fever and chills, and blood cultures were performed and found to be positive for *Staphylococcus aureus*. As the platelet count was normal, the plasma exchange was stopped and the central venous catheter was removed when antibiotic treatment was begun. Further investigations revealed the presence of antibodies to hepatitis A, hepatitis B, and human immunodeficiency virus (HIV) was documented while no antibodies to hepatitis C were found. Highly active antiretroviral therapy (HAART) was begun but shortly afterwards discontinued by the patient. The second episode of TTP occurred in February 2000 with the presenting sign of gross hematuria but no neurological signs. No renal or urinary tract abnormalities were discovered on this or any of his subsequent admissions to explain hematuria. During this episode, fever, chills, and blood cultures positive for *S. aureus* again recurred on the 12th day of plasma exchange. His third acute TTP episode occurred in July 2000, again with the presenting symptom of gross hematuria accompanied by extreme generalized weakness without further neurological abnormalities. No prednisone was given because of the infectious complications during his previous two

Table 1. Clinical presentation, treatment and laboratory findings at consecutive episodes of acute TTP. In all six episodes there were signs of microangiopathic hemolytic anemia and thrombocytopenia, but apart from hematuria and proteinuria only slight renal function impairment. The results for HIV RNA copies/mL (viral load) in plasma revealed the HIV positive state of the patient. HIV treatment was started several times subsequently but always discontinued by the patient within a few weeks.

Age at episode (years)	1st episode 41	2nd episode 42	3rd episode 43	4th episode 44	5th episode 46	6th episode 49
Clinical presentation						
Presenting symptoms	abdominal pain, nausea, vomiting	hematuria	weakness, hematuria	weakness, hematuria	hematuria	weakness, hematuria
Fever	no	no	no	no	no	no
Neurological symptoms	expressive aphasia, left-sided weakness	none	none	none	confusion	none
TTP treatment PEX sessions	6 in 6 days	23 in 41 days	13 in 21 days	12 in 21 days	14 in 15 days	9 in 15 days
steroids	none	yes	none	none	yes	yes
HIV treatment	none	none	none	none	none	none
Laboratory findings (normal range)						
Hemoglobin (136-172 g/L)	57	54	57	81	69	60
Platelet count (130-400 $\times 10^9$/L)	5	5	10	12	18	24
Leukocyte count (3.2-9.8 $\times 10^9$/L)	n.a.§	6.9	8.8	6.3	6.4	5.6
Lactate dehydrogenase (90-200 U/L)	1946	1200	1688	1056	2414	635
Bilirubin (0.1-1.0 mg/dL)	3.6	n.a.§	4.1	2.3	3.1	n.a.
Creatinine (0.6-1.2 mg/dL)	1.2	1.9	1.3	1.0	1.8	1.2
$CD4^+$ cell count (<0.35 $\times 10^9$/L)*	0.26	0.216	0.154	0.19	0.079	0.168
HIV RNA copies / mL (>50,000)*	110,000	72,054	n.a.§	n.a.§	>750,000	555,000

** treatment indication limit ; §n. a. not available; PEX. plasma exchange.*

episodes. Nevertheless, this episode was complicated by femoral vein thrombosis at the site of his central venous catheter which occurred 10 days after plasma exchange was started. On the same day the patient presented with fever and chills, and blood cultures were positive for *S. aureus*. His fourth episode of TTP occurred in April 2001 and was uncomplicated by thrombosis or infection. His fifth TTP episode occurred in December 2003; the presenting sign was again gross hematuria. A total of 14 plasma exchange sessions were necessary to achieve remission. As with his first three episodes *S. aureus* sepsis complicated this course with positive blood cultures on the 7th, 10th, and 12th day of plasma exchange. The patient was readmitted 2 weeks after discharge with fever and back pain. His platelet count and hematocrit were normal and there was no evidence of recurrent TTP. Blood cultures were positive for *S. aureus*, an epidural abscess at L4-L5 with adjacent osteomyelitis was diagnosed and subsequently aortic valve vegetations consistent with bacterial endocarditis were documented. The patient required hospitalization for 3 months to treat the infection effectively and provide rehabilitation. He had intermittent *S. aureus*-positive blood cultures during this period spent in hospital but no signs of recurrent TTP.

The patient experienced his sixth episode of acute TTP 3 years later in December 2006. Leading symptoms were weakness and hematuria. The course was again complicated by *S. aureus* infection, which manifested with fever, chills and positive blood cultures 6 days after plasma exchange was begun. In spite of lacking treatment for his HIV infection during the previous 8 years, his $CD4^+$ lymphocyte count was 168/μL and HIV RNA 555,000 copies/mL. He had had no AIDS-defining disorders and no systemic infections other than the occurrence of *S. aureus* sepsis, attributed to the central venous catheters, with five of his six TTP episodes. The patient never received rituximab. Three months after recovery from this last episode the patient died at home. An autopsy revealed systemic infection with Gram-positive cocci without evidence of recurrent TTP.[5] It was learned after his death that he had regularly used illegal intravenous drugs, including usage of his central venous catheter for drug injections during hospitalizations; this was assumed to be the origin of his recurrent systemic *S. aureus* infections.

The Oklahoma TTP-HUS Registry is approved by the institutional review boards of the University of Oklahoma Health Sciences Center and each participating community hospital. The patient gave written informed consent to the study.

Determination of ADAMTS13 activity and antigen

ADAMTS13 activity was determined by three different assays: (i) a quantitative immunoblotting assay, as previously described.[6,7] In this end-point assay, ADAMTS13 in a diluted test sample is given 18-20 h to proteolyse the substrate, full-length von Willebrand factor purified from plasma, in the presence of 1.5 M urea under static conditions; (ii) a FRETS-VWF73 assay[8] with the reported modifications of prediluting citrated normal human plasma standards in heat-inactivated normal human plasma and adding 1 mM Pefabloc SC (Boehringer, Mannheim, Germany) to the assay mixture.[9] Further refinements of this assay were as follows: the number of calibration samples was extended in the low ADAMTS13 activity range adding standards of normal human plasma prediluted 1:50 and 1:100 in heat-inactivated normal human plasma corresponding to 2% and 1% of ADAMTS13 activity, respectively. All calibration and test samples were diluted 1:25 in assay buffer, mixed with the FRETS-VWF73 peptide substrate at a final concentration of 2 μM and fluorescence evolution was recorded every 5 min for 42 consecutive cycles in a microplate fluorescence reader (Tecan, Zürich, Switzerland). The reaction rate was calculated by linear regression analysis of delta fluorescence over delta time from 5 to 60 min (cycles 2-13).[9] For samples with an ADAMTS13 activity of 25% or less additional reaction rates were calculated over cycles 6-42 (minutes 20–205) and cycles 26-37 (minutes 125–180). This extended fluorescence recording over 3.5 hours was initially set up for the accurate measurement of ADAMTS13 activities between 0-5% to identify patients with Upshaw-Schulman syndrome with residual ADAMTS13 activity in this very low range.[10] The occurrence of photo-bleaching and/or substrate exhaustion for samples with an ADAMTS13 activity of 30% or less in the standard assay was excluded by an extended run of 120 cycles and an increase in the number of light flashes from 10 to 100 per cycle; (iii) a flow-based assay, as described in detail elsewhere.[11-13] In this assay, the extent of cleavage of platelet-covered, endothelial cell-derived ultra-large VWF strings by ADAMTS13 is evaluated under fluid shear conditions. Mixtures of normal human plasma and heat-inactivated normal human plasma corresponding to ADAMTS13 activities of 100%, 50%, 25%, and 10% were used for semi-quantitative calibration.

All the patient's samples were analyzed in duplicate in the immunoblotting and FRETS-VWF73 assay, and twice, on consecutive days, with the flow-based assay.

ADAMTS13 antigen was assessed by a commercially available enzyme-linked immunosorbent assay (ELISA) (TECHNOZYM® ADAMTS-13 antigen, Technoclone, Vienna, Austria) according to the manufacturer's recommendations.

Functional ADAMTS13 inhibitors and anti-ADAMTS13 autoantibodies

Functional ADAMTS13 inhibitors were assessed by mixing heat-inactivated patient's serum 1:1 (v:v) with normal human plasma, followed by incubation for 2 h at 37°C.[14] Residual ADAMTS13 activity was then assessed by immunoblotting and the modified FRETS-VWF73 assay.

The presence of anti-ADAMTS13 autoantibodies of IgG class was assessed by two commercially available ELISA (TECHNOZYM® ADAMTS-13 INH, Technoclone; and IMUBIND® ADAMTS13 Autoantibody ELISA, American Diagnostica Inc., Stamford, CT, USA). According to the manufacturers' specifications a titer of greater than 15.1 arbitrary units (AU)/mL (TECHNOZYM®) and greater than 9.6 AU/mL (IMUBIND®), respectively, represents a positive anti-ADAMTS13 autoantibody result.

In addition, a semi-quantitative dot immunobinding assay for the detection of anti-ADAMTS13 autoantibodies was performed. Aliquots of 20 μL containing 200, 100, 50, 10, 5 and 1 ng of recombinant ADAMTS13 (a gift from F. Scheiflinger, Baxter Innovations, Vienna, Austria) in 0.01M Tris, 0.14 M NaCl, pH 7.4, containing 2 mg/mL bovine serum albumin (TBS-BSA) and 20 μL of TBS-BSA (negative control) were applied to nitrocellulose membranes using a Minifold I filtration apparatus (Schleicher & Schuell Bioscience GmbH, Dassel, Germany). The nitrocellulose membranes were fixed in 10% (v:v) acetic acid, 25% (v:v) isopropanol and blocked with BLOTTO (bovine lacto transfer technique optimizer[15]). The patient's serum samples or normal human plasma diluted 1:100 in BLOTTO were used as the source of primary antibodies, followed by an alkaline phosphatase conjugated rabbit anti-human IgG (D0336, DAKO, Glostrup, Denmark) as the secondary antibody.

von Willebrand factor antigen and multimers

VWF antigen (VWF:Ag) was determined by an in-house ELISA as described elsewhere.[16] The normal range of VWF:Ag was determined as 0.42-1.36 U/mL in 100 healthy controls.

VWF multimer analysis was performed on 1.7% sodium-dodecyl sulfate-agarose (Type VII, LGT; Sigma-Aldrich, St Louis, MO, USA) gels according to Budde *et al.*[17] The patient's multimer patterns were compared to the reference pattern of normal human

plasma run on the same gel by scanning densitometry[18] and visual comparison by one of us (UB), blinded for corresponding ADAMTS13 activity and disease state. To assess endogenous VWF proteolysis semi-quantitatively, VWF satellite bands were classified as being increased, normal, decreased or absent.

Epitope mapping of anti-ADAMTS13 autoantibodies

Epitope mapping of anti-ADAMTS13 autoantibodies was performed by immunoprecipitation using recombinant fragments of different ADAMTS13 protein domains (PMDTCS-13: ADAMTS13 propeptide/metalloprotease/disintegrin/thrombospondin type 1 repeat number 1/cysteine-rich and spacer domains; T 2-8: ADAMTS13 thrombospondin type 1 repeat number 2-8; CUB 1-2: ADAMTS13 CUB domains 1 and 2) as well as a hybrid protein fragment, PMDTCS-1, which corresponds to PMDTCS-13 except for the spacer domain which is replaced by the ADAMTS1 spacer domain, as previously described.[19,20] All of the above mentioned recombinant truncated ADAMTS13 proteins contained a C-terminal V5 tag. Normal human plasma served as an autoantibody-negative control and a commercially available monoclonal anti-V5 antibody (Invitrogen, Carlsbad, CA, USA) as a positive control.

Adsorption of patient's serum samples on protein G sepharose

To remove possible immune complexes containing ADAMTS13, 50 μL of the patient's serum samples were mixed with an equal volume of 10 mM Bis-Tris, pH 7.0 supplemented with 2 mM Pefabloc SC (Boehringer, Mannheim, Germany) and 2 mg/mL BSA (adsorption buffer) and incubated with 50 μL (bed-volume) of protein G sepharose 4 Fast Flow (GE Healthcare Life Sciences, Uppsala, Sweden) by end-over-end rotation for 2 h at room temperature. The supernatant antibody-depleted serum was collected after centrifugation. Original serum samples were diluted 1:3 in adsorption buffer and subsequently analyzed in parallel with antibody-depleted serum samples by the modified FRETS-VWF73 assay at a final dilution of 1:50 in the assay. As a control, normal human plasma was carried along in all experimental steps.

Results

ADAMTS13 activity and related parameters

At presentation with his first acute TTP episode, normal ADAMTS13 activity was measured by all three conceptually different ADAMTS13 assays employed (full-length VWF *versus* VWF A2 domain peptide as substrate, flow-based *versus* static assays, endpoint *versus* kinetic assays) (Table 2). At subsequent acute TTP episodes severely deficient ADAMTS13 activity was documented first by the flow-based assay, followed by the FRETS-VWF73 assay and with the onset of the fifth episode all three assays showed severely deficient ADAMTS13 activity.

In parallel with the results of the flow-based ADAMTS13 activity assay we documented severely impaired proteolysis of endogenous VWF as evidenced by the absence of satellite bands on VWF multimer analysis.

Notably, analysis using the modified FRETS-VWF73 assay with recording over 3 h revealed that fluorescence evolution was not linear but followed a polynomial function (2nd degree polynomial function, $R^2 = 0.9981$) at presentation with the third and fourth episodes and ADAMTS13 initial rate activity increased over time (Figure 1). Computation of ADAMTS13 activity for the first (cycles 2-13) and the third hour (cycles 26-37) separately, gave ADAMTS13 activities of 12% and 38% for the third episode and of less than 1% and 9% for the fourth episode.

Functional ADAMTS13 inhibitors were detected by the FRETS-VWF73 assay from the third episode onwards, and from the fourth episode onwards also by the immunoblotting assay (Table 2). Moreover, anti-ADAMTS13 IgG were found by an ELISA as well as by a dot immunobinding assay already at the first TTP episode even though the titers increased greatly during subsequent episodes (Table 2; Figure 2A).

Epitope mapping of anti-ADAMTS13 autoantibodies

The principal antigenic epitope of the disease-associated anti-ADAMTS13 autoantibodies of all acute TTP episodes was located in the ADAMTS13 spacer domain, despite the antibodies' otherwise variable characteristics. Antibodies of all acute episodes recognized the ADAMTS13 PMDTCS-13 fragment (Figure 2B), while interactions of the antibodies with the hybrid fragment PMDTCS-1, where the ADAMTS13 spacer domain is replaced by the spacer domain of ADAMTS1, as well as with the ADAMTS13 CUB 1-2 fragment were weak in all episodes. In none of the acute episodes did we detect antibodies reacting with the construct consisting of the C-terminal thrombospondin repeats (T2-8).

Table 2. **ADAMTS13-related parameters at presentation with consecutive acute TTP episodes. ADAMTS13 activity was determined by three different ADAMTS13 assays: the quantitative immunoblotting assay (IB), the 1 h FRETS-VWF73 assay (FRETS) and a flow-based assay (see *Design and Methods* section for details). No sample was available from the second acute TTP episode, and the amount of serum available from the sixth episode was not sufficient to perform all assays.**

TTP episode	ADAMTS13 activity			Anti-ADAMTS13 autoantibodies					ADAMTS13 Antigen (%)	VWF Antigen (%)	Endogenous VWF proteolysis (semi-quant.)†
				Functional inhibitor (BU/mL)		IgG-Antibodies (AU/mL)					
	IB (%)	FRETS (%)	Flow-based (semi-quant.)	IB	FRETS	Technozym	IMUBIND	Dot*			
1st	60%	53%	normal	none	none	26.8	13.5	weak	52	326	normal
3rd	50%	15%	severely deficient	none	1.4	73.5	31.6	strong	97	148	absent
4th	6%	<5%	severely deficient	traces	0.8	>88.3	25.9	strong	50	343	absent
5th	<5%	<5%	severely deficient	1	1.1	>88.3	26.7	strong	47	301	decreased
6th	<5%	<5%	n.d.§	1-2	1.4	88.2	n.d.	n.d.	63	291	decreased

**Dot immunobinding assay (qualitative information only); †Proteolysis of endogenous VWF was assessed by rating the presence of the VWF satellite bands on VWF multimer analysis as being increased, normal, decreased or absent. §n. d. : not done.*

Adsorption of patient's serum on protein G sepharose

Given the variable ADAMTS13 antigen values of 47% to 97% and simultaneous ADAMTS13 initial rate activity values by FRETS-VWF73 assay of less than 5% to 53% at consecutive acute TTP episodes (Table 2), we explored the possibility that ADAMTS13 was complexed with inhibiting autoantibodies. Furthermore, we investigated the hypothesis that ADAMTS13 dissociated from its inhibitors during the course of the FRETS-VWF73 assay. Removing the entire antibody fraction from the serum samples by adsorption on protein G sepharose revealed that ADAMTS13 activity in IgG-depleted serum of the first acute TTP episode was almost identical to the activity in unadsorbed serum, while IgG-depleted serum of the third and the sixth episodes was virtually devoid of ADAMTS13 activity with higher activity in the respective unadsorbed serum sample (Figure 3).

Discussion

This study illustrates several important aspects concerning development of autoimmunity towards ADAMTS13, pathogenesis of TTP in an HIV-positive patient and information provided by different ADAMTS13 activity assays. The patient presented with a first acute TTP episode in 1998. At this time ADAMTS13 activity was normal, as determined by three conceptually very different assays. Over a period of 8 years the patient experienced five relapses and developed inhibitory anti-ADAMTS13 antibodies leading to a severe ADAMTS13 deficiency at presentation of episodes 4 through 6.

Anti-ADAMTS13 autoantibodies were found in samples taken during all of the acute TTP episodes. The main antigenic epitope recognized by these anti-ADAMTS13 autoantibodies was found to reside in the ADAMTS13 spacer domain (Figure 2B), which has been reported to contain the primary antigenic epitope of inhibitory anti-ADAMTS13 antibodies.[19,21-23] This suggests a pathophysiological role of the anti-ADAMTS13 autoantibodies present in low titers during the first acute episode despite normal *in vitro* ADAMTS13 activity documented by three different assays.

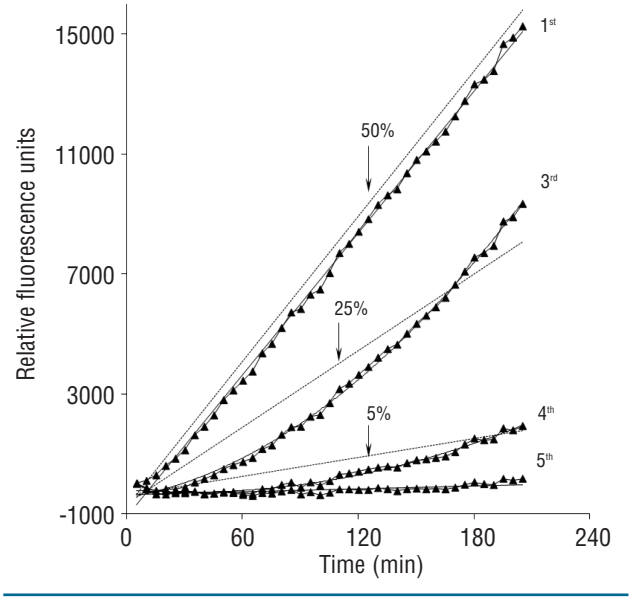

Figure 1. Fluorescence evolution over time in the modified FRETS-VWF73 assay for patient's serum samples withdrawn at the onset of the 1st, 3rd, 4th and 5th acute TTP episodes. Dotted lines represent the fluorescence evolution in standard curve samples (defined mixtures of normal human plasma and heat-inactivated normal human plasma, see *Design and Methods* section for details) run in the same assay.

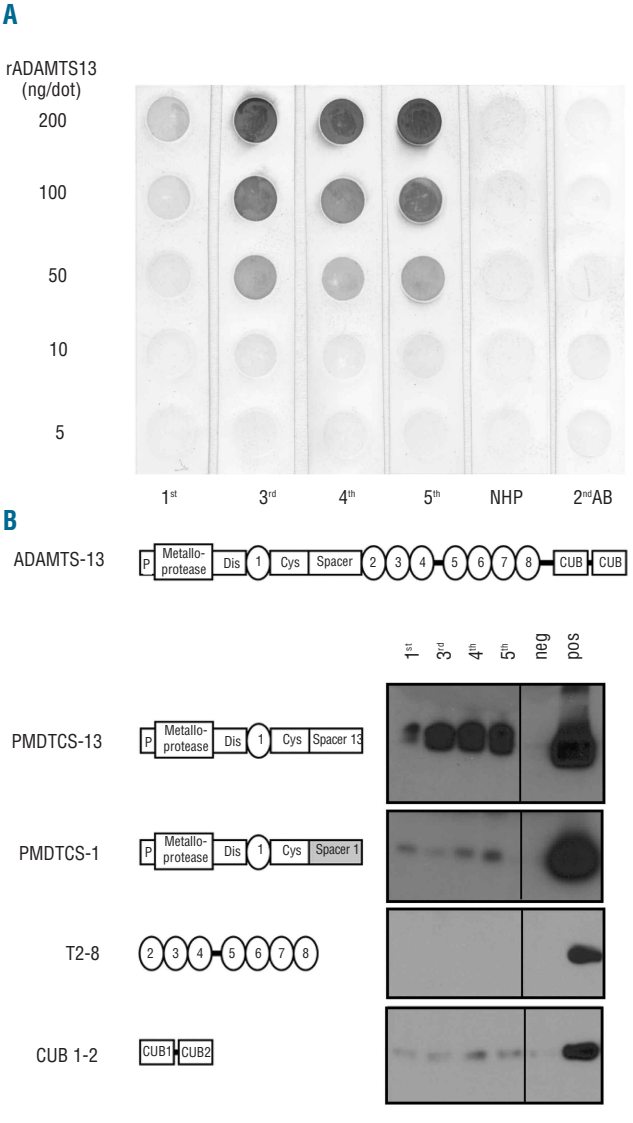

Figure 2. *In vitro* characterization of anti-ADAMTS13 autoantibodies. (A) Dot immunobinding assay. Recombinant ADAMTS13 was spotted at different concentrations onto nitrocellulose membranes and 1:100 (v:v) diluted patient's serum samples from different acute TTP episodes (1st – 5th) was used as the source of the primary antibody. A 1:100 diluted normal human plasma pool (NHP) and the secondary alkaline phosphatase-conjugated rabbit anti-human IgG antibody used as primary antibody sources served as controls. (B) Epitope mapping of anti-ADAMTS13 autoantibodies by immunoprecipitation, and a schematic overview of ADAMTS13 domain structure and of different ADAMTS13 constructs used for analysis (PMDTCS-13, consisting of ADAMTS13 propeptide – *met*alloprotease – *d*isintegrin – thrombospondin type I repeat number 1 – *cys*-rich and spacer domain; PMDTCS-1, the same as PMDTCS-13, however with replacement of ADAMTS13 spacer by the spacer domain of ADAMTS1 (highlighted); T 2-8, ADAMTS13 thrombospondin type I repeats number 2 – 8; CUB 1-2, ADAMTS13 CUB domains 1-2). ADAMTS13 constructs all containing a carboxy-terminal V5 tag,[19,20] were used as antigen. A normal human plasma pool served as a negative control, and a commercially available monocloncal anti-V5 antibody as a positive control.

The study illustrates that discrepant ADAMTS13 activity results obtained by different methods may have a pathophysiological basis.[3] Understanding the limitations and the strengths of the assays opens up the possibility of detecting different ADAMTS13 pathologies with different assays. ADAMTS13 assays representing the *in vivo* situation as closely as possible, including a short interaction time between ADAMTS13 and its substrate, endothelium-adherent long VWF strings, in the presence of platelets and shear stress, have been eagerly sought after during the last decade but have not reached ordinary laboratories so far. The flow-based assay used here[11] is an example of such an assay. It detected a severe ADAMTS13 deficiency consistent with the clinical picture of acute TTP in our patient already in the third episode, when the two other assays still measured an ADAMTS13 activity of 50% and 15%. However even this assay failed to detect deficient ADAMTS13 activity at the time of this patient's initial presentation.

The modified FRETS-VWF73 assay reported in detail here is set as a kinetic assay allowing the recording of substrate turnover over a prolonged time (Figure 1). From the polynomial fluorescence evolution over time in the third and fourth TTP episodes, it is evident that substrate turnover per unit of time reflecting ADAMTS13 initial rate activity is lower in the first minutes than later on. This may explain the higher activity in an endpoint assay, particularly in the immunoblotting assay in which patient's ADAMTS13 and the VWF substrate are incubated for 18-20 h. During prolonged incubation ADAMTS13 activity is at least in part set free from circulating ADAMTS13 containing immune complexes (Figure 3), which may also influence the results in other ADAMTS13 assays with prolonged enzyme-substrate interaction times.

Polynomial fluorescence evolution in the FRETS-VWF73 assay is a phenomenon that we have encountered only in the presence of anti-ADAMTS13 autoantibodies and it is often particularly prominent in patients during follow-up after an acute TTP episode with acquired severe ADAMTS13 deficiency when anti-ADAMTS13 autoantibodies reappear (*unpublished observations*).

Systemic infections can mimic all of the clinical features of TTP.[24] Moreover, reduced and even severely deficient ADAMTS13 activities have been reported in severe sepsis and septic shock.[16,25] Although *S. aureus* infections were documented during five of the six acute TTP episodes, clinical and laboratory signs of systemic infections, including positive blood cultures, developed at least 5-12 days after beginning plasma exchange, while at presentation with all six TTP episodes when ADAMTS13 activity was measured, the patient was afebrile with no clinical or laboratory signs of sepsis. We, therefore, judged the *S. aureus* infections as complications of his central venous catheter.

Diagnostic work-up during the first acute episode revealed that the patient was HIV-positive. Whether and, if so, to what extent the patient's uncontrolled HIV infection, as evidenced by the high numbers of HIV RNA copies as well as the low CD4$^+$ lymphocyte counts documented on several occasions (Table 1), contributed to the development of TTP and the evolution of pathogenic anti-ADAMTS13 autoantibodies remains unanswered. In HIV, neither the cellular nor the humoral immune system is able to control the infection which ultimately results in major immunological defects in the T-cell as well as the B-cell compartments. This is illustrated by spontaneous antibody production of cultured peripheral lymphocytes, hypergammaglobulinemia, the presence of autoantibodies and of circulating immune complexes.[26-29] Chronic activation of the immune system in this case likely fostered the generation of anti-ADAMTS13 autoantibodies, which were already found during the first acute TTP episode but increased in titer over time and whose functional properties changed by gaining inhibitory capacity (Table 2, Figure 2A). This implies affinity maturation by somatic hypermutation of the patient's immunoglobulin genes, a process dependent on antigen presentation and CD4$^+$ T helper cells. The effect of HIV infection on number and function of these cells may explain the relatively slow evolution of the antibody response over the course of six TTP episodes during 8 years.

In summary, the course of TTP in the patient described is remarkable, as we were able to observe the evolution of pathogenic anti-ADAMTS13 autoantibodies over a period of 8 years, a process which was likely fostered by the chronic activation of the immune system in the context of his HIV infection. In addition, we have presented the first suggestion that low titers of anti-ADAMTS13 autoantibodies – even in the presence of normal ADAMTS13 activity as measured by two routine and one research assays – may have a pathophysiological role. Consequently, treatment decisions should not be based solely on activity assay results. Finally, the study illustrates that current ADAMTS13 assays – although possibly far from ideal - may already provide helpful and important information when their potential is recognized and consequently exploited, such as by observing fluorescence evolution over a prolonged time as in the 3.5 h FRETS-VWF73 assay.

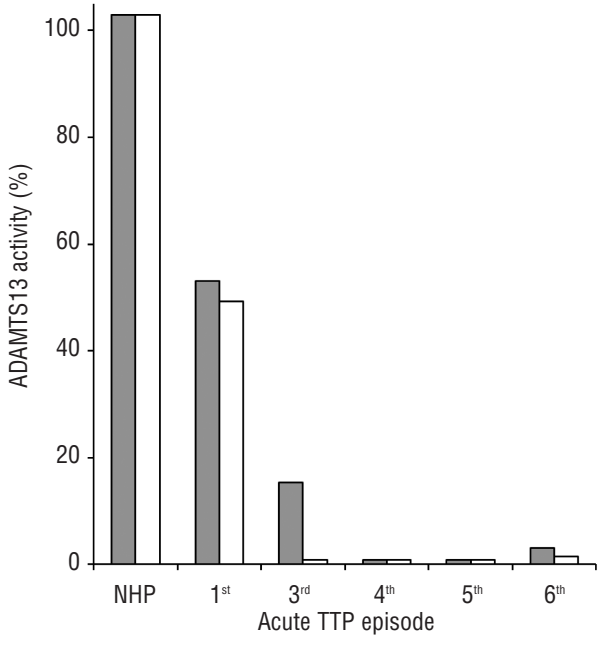

Figure 3. Adsorption of the patient's serum samples on protein G sepharose. Antibodies and immune complexes were removed by protein G sepharose from the patient's serum samples taken during the 1st, 3rd, 4th, 5th and 6th episodes. ADAMTS13 activity was determined in serum before (gray columns) and after IgG-depletion using protein G sepharose (white columns).

Authorship and Disclosures

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