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Epitope mapping of ADAMTS13 autoantibodies in acquired thrombotic thrombocytopenic purpura

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Abstract

A severe deficiency of the von Willebrand factor (VWF)-cleaving protease, ADAMTS13, can lead to thrombotic thrombocytopenic purpura (TTP), a disease associated with the widespread formation of platelet-rich thrombi in many organs. Autoantibodies that inactivate ADAMTS13 are the most frequent cause of acquired TTP. Little is known about epitope specificity and reactivity of anti-ADAMTS13 antibodies. In this study a series of ADAMTS13 domains were expressed in *Escherichia coli* and the reactivity of purified recombinant fragments with anti-ADAMTS13 autoantibodies from 25 patients with severe ADAMTS13 deficiency was evaluated in vitro. All TTP plasmas contained antibodies directed against the cysteine-rich and the spacer domain (cys-rich/spacer) of ADAMTS13. In the plasmas of 3 patients antibodies were detected that reacted exclusively with the cys-rich/spacer domain, underscoring the importance of this region for functional activity of ADAMTS13. In 64% of the plasmas antibodies reacted with the two CUB domains and in 56% with the isolated first thrombospondin type 1 repeat (TSP-1) and with the compound fragment consisting of the catalytic, the disintegrin-like and the TSP1-1 domain. Less frequent, in 28%, antibodies reacted with the TSP1 repeats 2-8. Unexpectedly, antibodies reacted with the propeptide region in 20% of the plasmas. In conclusion, this study shows that even though anti-ADAMTS13 autoantibodies react with multiple domains of the protease, the cys-rich/spacer domain is consistently involved in antibody reactivity.

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Introduction

Abnormalities of von Willebrand factor (VWF) have a prominent role in the pathogenesis of thrombotic thrombocytopenic purpura (TTP), as indicated by the excessive accumulation of VWF in microvascular platelet thrombi found in the vascular lesions characteristic for TTP [1] and by unusually large VWF (ULVWF) multimers detected in patients with the chronic relapsing form of the disease [2]. ULVWF was shown to interact very efficiently with the platelet glycoprotein Ibα (GpIbα), a component of the GpIb/IX/V receptor for VWF expressed on platelets [3,4]. Physiologically, efficient VWF-platelet interaction is of major importance for the recruitment of platelets to the site of vascular injury, but a prolonged uncontrolled presence of ULVWF may cause platelet thrombus formation in the microcirculation, particularly in conditions of high shear stress.

The currently most favored hypothesis of the pathophysiology of TTP proposes that a still unknown precipitating event causes the excessive accumulation of ULVWF multimers on endothelial cell surfaces and in plasma that, in the absence of VWF-cleaving protease activity, leads to ULVWF-mediated platelet thrombus formation and ischemic symptoms in the circulation of multiple organs.

A VWF-cleaving protease, first isolated and characterized by Tsai [5] and by Furlan et al. [6], was shown to cleave VWF at position Tyr[1605] - Met[1606], the peptide bond physiologically cleaved in vivo [7]. Protein purification and N-terminal sequencing as well as positional cloning identified the protease as a new member of the ADAMTS family of metalloproteases [8-10].

This new protease was called ADAMTS13 (A disintegrin and metalloprotease with thrombospondin type 1 repeats) [11]. A complete lack of ADAMTS13 activity coupled with the presence of ULVWF multimers was first found in 4 patients with chronic relapsing TTP [12]. Autoantibodies inactivating ADAMTS13 activity were subsequently described in a patient with acute acquired TTP [13] and confirmed in a large proportion of patients diagnosed with this form of the disease [14,15].

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Autoantibodies inhibiting ADAMTS13 activity have since been detected in the majority of patients with acquired TTP [14-19]. However, acute TTP causing antibodies might not interfere with the activity assays [20], or may be directed against molecules other than ADAMTS13, although physiologically interacting with ADAMTS13 activity [21]. For instance, antibodies against CD36 (thrombospondin receptor) have been found in 23 out of 27 TTP plasmas analyzed [22] and 8 of 11 TTP patient plasmas reacted with a 85 kDa form of CD36 [23].
ADAMTS13 inhibitors have not been fully characterized at the molecular level. Recent publications pointed out the critical importance of the cysteine-rich and the spacer region for substrate recognition and VWF cleavage [24,25]. Competitive inhibition using a series of peptides derived from different ADAMTS13 regions further revealed that the C-terminus of ADAMTS13, including the thrombospondin type 1 repeats (TSP1) 2-8 and the CUB domains, are necessary for ADAMTS13 to bind to VWF under flow conditions [26,27]. Against this background we were interested in a more detailed analysis of the autoimmune response leading to severely deficient ADAMTS13 activity in patients with acquired TTP. To map major epitopes of the human anti-ADAMTS13 antibody response in vivo, we expressed ADAMTS13 domains in *E. coli,* either individually or as units of consecutive domains and used the purified recombinant polypeptides as antigens in a series of Western blots.

Material and Methods

Expression and purification of ADAMTS13 fragments in *E. coli*

DNA fragments coding for the various ADAMTS13 domains were generated by PCR using Hot Star Taq DNA polymerase (Qiagen, Hilden, Germany) and the wild-type ADAMTS13 cDNA [28] as a template. Primer sequences used for the amplification of the desired nucleotide regions are available on request. The resultant PCR fragments were cloned into the pBAD/Thio-TOPO expression vector using the TOPO cloning technology according to the

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suppliers' instructions (Invitrogen, Lofer, Austria). Fragments chosen to be expressed were: 1) the propeptide domain, 2) the compound fragment containing the entire catalytic domain, the disintegrin-like domain and the first thrombospondin type 1 repeat (TSP1-1) element (cat/dis/tsp1-1), 3) the isolated TSP1-1 domain (tsp1-1), 4) the cysteine-rich and the spacer domain (cys-rich/spacer), 5) a fragment containing TSP1 repeats 2-8 (tsp1/2-8), 6) the two CUB domains (cub1+2) and, as a negative control, the his-tagged thioredoxin (thio/his) fragment. Upon transformation into *E. coli* TOP10 (Invitrogen) ADAMTS13 fragments were produced as thioredoxin fusion proteins with a C-terminal $(his)_6$-tag attached to facilitate detection and purification. After induction of protein expression with 0.002% arabinose for 4 hours at 37°C, bacterial cells were collected and lysed in 300 mM NaCl, 50 mM sodium phosphate pH 8.0, and 10 mM imidazole containing 1 mg/mL lysozyme. The recombinant fragments consisting of the propeptide, the tsp1-1 and the thio/his fusion part (from the empty parental vector) were obtained from the soluble fraction, whereas the larger compound fragments cat/dis/tsp1-1, cys-rich/spacer, tsp1/2-8 and cub 1+2 were from the insoluble fraction. Soluble fragments were purified under native conditions using Ni^{2+}-charged HiTrap chelating HP columns (Amersham Biosciences, Freiburg, Germany) and increasing imidazole concentrations. Fragments obtained from inclusion bodies were treated with solubilization buffer (8 M urea, 20 mM sodium phosphate pH 7.5, 0.5 M NaCl, 30 mM imidazole), applied to the HP columns and eluted with increasing concentrations of imidazole. The cat/dis/tsp1-1 fragment was further purified by anion-exchange chromatography using Q Sepharose XL (Amersham Biosciences). The purity of the isolated recombinant fragments was judged by SDS-PAGE electrophoresis and silver staining.

Plasma samples

Plasma samples 1-10 were collected at the Central Hematology Laboratory, University of Bern in Switzerland and samples 11-25 at the Angelo Bianchi Bonomi Hemophilia and

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Thrombosis Center, Milan, Italy. Measurement of ADAMTS13 activity was carried out according to Studt et al. [29] (plasmas 1-10) and to Gerritsen et al. [30] (plasmas 11-25). The inhibitory titer expressed in Bethesda units (BU), was determined as described by Knöbl et al. [31] for plasma 1-10 and by Lattuada et al. [32] for plasma 11-25. A pool of normal human plasma (NHP; Baxter AG, Vienna, Austria) with no detectable ADAMTS13 inhibitor was used as a negative control.

Western blot analysis

Equivalent amounts of purified recombinant protein were reduced, denatured, subjected to 12% SDS-PAGE electrophoresis and transferred to nitrocellulose membranes as described [33]. Purified recombinant ADAMTS13 (rADAMTS13), derived from mammalian cell culture [28], and the thioredoxin-his fusion part, derived from *E. coli* and expressed from the empty parental vector, were used as positive and negative controls, respectively.

Rabbit anti-human-rADAMTS13 antiserum was generated by repeated immunization of one New Zealand White rabbit (Charles River, Kisslegg, Germany) with purified, recombinant C-terminally his-tagged human ADAMTS13 (rADAMTS13-his). The first immunization was carried out using 20 µg rADAMTS13-his in complete Freund's adjuvant whereas the booster immunization was done with incomplete Freund's adjuvant.

Citrated plasma samples, collected from patients with acute acquired TTP, were used as a source of anti-ADAMTS13 antibodies (primary antibody, diluted 1:500 in 10 mM Tris pH 8.0, 150 mM NaCl, 0.05% Tween 20 containing 2% skim milk and 5% TOP10 lysate). Bound human anti-ADAMTS13 antibodies were visualized using goat anti-human Ig specific for the α, γ, µ chain (Sigma, Cambridge, UK) coupled to alkaline phosphatase (AP) and BCIP/NBT (Promega, Madison, WI, USA) as substrate. His-tagged proteins were visualized using murine anti-his monoclonal antibody (Qiagen) and secondary AP-conjugated anti-mouse IgG (Sigma) antibody and BCIP/NBT.

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The rabbit anti-ADAMTS13-his antiserum was used in a 1:1000 dilution and bound antibodies were detected by an AP-conjugated goat anti-rabbit IgG antibody (1:10.000, Promega) and BCIP/NBT.

Results

Expression of ADAMTS13 domains in *E. coli*

The ADAMTS13 fragments used for Western blot analysis spanned the propeptide region (amino acid residues 30-74, numbering of amino acids according to Zheng et al. [11]), the cat/dis/tsp1-1 region (amino acid residues 75-439), the central tsp1-1 fragment (amino acid residues 386-439), the cys-rich/spacer domain (amino acid residues 440-685), the series of C-terminal TSP1 repeats 2-8 (amino acid residues 686-1131) and the cub1+2 domains corresponding to amino acid residues 1132-1427 (figure 1A, table 1). The predicted molecular weights of the various ADAMTS13 and thioredoxin-his fusion proteins are listed in table 1.

	Protein fragment (aa)	MW (including thio/his)	Region in ADAMTS13
I	30(P) - 74 (R)	21.3 kDa	propeptide
II	386 (R) - 439 (E)	22 kDa	tsp1-1
III	75(A) - 439(E)	55 kDa	cat/dis/tsp1-1
IV	440(K) - 685(A)	43.7 kDa	cys-rich/spacer
V	686(W) - 1131(V)	63.2 kDa	tsp1/2-8
VI	1132(G) – 1427(T)	48.3 kDa	cub1+2

Table 1 . Summary of the domains cloned for expression in *E. coli*. The positions of the amino acids spanning the selected domain(s) and the calculated molecular weight (MW) of the predicted his-tagged thioredoxin fusion protein are indicated.

Because many human plasma samples contain anti-*E. coli* antibodies, the possibility that contaminating *E. coli* proteins could give rise to false positive results was taken into

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consideration. All ADAMTS13 fragments were purified to homogeneity and were shown by silver staining to be free from contaminating *E. coli* cell debris (figure 1B). All the fusion proteins, with one notable exception, were expressed and purified as single bands. The exception was the fragment tsp1/2-8, which was somewhat difficult to purify. Despite many efforts, we were unable to eliminate three contaminating protein bands of approximately 44 kDa, 36 kDa and 25 kDa, apparently derived from *E. coli* (figure 1B). Western blot analysis using an anti-his-tag antibody confirmed that the purified ADAMTS13 fragments were isolated as single undegraded bands (figure 1C) with the expected molecular weight.

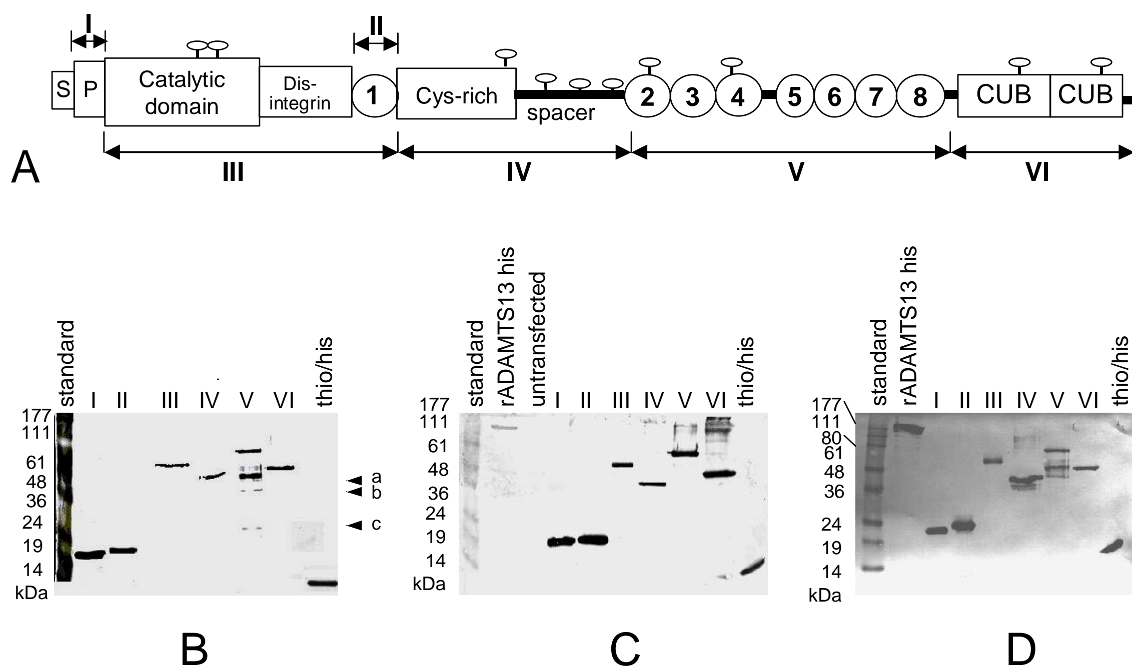

Figure 1. A) Domain organization of ADAMTS13 and a map of the ADAMTS13 fragments (I, II, III, IV, V and VI) expressed in *E. coli*. Indicated are the signal peptide (S), the propeptide (P), the catalytic, the disintegrin-like, the cysteine-rich (Cys), the spacer domain, the two CUB domains as well as the 8 TSP type 1 repeats (1-8) (according to Zheng et al. [11]). **B**) Silver staining of purified recombinant ADAMTS13 fragments expressed in *E. coli*. (a), (b) and (c) mark contaminating bands derived from *E. coli*, which could not be removed

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during the purification process. **C**) Western Blot analysis of the purified recombinant ADAMTS13 fragments using an anti-his-tag antibody. **D**) Western Blot analysis of the purified recombinant ADAMTS13 fragments using a polyclonal rabbit anti-human ADAMTS13-his antiserum. Abbreviations used are: fragment I : propeptide, fragment II : tsp1-1, fragment III : cat/dis/tsp1-1, fragment IV : cys-rich/spacer, fragment V : tsp1/2-8, fragment VI: cub1+2.

In addition, all the purified fragments strongly reacted with a high-titer rabbit anti-human ADAMTS13 antiserum (figure 1D) as single bands and no cross reaction with contaminating *E. coli* material (i.e. bands a, b, and c) was seen. The thio/his fragment most likely cross-reacts in this assay because it contains a his-tag and the rabbit anti-ADAMTS13 antiserum contains anti-his tag antibodies too.

Western Blot analysis of the patients' plasma samples

Twenty-five plasma samples, from patients with idiopathic acquired TTP (table 2) were selected for analysis. The patients had severe to moderately severe ADAMTS13 deficiency with a residual ADAMTS13 activity varying from less than 3% to 10 % of normal (table 3). The titers of inhibitory antibodies were in the range of 1 to 40 BU/mL.

In this study we found no obvious correlation between the ADAMTS13 inhibitor titer of an individual plasma, the residual ADAMTS13 activity and the number or amino acid sequence of ADAMTS13 fragments recognized. A typical result of the Western blot analysis is shown in figure 2 A using plasma #14 (table 3) with a very high ADAMTS13 inhibitory titer of 32 BU/mL. In this plasma we found anti-ADAMTS13 antibodies reactive with the tsp-1, the cat/dis/tsp1-1, the cys-rich/spacer and the cub1+2 region.

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TTP Plasma #	Age	Sex	Clinical Characteristics
1	57	m	idiopathic, acute
2	38	m	idiopathic, relapse
3	43	m	idiopathic, acute
4	25	m	idiopathic, acute
5	27	f	idiopathic, relapse
6	39	m	idiopathic (previous drug-intake not excluded)
7	49	m	idiopathic, acute
8	49	f	relapse (first episode during pregnancy at age 35)
9	29	f	idiopathic, relapse
10	63	f	idiopathic, relapse
11	37	f	idiopathic, in remission
12	36	f	idiopathic, in remission
13	35	f	idiopathic, recurrent
14	43	m	idiopathic, acute
15	27	f	idiopathic, in remission after first episode of acute TTP
16	52	f	idiopathic, in remission after second episode of acute TTP
17	29	f	idiopathic, acute
18	20	f	idiopathic, acute
19	27	f	idiopathic, acute
20	24	f	idiopathic, in remission after splenectomy
21	25	m	idiopathic, in remission after second episode of acute TTP
22	33	f	idiopathic, in remission after second episode of acute TTP
23	37	m	idiopathic, recurrent
24	n.a.	f	idiopathic, acute
25	46	m	idiopathic, relapse

Table 2 . TTP patients characteristics. m, male; f, female.

All plasmas reacted with the full-length ADAMTS13 molecule (figure 2, table 3). More specifically, all of the plasmas tested contained antibodies directed against the cys-rich/spacer region. In three plasmas (#1, #3 and #5; table 3) antibodies reacted only with this region (figure 2D), identifying it as essential for ADAMTS13 activity in vitro. Sixteen of the 25 plasmas (64%) contained antibodies directed against the polypeptide spanning the CUB1 and the CUB2 domain, making this the second most frequently recognized region. Fourteen plasmas (56%) contained antibodies against the composite fragment cat/dis/tsp1-1. Ten of the plasmas reactive with cat/dis/tsp1-1 also reacted with the isolated tsp1-1 fragment, indicating that they contained antibodies against this particular region. However, 4 plasmas (#22, #23, #24, #25, table 3) contained antibodies strongly reactive with the isolated tsp1-1 fragment, but

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not with the tsp1-1 region when the domain was presented in the context of the catalytic and the disintegrin-like domain in the cat/dis/tsp1-1 fragment (table 3, figure 2C).

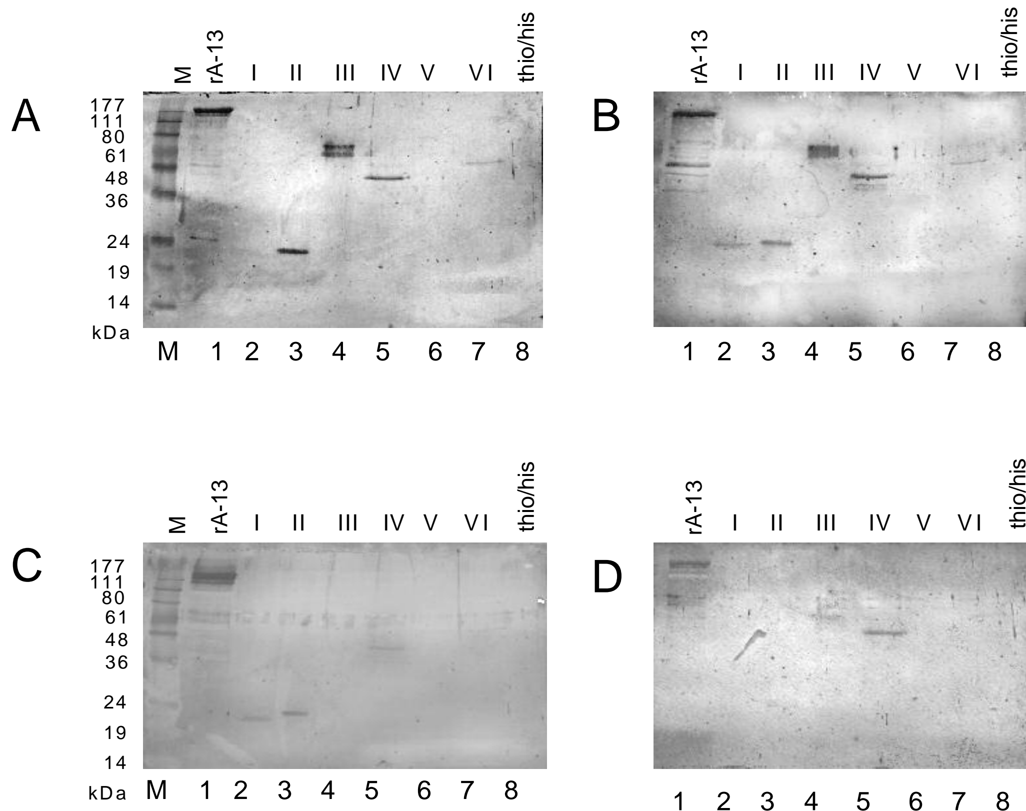

Figure 2. Immunoblot analysis showing the domain-specific reactivity of TTP patient plasmas with different anti-ADAMTS13 inhibitor titers. Equivalent amounts of recombinant ADAMTS13 fragments were resolved on a 12% SDS-PAGE and electroblotted. Patient plasma sample #14 (32 BU/mL) (A), #18 (6 BU/mL) (B), # 22 (10 BU/mL) (C) and #3 (3 BU/mL) (D) were used as a source of primary antibody, AP-conjugated anti-human Ig and BCIP/NBT was used for visualization. Recombinant protein, lane 1: recombinant ADAMTS13 (rA-13) derived from HEK 293 cells. Purified ADAMTS13 fragments, lanes 2-7: propeptide (lane 2, I), tsp1-1 (lane 3, II), cat/dis/tsp1-1 (lane 4, III), cys-rich/spacer (lane 5, IV), tsp1/2-8 (lane 6, V) and cub1+2 (lane 7, VI). As negative control the thio/his fragment was applied (lane 8). M, Molecular weight markers.

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Only 7 plasmas (28%) contained antibodies reactive against ADAMTS13 fragment tsp1/2-8. Antibodies directed against the ADAMTS13 propeptide were detected in 5 out of 25 plasmas (20%), (figure 2, B and C). Most plasmas contained antibodies reactive with 3-5 fragments, suggesting that the antibody response is directed against various epitopes located across the entire molecule.

				ADAMTS13 fragments (prokaryotic)					
TTP Plasma #	ADAMTS13 activity (%)	anti-ADAMTS13 Inhibitor titer [BU/mL]	rADAMTS13 (eukaryotic)	pro-peptide	tsp1-1	cat/dis/tsp1-1	cys-rich/spacer	tsp1/2-8	cub1+2
1	~6	3	+				+		
2	<5	3	+			+	+		+
3	<3	3	+				+		
4	<3	8	+				+		+
5	<3	9	+				+		
6	3-5	8	+				+		+
7	<3	6	+		+	+	+	+	+
8	<3	1	+				+	+	+
9	<3	10	+				+		+
10	<5	40	+	+	+	+	+	+	+
11	<6	16	+			+	+	+	+
12	<6	2	+		+	+	+		+
13	<6	8	+		+	+	+		+
14	<6	32	+		+	+	+		+
15	<6	18	+			+	+		
16	9	1	+		+	+	+		
17	<6	1	+		+	+	+		+
18	<6	6	+	+	+	+	+		+
19	<6	4	+			+	+		+
20	<6	4	+		+	+	+		
21	<6	2	+		+	+	+	+	
22	<6	10	+	+	+		+		
23	10	2	+	+	+		+		
24	10	2	+	+	+		+	+	+
25	<6	5	+		+		+	+	+
			25/25	**5/25**	**14/25**	**14/25**	**25/25**	**7/25**	**16/25**
			100%	**20%**	**56%**	**56%**	**100%**	**28%**	**64%**

Table 3. Summary of the results obtained with immunoblot analyses using TTP patient plasma. (+) denotes a positive reactivity towards the respective ADAMTS13 fragment.

To summarize, in plasma from 25 acute TTP patients with severe ADAMTS13 deficiency, we found reactivity against the various ADAMTS13 fragments in the following order: cys-rich/spacer: 100%, cub1+2: 64%, cat/dis/tsp1-1: 56%, tsp1-1: 56%, tsp1-2/8: 28% and propeptide: 20%.

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Discussion

The multidomain structure of ADAMTS13 is highly conserved across mammals, birds and fish [34] implying preserved and essential functions for each of the structural elements. Although the physiological function of most of the domains is currently unknown, they obviously act together in a concerted way to mediate ADAMTS13 function in vivo. The inhibition or depletion of ADAMTS13 activity, which is the cause, or at least a major risk factor for the development of acquired TTP [35], may therefore be attributable to various mechanisms, dependent on the epitope bound by the autoimmune antibodies.

Autoantibodies inactivating ADAMTS13 activity are considered the most frequent cause of acute idiopathic TTP [14-16,18]. However, the epitope specificity of such antibodies has not been analyzed in detail. We used Western blot to analyze plasmas from patients with TTP due to acquired ADAMTS13 deficiency. The major limitations of Western blotting are that it does not address the ability of the detected antibodies to inhibit ADAMTS13 or reveal the extent to which the different antibodies contribute to the overall plasmatic inhibitor titer. The method does, however, give a good overview of the regions and domains hit by an autoimmune response and allows major immunogenic regions to be identified.

Our result that all TTP plasma samples contained detectable antibodies to the cys-rich/spacer region accords with recent data from Soejima et al. [24], showing that a major epitope of anti-ADAMTS13 inhibitors resides within the cys-rich/spacer domain of ADAMTS13. Moreover, our results suggest that antibodies against the cys-rich/spacer region are necessary and sufficient to induce acquired ADAMTS13 deficiency because we found no patient without antibodies against the cys-rich/spacer region and 3 of our patients had antibodies that reacted with this particular region only. On the other hand bearing in mind that Western blots exclusively detect antibodies directed against linear epitopes, it cannot be excluded that these 3 latter patients mounted an antibody response against 3-dimensional epitopes exhibited on other parts of ADAMTS13 or, apart from ADAMTS13, against binding partners of

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physiological significance which would not be picked up in our experiments. Hence, our results are in good agreement with the ADAMTS13 truncation experiments indicating that the cys-rich/spacer domains are necessary for VWF-cleaving activity [24,25].

The importance of the cys-rich/spacer region for ADAMTS13 activity in vivo is also underscored by the relatively large number of ADAMTS13 missense mutations that map to this region associated with congenital TTP [10,36-39]. A RGD motif potentially providing an integrin binding site is found within the cys-rich/spacer domains. Although mutation of the RGD sequence to RGE does not alter ADAMTS13 activity in vitro [24], anti-cys-rich/spacer antibodies might block specific RGD-motif-mediated ADAMTS13 interactions and therefore contribute to TTP development.

The majority (64%) of patients' plasmas reacted also with the two CUB domains. CUB domains, uniquely found in ADAMTS13 among the known ADAMTS family members, but present in other proteins, some of which are involved in developmental processes (e.g. embryogenesis, organogenesis, innervation), are thought to mediate protein-protein interactions [40]. Competitive binding studies using short peptides derived from different domains of ADAMTS13 suggested that C-terminal regions downstream from the sixth TSP1 repeat mediate the binding of ADAMTS13 to VWF [26,27]. In contrast, Banno et al. [41] showed recently that several in-bred strains and out-bred strains of mice contain an insertion in the ADAMTS13 gene resulting in a C-terminal truncation of ADAMTS13 after TSP1 repeat 7 suggesting that the CUB domains, at least in the mouse, are dispensable for ADAMTS13 function in vivo. Anti-CUB domain antibodies might therefore interfere with the substrate recognition process, or alternatively, might lead to an ADAMTS13 depletion by the formation of antibody antigen complexes.

Other epitopes frequently recognized in this study reside in the isolated central tsp1-1 domain (56%) and in the fragment that includes the entire catalytic domain, the disintegrin-like domain and central tsp1-1 domain. Both recombinant proteins were detected by 56% (14 of

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25) plasmas. Of the 14 plasmas reactive with cat/dis/tsp1-1, 10 crossreacted with the isolated tsp1-1 fragment, indicating that they might have contained antibodies reactive with the full length fragment or, alternatively, with the tsp1-1 portion only. Conversely, 4 of the plasmas containing antibodies binding to the isolated tsp1-1 fragment had no binding to the compound fragment cat/dis/tsp1-1. All fragments were used at the same concentration of approximately 200 ng per lane. This corresponds, on a molar basis, to a concentration of the isolated tsp1-1 fragment that is 3-fold higher than that of tsp1-1 in the compound fragment cat/dis/tsp1-1. If anti-tsp1-1-antibodies were present in low concentrations they might be more easily picked up by the more concentrated isolated fragment.

The tsp1-1 fragment is the major antigenic region on ADAMTS13, apart from the cys-rich/spacer and the CUB domains. TSP type 1 motifs have been identified in many different protein families and function as attachment sites for different cell types, proteins and extracellular matrix components [42,43]. The central TSP1 repeat contains a complete WXXW motif and a CSXS/TCG sequence, possibly facilitating interactions with the CD36 cell receptor expressed on a variety of cell types including vascular endothelial cells [44,45]. Soluble purified TSP1 has been shown to completely block ULVWF cleavage on the surface of endothelial cells, but anti-CD36 antibodies did not, suggesting that ADAMTS13 might use different cell receptors for endothelial attachment [46,47]. Antibodies directed against the tsp1-1 domain might interfere with the interaction of ADAMTS13 and binding partners important for its in vivo activity.

Only 28% of the TTP plasma samples recognized the fragment spanning TSP1 repeats 2 to 8, suggesting that this region is less immunogenic than, the cys-rich/spacer domains. Alternatively, antibodies against tsp1/2-8 might be directed mostly against conformational epitopes or be present in such low concentrations that we were unable to detect them. Interestingly, 20% of the tested plasma samples reacted with the propeptide fragment, suggesting that either the unprocessed ADAMTS13 containing the propeptide or soluble

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propeptide is sometimes present in plasma. Recently, the ADAMTS13 precursor, generated by mutation of the furin cleavage site or by expression in furin-deficient cells, was shown to be functionally active [48].

In conclusion, this epitope mapping study provides detailed information about the specificity of ADAMTS13-inhibiting antibodies. We propose that the cysteine-rich and spacer domain along with the CUB domains and the first TSP1 repeat constitute major epitopes for inhibitory ADAMTS13 autoantibodies in patients with TTP.

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Epitope mapping of ADAMTS13 autoantibodies in acquired thrombotic thrombocytopenic purpura

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