Novel cryptic ADAMTS13 epitopes uncover a distinct open ADAMTS13 conformation in immune-mediated TTP

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Abstract

Open ADAMTS13 conformation is gaining clinical interest as a biomarker for diagnosing immune-mediated thrombotic thrombocytopenic purpura (iTTP) and monitoring patients in remission for increased risk of relapse. Nevertheless, little is known about how open the structure of ADAMTS13 is exactly in iTTP patients. In this study, we aimed to assess the uniformity of open ADAMTS13 across iTTP patients. To do this, we identified four monoclonal antibodies that recognize epitopes that are cryptic in closed ADAMTS13 from healthy donors but accessible upon antibody-mediated ADAMTS13 opening. Distributed across the D, T7, T8 and CUB1 domains, these cryptic epitopes indicate ADAMTS13 closure through multiple interdomain contacts extending beyond the well-described S-CUB interaction. Interestingly, all acute iTTP patients consistently present one distinct open ADAMTS13 in which all novel cryptic epitopes are accessible. During remission, closed ADAMTS13 with all epitopes being cryptic is predominantly found in patients with restored activity, whereas distinct open ADAMTS13 is present in patients with subclinical disease. Furthermore, IgG from iTTP patients opened the conformation of ADAMTS13, corroborating the role of pathogenic autoantibodies in opening ADAMTS13 in iTTP. These new cryptic epitope-recognizing monoclonal antibodies hold promise to further enhance our understanding of compactly closed conformation of ADAMTS13 and may support the prediction of early relapses in the future.

Introduction

Plasma ADAMTS13 circulates as a multi-domain enzyme consisting of an N-terminal active site-bearing metalloprotease (M) domain, a disintegrin-like (D) domain, a first thrombospondin type-1 repeat (T1), a cysteine-rich (C) domain and a spacer (S) domain. The C-terminal tail of ADAMTS13 consists of seven more thrombospondin type-1 repeats (T2-T8) and two CUB (complement C1r/C1s, Uegf, BMP-1; CUB1-2) domains.[1,2] Over the last decades, crystal structures were resolved for the DTCS, MDTCS and CUB1-2 domains of ADAMTS13, and the interdomain interactions between the S and CUB1-2 domains have been extensively investigated.[3-5] Nevertheless, the structure of full-length ADAMTS13 remains elusive. Recent AlphaFold predictions suggested that full-length ADAMTS13 folds compactly through additional interdomain contacts besides the well-described S-CUB interaction.[6,7] However, as AlphaFold is a weak predictor of fold-switching proteins,[8] structural differences between closed and open ADAMTS13 remain poorly understood.

In immune-mediated thrombotic thrombocytopenic purpura (iTTP), pathogenic immunoglobulin G (IgG) autoantibodies induce an open ADAMTS13 conformation.[9,10] As a biomarker for acute and subclinical iTTP, open ADAMTS13 is gaining clinical interest as a way of assisting the diagnosis of patients with borderline ADAMTS13 activity as well as predicting relapse risks during remission.[9-13] Upon disruption of the S-CUB interaction, open ADAMTS13 exposes an S domain epitope, which normally remains cryptic in closed ADAMTS13.[9] Similarly, we showed that binding of our anti-CUB1 monoclonal antibody (mAb) 17G2 opens closed ADAMTS13 from healthy donor plasma, leading to exposure of the cryptic S domain epitope recognized by our mAb

1C4.[9,14,15] Besides conformationally opening ADAMTS13, 17G2 binding allosterically activates the M domain, resulting in the exposure of a cryptic epitope recognized by our anti-M mAb 6A6.[14-16]

With growing interest in open ADAMTS13 as a diagnostic marker, a thorough understanding of its conformation is essential. Therefore, the aim of this study was to characterize the uniformity of open ADAMTS13 conformation across iTTP patients. Through screening of novel cryptic epitopes, our main finding is that one distinct open ADAMTS13 conformation with exposure of multiple cryptic epitopes distributed across several domains characterizes acute iTTP. In addition, IgG isolated from acute iTTP plasma samples induce this distinct open ADAMTS13 conformation.

Methods

Patients' samples

Citrated plasma samples from 53 patients with acute iTTP, 30 patients with iTTP in remission, and 25 healthy donors were available for ADAMTS13 analysis. By pooling plasma from more than 20 healthy donors, a normal human plasma pool was generated. All plasma samples were obtained according to the Declaration of Helsinki, and their use was approved by local ethic committees (N°#2007/23, Marseille, France or S62889 and S66725, UZ/KU Leuven, Belgium).

Monoclonal and total IgG antibodies

To identify novel cryptic epitope-recognizing mAb, our antibody screen included a total of 60 different mAb of which some were newly produced as described before, whereas most had already been described.[9,14,16-18] In various enzyme-linked immunosorbent assay (ELISA) setups, the mAb 3H9, 6A6, 1D5, 1C4, 9C12, 19H4 and 10D2 were used as coating antibodies, whereas mAb 17G2 conformationally opens ADAMTS13 enabling detection of captured ADAMTS13 using biotinylated mAb 15D1, 19H4 or 17G2.[9,14,16-18] These murine anti-human ADAMTS13 mAb were produced and purified as previously described.[9,14,16-18] Total IgG (auto)antibody fractions were purified from acute iTTP patients' plasma (samples 2, 5, 9, 12 and 20 of the MF-KB-TTPxx-A cohort) and plasma from healthy donors as previously described.[10,19]

Screening of monoclonal antibodies that recognize conformation-sensitive epitopes

Based on our previously described Open/Closed ELISA, 60 anti-ADAMTS13 mAb were screened for their potential to recognize cryptic epitopes that become exposed upon opening of closed plasma ADAMTS13.[9,14,16-18] Epitopes were considered cryptic when mAb could not capture closed plasma ADAMTS13 but could capture plasma ADAMTS13 upon opening using mAb 17G2. In brief, candidate mAb were coated (5 μg/mL in 0.05 M carbonate/bicarbonate buffer at pH 9.6), after which 1/4-diluted samples from the normal human plasma pool were added to capture plasma ADAMTS13 that was preincubated in the absence (closed ADAMTS13) or presence (open ADAMTS13) of 17G2 (2.5 μg/mL in 0.3% milk powder in phosphate-buffered saline [PBS]). Captured, open ADAMTS13 was detected using the biotinylated anti-S mAb 15D1 (1.5 μg/mL in 0.3% milk powder in PBS) and horseradish peroxidase (HRP)-labeled high-sensitivity streptavidin (1/10,000 in 0.3% milk powder in PBS; Pierce™, Waltham, USA). As a positive control, mAb 1C4 was used, which is known to recognize a cryptic S domain epitope in ADAMTS13. In this case, the biotinylated anti-M mAb 3H9 was used for detection as previously described.[9,10]

ADAMTS13 antigen enzyme-linked immunosorbent assay

To quantify ADAMTS13 antigen levels in plasma from patients or healthy donors, an in-house ELISA was used.[20] The anti-M mAb 3H9 (5 μg/mL in 0.05 M carbonate/bicarbonate buffer at pH 9.6) was coated onto a 96-well plate to capture plasma ADAMTS13. Starting at a 1/12.5 or 1/100 dilution for patients or healthy donors, respectively, plasma samples were serially diluted in a 1.5/2.5 ratio. Captured plasma ADAMTS13 was detected using the biotinylated anti-T8 19H4 and anti-CUB1 17G2 mAb (each at 1.5 μg/mL in 0.3% milk powder in PBS) and HRP-labeled streptavidin (1/10,000 in 0.3% milk powder in PBS; Roche, Basel, Switzerland). To calculate ADAMTS13 antigen present in samples from patients or healthy donors, antigen from the normal human plasma pool served as a 1 μg/mL reference.[20]

Evaluation of ADAMTS13 conformation

Cryptic epitope exposure was assessed in samples from patients or healthy donors based on our Open/Closed ELISA.[9,10] In brief, cryptic epitope-recognizing mAb were individually coated (5 μg/mL in carbonate/bicarbonate buffer at pH 9.6), after which a plasma dilution series was added. Plasma samples were first preincubated in either the absence or presence of the opening mAb 17G2 (2.5 μg/mL in 0.3% milk powder in PBS). Plasma from the normal human plasma pool preincubated with the opening mAb 17G2 was used as an intra-assay reference. To verify the trigger for ADAMTS13 opening, a 1/8 dilution of the normal human plasma pool (containing closed plasma ADAMTS13) was preincubated with the total IgG fraction purified from patients with acute iTTP (samples 2, 5, 9, 12 and 20 of the MF-KB-TTPxx-A cohort) or healthy donors.[10,19] Detection of captured, open ADAMTS13 occurred as described above. When mAb 1C4 was coated, the biotinylated anti-M mAb 3H9 was used, whereas the biotinylated anti-S mAb 15D1 was used for detection when all other cryptic epitope-recognizing mAb were coated. Cryptic epitope exposure was determined from ADAMTS13's conformation index (CI), which is calculated by correcting optical density values for plasma antigen and normalizing for the intra-assay normal

human plasma pool control.[21] As previously described, a CI above 0.50 indicates accessible cryptic epitopes (open ADAMTS13), whereas a CI equal or below 0.50 represents inaccessible cryptic epitopes (closed ADAMTS13).[9,10]

Results

Anti-ADAMTS13 antibodies reveal novel cryptic epitopes in plasma ADAMTS13

To extend our understanding of open ADAMTS13, we first aimed to identify specific epitopes within different ADAMTS13 domains that only become accessible upon transition from its closed to its open conformation. For this purpose, we screened 60 mAb from our anti-ADAMTS13 mAb library for their capacity to only capture mAb 17G2-induced open ADAMTS13 but not closed ADAMTS13. Alongside the mAb 1C4 (anti-S)[9] and 6A6 (anti-M),[16] we identified four mAb that specifically recognized cryptic epitopes exposed in open ADAMTS13. These were mAb 1D5, 9C12, 19H4 and 10D2 targeting, respectively, the D, T7, T8 and CUB1 domains of ADAMTS13.[14,16,17,22] Indeed, all six antibodies showed residual to no binding to closed ADAMTS13 (Figure 1, filled bars). Only upon 17G2-induced opening, did all six antibodies show distinct binding to open ADAMTS13 (Figure 1, open bars), suggesting a significant role for the M, D, S, T7, T8 and CUB1 domains in maintaining ADAMTS13 closed. Importantly, about 90% of our 60 anti-ADAMTS13 mAb were found to distinctly capture closed plasma ADAMTS13, suggesting that most antibody epitopes are only partially cryptic or non-cryptic (*Online Supplementary Figures S1-S5*).

Closed ADAMTS13 in healthy donors

After identifying this new set of cryptic epitope-recognizing mAb, we screened a cohort of 25 healthy donors to verify the CI cut-off that distinguishes between open ADAMTS13 with accessible epitopes or closed ADAMTS13 with cryptic epitopes. Previously, we identified a CI cut-off of 0.50 in our Open/Closed ELISA to evaluate the accessibility of the cryptic epitope recognized by the anti-S mAb 1C4.[9,10] Values above this threshold indicated open ADAMTS13 with accessible 1C4 epitope, while those equal or below the CI cut-off indicated closed ADAMTS13 with a cryptic 1C4 epitope. Similarly, we found that this same CI cut-off of 0.50 can be used to determine whether the cryptic epitopes recognized by mAb 6A6, 1D5, 9C12, 19H4 and 10D2 are accessible (>0.50) or cryptic (≤0.50). Indeed, the CI values for closed ADAMTS13 (absence of 17G2) were below 0.50 in 22 out of 25 plasma samples from healthy donors (range CI_{6A6}=0.06-0.35, range CI_{1D5}=0.05-0.46, range CI_{9C12}=0.04-0.38, range CI_{19H4}=0.07-0.35 and range CI_{10D2}=0.02-0.15), whereas those for open ADAMTS13 (presence of 17G2) were all above 0.50 (range CI_{6A6}=0.83–2.03, range CI_{1D5}=0.66–1.54, range CI_{9C12}=0.58–1.33, range CI_{19H4}=0.67–1.79 and range CI_{10D2}=0.58–1.39) (Figure 2). Of note, three healthy donors had a CI_{6A6}, and CI_{9C12} above 0.50 indicating that both M and T7 epitopes were accessible in these three healthy donors while the 1D5, 1C4, 19H4 and 10D2 epitopes within the D, S, T8 and CUB1 domains remained cryptic.

Distinct open ADAMTS13 in acute phase immune-mediated thrombotic thrombocytopenic purpura

Previously, we showed that patients with acute phase iTTP

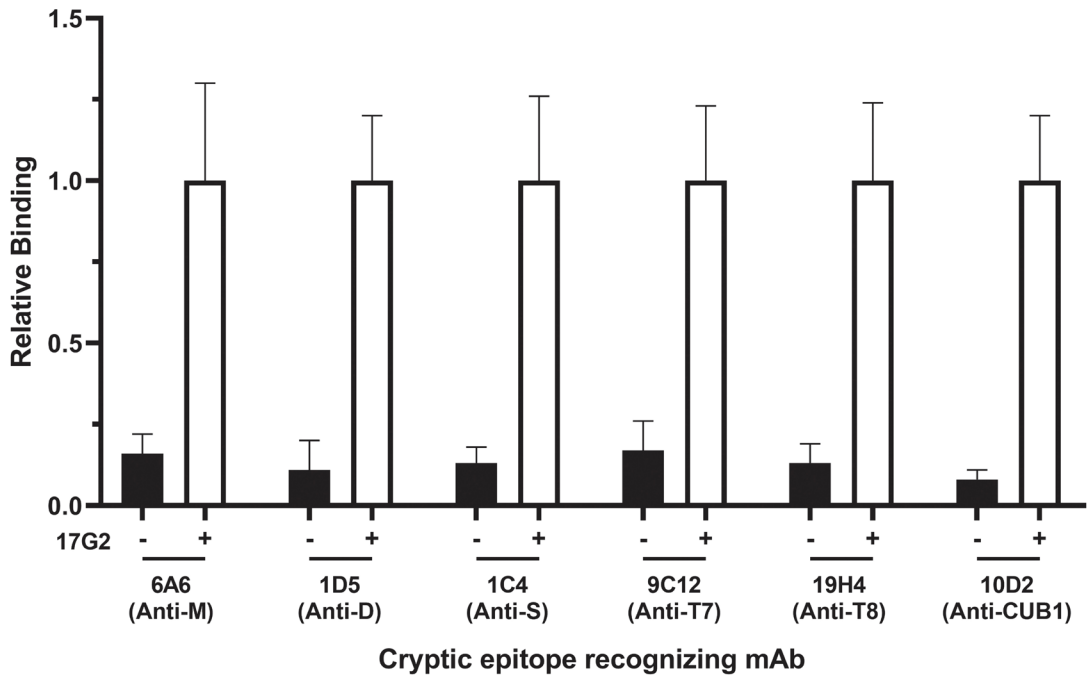

Figure 1. Open ADAMTS13 reveals cryptic epitopes across various domains. Closed plasma ADAMTS13 was incubated without (black bars) or with (white bars) the anti-CUB1 monoclonal antibody (mAb) 17G2, which conformationally opens ADAMTS13 before loading on a 96-well plate coated with the previously described mAb 1C4 and 6A6,[9,14,16] and the new mAb 1D5, 9C12, 19H4 and 10D2, directed against epitopes in the S, M, D, T7, T8 and CUB1 domains, respectively. All coated mAb could capture open ADAMTS13 (white bars) but not closed ADAMTS13 (black bars), indicating that these mAb recognize cryptic epitopes that become exposed upon mAb 17G2-induced conformational opening of ADAMTS13. For each mAb, data are expressed as relative binding to open ADAMTS13 (mean ± standard deviation, N=3 independent experiments).

presented open ADAMTS13 with an accessible 1C4 S domain epitope. Using our novel cryptic epitope-recognizing mAb, we then wanted to evaluate whether acute phase iTTP patients present one distinct or multiple variable open ADAMTS13 conformations. We, therefore, screened the accessibility of each of the novel cryptic epitopes in plasma from 53 acute phase iTTP patients. For 14 patients, CI values could not be calculated as ADAMTS13 antigen was undetectable (i.e. ≤0.03 μg/mL).[10,20] Interestingly, for all other acute phase iTTP patients, we found that all cryptic epitopes in the M, D, S, T7, T8 and CUB1 domains were uniformly exposed, regardless of the presence of 17G2 (Figure 3). Indeed, all acute iTTP patients presented CI values above 0.50 (range CI_{6A6}=0.91-18.27, range CI_{1D5}=0.58-9.47, range CI_{9C12}=0.63-25.50, range CI_{19H4}=2.50-14.59, range CI_{10D2}=0.51-9.42). As expected, the conformation remained open in all iTTP samples when preincubated in the presence of 17G2 (range CI_{6A6}=1.33-16.74, range CI_{1D5}=0.73-9.52, range CI_{9C12}=0.58-20.58, range CI_{19H4}=3.07-18.65, range CI_{10D2}=0.62-9.39). Of note, one patient with acute phase iTTP had a cryptic CUB1 epitope in the presence of 17G2 (CI_{10D2}=0.48). Overall, these results indicate that regardless of mAb 17G2 addition, acute phase iTTP patients consistently present one distinct open ADAMTS13 conformation in which all newly identified mAb epitopes are exposed.

Distinct open ADAMTS13 in subclinical immune-mediated thrombotic thrombocytopenic purpura

Despite treatment response and clinical recovery, remission phase iTTP patients remain at risk of clinical and/or ADAMTS13 relapses.[11,23] Reappearance of anti-ADAMTS13 autoantibodies and a drop in ADAMTS13 activity represents ongoing subclinical iTTP, which is linked to open ADAMTS13 with accessible 1C4 S domain epitope.[10] Here, we evaluated the accessibility of the novel cryptic epitopes in remission phase iTTP patients with ADAMTS13 activity above (N=15) or below (N=15) 50% of its normal activity (Figure 4). All samples from patients in remission showed detectable ADAMTS13 antigen enabling CI calculation for each cryptic epitope-recognizing antibody. In remission patients with ADAMTS13 activity restored to greater than 50%, about half of the plasma samples (53%, 8/15) indicated a closed ADAMTS13 with cryptic D, S, T7, T8 epitopes. Interestingly, the M domain epitope recognized by mAb 6A6 was found to remain cryptic less frequently (27%, 4/15), whereas the CUB1 domain epitope recognized by mAb 10D2 remained cryptic more frequently (80%, 12/15) in these patients with ADAMTS13 activity restored to above 50%. Upon addition of the mAb 17G2, closed ADAMTS13 could be opened as reflected by the exposure of all six domain epitopes. In remission patients with ADAMTS13 activity below 50%, almost all patients (93%, 14/15) presented the same distinct open ADAMTS13 conformation with all M, D, S, T7, T8 and CUB1 domain epitopes being accessible, regardless of mAb 17G2 addition. Of note, in one patient presenting closed ADAMTS13 with inaccessible D, S, T7, T8 and CUB1 domain epitopes, its M domain epitope was accessible for 6A6

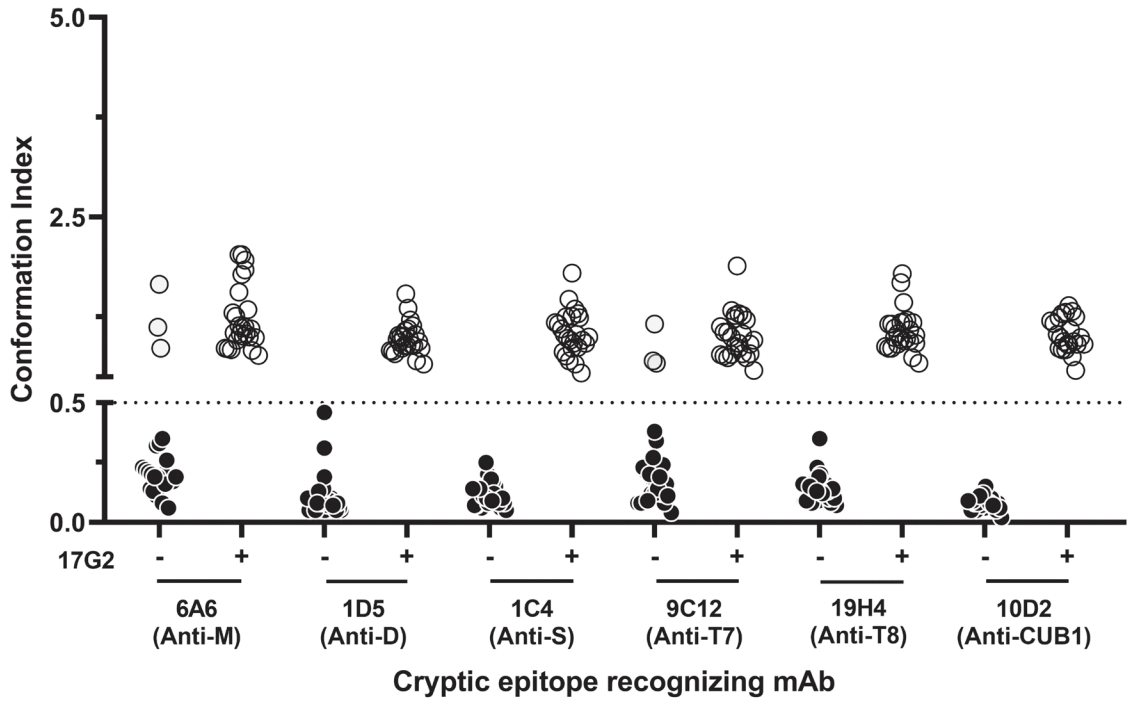

Figure 2. ADAMTS13 is closed in healthy donors. Microtiter plates were coated with monoclonal antibodies (mAb) to screen cryptic ADAMTS13 epitope exposure in plasma from healthy donors in the absence or presence of the anti-CUB1 mAb 17G2. Nearly all plasma samples from healthy donors (22/25) showed natively closed ADAMTS13 in which all cryptic epitopes were inaccessible (filled circles) but could become exposed upon incubation with mAb 17G2 (open circles). Three healthy donors revealed accessible 6A6 and 9C12-recognized epitopes in the M and T7 domains, respectively (open circles), whereas their 1C4, 1D5, 19H4 and 10D2-recognized epitopes in the S, D, T8 and CUB1 domains remained cryptic (filled circles). For each healthy donor, data are expressed as a conformation index by correcting enzyme-linked immunosorbent assay absorbance values for plasma antigen levels and normalizing for the intra-assay normal human plasma pool control.[21] The dotted line represents the cut-off that differentiates accessible (>0.50) from inaccessible (≤0.50) ADAMTS13 epitopes, representing open and closed ADAMTS13, respectively.

recognition. On the other hand, in another patient presenting open ADAMTS13 with accessible M, D, S, T7 and T8 domain epitopes, its CUB1 domain epitope remained cryptic and inaccessible for 10D2 recognition.

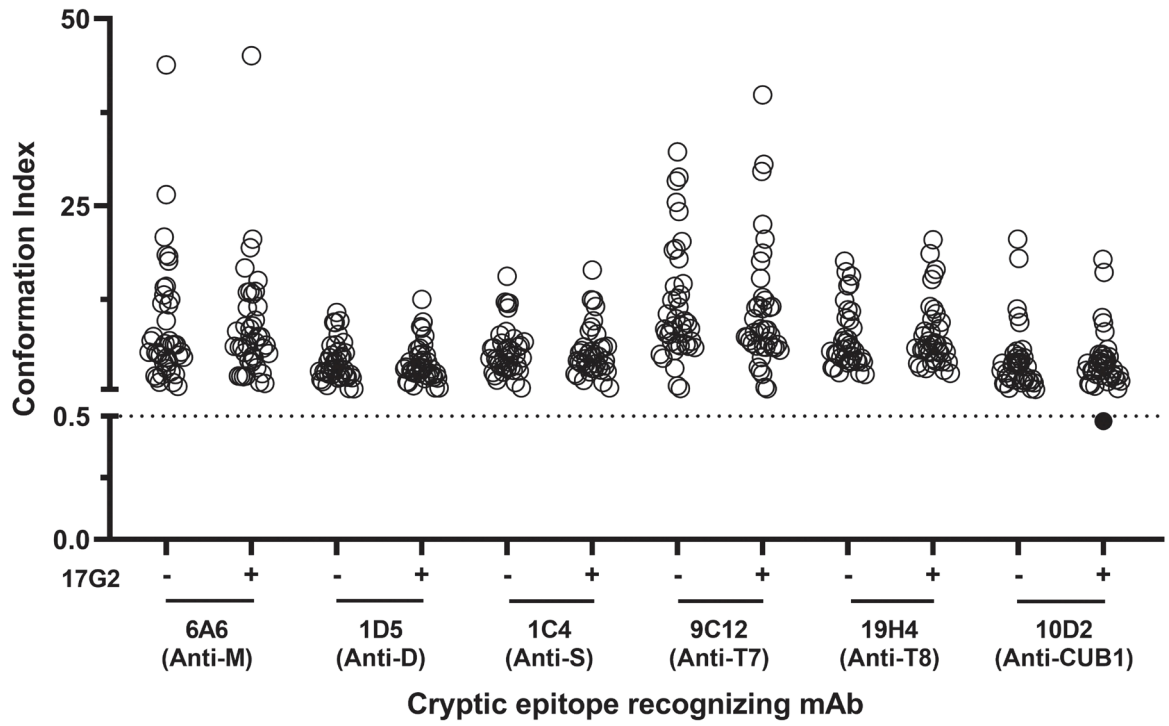

Figure 3. One distinct open ADAMTS13 conformation in acute immune-mediated thrombotic thrombocytopenic purpura. Microtiter plates were coated with monoclonal antibodies (mAb) to screen cryptic ADAMTS13 epitope exposure in plasma from patients with acute immune-mediated thrombotic thrombocytopenic purpura (iTTP) in the absence or presence of the anti-CUB1 mAb 17G2. Plasma samples from 39 acute iTTP patients were eligible for screening of their exposure of novel cryptic ADAMTS13 epitopes in an enzyme-linked immunosorbent assay. Regardless of mAb 17G2 addition, all patients presented one distinct open ADAMTS13 conformation (open circles) with exposure of each cryptic epitope. The dotted line represents the cut-off that differentiates accessible (>0.50) from inaccessible (≤0.50) ADAMTS13 epitopes.

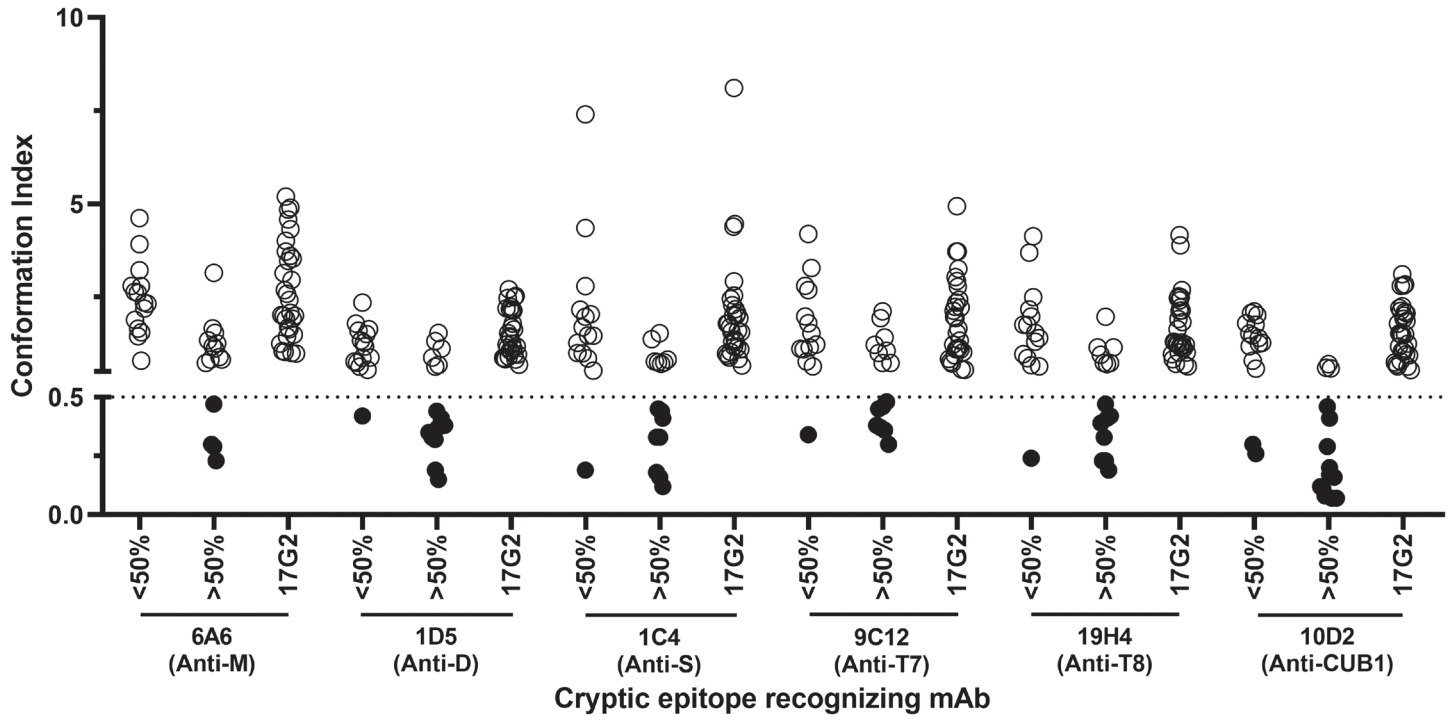

Figure 4. One distinct open ADAMTS13 conformation in subclinical immune-mediated thrombotic thrombocytopenic purpura. Microtiter plates were coated with monoclonal antibodies (mAb) to screen for cryptic ADAMTS13 epitope exposure in plasma from patients with immune-mediated thrombotic thrombocytopenic purpura (iTTP) in remission in the absence or presence of the anti-CUB1 mAb 17G2. Plasma samples from 30 remission iTTP patients were available for cryptic epitope screening in enzyme-linked immunosorbent assays. In patients with ADAMTS13 activity >50%, over half of the patients (8/15) had closed ADAMTS13 with cryptic D, S, T7, T8 epitopes (closed circles). The M domain epitope recognized by mAb 6A6 remained less frequently cryptic (4/15), whereas the CUB1 domain epitope recognized by mAb 10D2 remained more frequently cryptic (12/15) in these patients. In patients with ADAMTS13 activity <50%, almost all patients (13/15) presented one distinct open ADAMTS13 conformation with all M, D, S, T7, T8 and CUB1 domain epitopes being accessible (open circles). Addition of mAb 17G2 opened ADAMTS13 in all patients (30/30) reflected by exposure of all six domain epitopes. The dotted line represents the cut-off that differentiates accessible (>0.50) from inaccessible (≤0.50) ADAMTS13 epitopes.

Pathogenic IgG trigger distinct open ADAMTS13

As pathogenic IgG autoantibodies from iTTP patients were previously found to expose the S domain epitope for 1C4 recognition,[10,19] we investigated whether such patients' autoantibodies also trigger the exposure of the M, D, T7, T8 and CUB1 domain epitopes. To do this, we incubated closed plasma ADAMTS13 with either iTTP patient-purified total IgG or healthy donor-purified total IgG (Figure 5). Incubation of a buffer condition (i.e., 'No IgG'), verified that plasma presented closed ADAMTS13 with all six epitopes being cryptic. The conformation of ADAMTS13 also remained closed, with all six epitopes being cryptic, following incubation of the total IgG fraction purified from two different healthy donors. Only upon incubation of the total IgG fraction purified from five different iTTP patients did ADAMTS13 adopt its distinct open conformation in which all M (range CI_{6A6}=2.63-3.75), D (range CI_{1D5}=0.95-1.65), S (range CI_{1C4}=0.89-1.85), T7 (range CI_{9C12}=1.68-3.75), T8 (range CI_{19H4}=1.20-2.06) and CUB1 (range CI_{10D2}=0.74-1.80) domain epitopes became accessible for mAb recognition. As anti-ADAMTS13 autoantibodies trigger the exposure of cryptic mAb epitopes in multiple ADAMTS13 domains, we studied whether the presence of anti-ADAMTS13 autoantibodies in the three healthy donors could explain the presence of variably open ADAMTS13 (with accessible M and T7 epitopes, but inaccessible D, S, T8 and CUB1 epitopes) in these individuals. When evaluating all three healthy donor plasmas using an in-house autoantibody ELISA,[24] no positivity for anti-ADAMTS13 IgG was found (*data not shown*), suggesting a different trigger to cause this M and T7 domain epitope exposure.

Discussion

In this study, we performed an antibody screen to identify mAb that selectively recognize previously unidentified cryptic epitopes across different domains in healthy donor plasma ADAMTS13. Besides the previously described mAb 6A6 (anti-M) and 1C4 (anti-S),[9,16] we here report that four mAb (1D5, 9C12, 19H4 and 10D2) specifically bind epitopes within the D, T7, T8 and CUB1 domains of open ADAMTS13, but do not bind to closed ADAMTS13.[14,16,17,22] We previously demonstrated that ADAMTS13 adopts an open conformation in acute iTTP, characterized by the exposure of a cryptic epitope within the spacer domain, recognized by monoclonal antibody 1C4.[9] In the present study, we showed that this open conformation also revealed cryptic epitopes within the D, T7, T8, and CUB1 domains. These findings indicate the presence of a distinct and consistent open ADAMTS13 conformation across all patients with acute iTTP. In addition, when ADAMTS13 activity recovers above 50% during remission, the enzyme predominantly adopts a closed conformation in most patients, rendering all epitopes cryptic. Interestingly, the same distinct open conformation of ADAMTS13 observed during the acute phase was also present in a subset of remission patients,

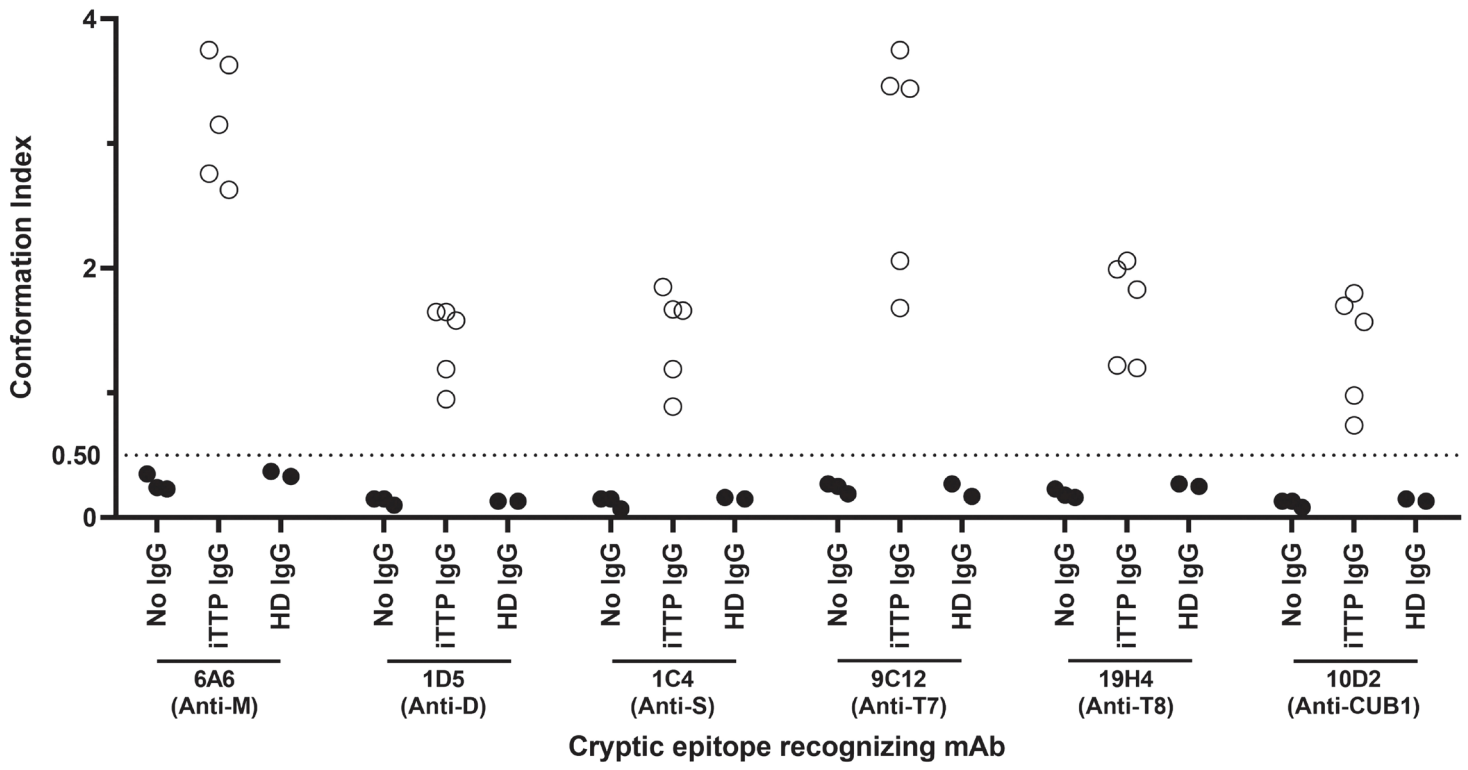

Figure 5. Pathogenic IgG trigger distinct open ADAMTS13. Microtiter plates were coated with monoclonal antibodies (mAb) to screen for cryptic ADAMTS13 epitope exposure in plasma from healthy donors in the absence or presence of the total immunoglobulin G (IgG) fraction purified from patients with acute immune-mediated thrombotic thrombocytopenic purpura (iTTP) or healthy donors (HD). In the absence of IgG, plasma ADAMTS13 was verified to be closed (closed circles). Following incubation of the total IgG fraction purified from HD, closed ADAMTS13 was indicated by inaccessible cryptic epitopes. Incubation with iTTP-purified IgG induced distinct open ADAMTS13 in which all epitopes became accessible (open circles). The dotted line represents the cut-off that differentiates accessible (>0.50) from inaccessible (≤0.50) ADAMTS13 epitopes.

in whom subclinical disease maintained ADAMTS13 activity below 50%. Finally, pathogenic IgG autoantibodies, isolated from acute iTTP plasma, were capable of shifting the closed ADAMTS13 conformation from healthy donors to a distinct open ADAMTS13 with all six epitopes being exposed. Thereby, we provide novel insight into the extent to which ADAMTS13 opens its conformation, and thus enhance our understanding of the role of ADAMTS13's conformation in the pathophysiology of iTTP. The mAb identified herein provide an interesting tool to further characterize iTTP disease progression. With open ADAMTS13 being a predictive marker for the risk of earlier relapse, our mAb might provide future value for the prediction and prevention of iTTP relapses.[12,13]

In the absence of pathological IgG, nearly all healthy donors adopted closed ADAMTS13 with cryptic M, D, S, T7, T8 and CUB1 domain epitopes. Hence, each of these domains appear to conformationally vary between closed and open ADAMTS13. Although it remains unclear whether these novel cryptic epitopes are exposed through inter- or intradomain changes, our findings support the hypothesis that closed ADAMTS13 could be compactly folded through multiple interdomain contacts extending beyond the well-described S-CUB interaction.[6,7] With cryptic epitopes in both proximal MDTCS and distal T2C2 domains, our findings for closed ADAMTS13 align with the condensed form of ADAMTS13 observed via electron microscopy, and with the more compact envelope found via small angle X-ray scattering (SAXS) and AlphaFold for full-length ADAMTS13 compared to truncated variants.[16,17,25] Intricately, the cryptic epitopes identified within the T7 and T8 domains corroborate their involvement in the minimal structure for allosterically regulated ADAMTS13 as proposed by SAXS, phylogenetic and functional analysis.[26,27] Curiously, no cryptic epitopes were found within the T3 to T6 domains that are dispensable in the allosteric regulation of ADAMTS13.[26,27] As we also found cryptic epitopes within the M and D domains, it is tempting to speculate that these might be shielded by distal domain interactions as predicted by AlphaFold simulations and thus might be involved in the allosteric regulation of ADAMTS13 by occluding the active or substrate-binding sites. Future resolving of the exact residues that shape each of these cryptic epitopes might provide valuable insight into how these domains likely engage in interdomain contacts to close ADAMTS13. Surprisingly, three out of 25 healthy donors exhibited closed ADAMTS13 with cryptic D, S, T8 and CUB1 epitopes, yet with accessible M and T7 epitopes. As all plasma samples tested negative for anti-ADAMTS13 IgG, the molecular basis for this variably closed ADAMTS13 remains unknown. Crystallization of the M domain has previously required structural stabilization by the mAb 3H9,[4] and hydrogen-deuterium exchange mass spectrometry has shown that mAb binding induces flexibility in the M domain.[14,28] Thus, the accessibility of the 6A6 epitope might be linked to the inherent molecular flexibility of the M domain. Nevertheless, it remains unclear whether the accessibility of both M and T7 epitopes occurred by chance or resulted from the disruption of an interdomain contact. Hence, this finding raises the possibility that the conformation of closed ADAMTS13 may be more variable than previously thought. As differences in closed ADAMTS13 are poorly explored, its incidence in the broader population, and significance for health or disease in circulation remains currently unknown.

In conclusion, our study provides new insights into open and closed ADAMTS13 in two ways. First, we demonstrated that open ADAMTS13 exposes multiple cryptic epitopes across various domains, extending beyond those of the S-CUB interaction. We here reveal that once opened, ADAMTS13 adopts one distinct open conformation that is consistently observed in patients with acute and subclinical iTTP. Secondly, we found that closed ADAMTS13 likely adopts a compact, folded structure stabilized by more interdomain interactions than previously recognized.

Disclosures

BSJ has participated in advisory boards for Sanofi, Takeda, Alexion and Werfen. PC is a member of clinical advisory boards for Alexion, Sanofi-Genzyme, Takeda and Janssen Pharmaceutica and has received fees from Alexion, Sanofi-Genzyme, Takeda, SOBI and Janssen Pharmaceutica. AV is a member of the French advisory board for caplacizumab (Sanofi-Genzyme) and for recombinant human ADAMTS13 (Takeda). KV has participated in advisory boards for Takeda and Werfen. The other authors have no conflicts of interest to disclose.

Contributions

QB conceptualized the study, designed and performed experiments, analyzed and discussed data, prepared figures and wrote the manuscript. FB, LDW, LV and IP performed experiments and assisted in data analysis. KK designed and produced proteins. ET and GK provided samples. CT, SFDM, BSJ, AV and PC discussed data and edited the manuscript. KV conceptualized the study, analyzed and discussed data and wrote the manuscript. All authors discussed results, supplied critical feedback and approved the final version of the manuscript.

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Data-sharing statement

All data are available upon reasonable request to the corresponding author.

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