Antibodies against the CUB1-2 domains of ADAMTS13 in a patient with benign monoclonal gammopathy: no causal relationship

We present a patient with a history of benign monoclonal gammopathy, who developed thrombotic thrombocytopenic purpura (TTP), initially presenting as bilateral serous retinal detachment. Plasma of the patient contained high titers of anti-ADAMTS13 antibodies that were directed towards the disintegrin/TSR1/cysteine-rich/spacer and CUB1-2 domains. ADAMTS13 activity was undetectable. Total IgG purified from plasma of the patient partially inhibited ADAMTS13 activity. In contrast, the isolated M-protein did neither bind to, nor inhibit activity of ADAMTS13. We conclude that in this patient the monoclonal gammopathy and TTP co-existed as distinct pathological entities.

Design and methods
ADAMTS13 activity was determined using a collagen binding assay (CBA) of degraded VWF essentially as described previously. Levels of ADAMTS13 activity in normal individuals range from 28-119% whereas ADAMTS13 activity levels are < 5% in patients with acquired or congenital TTP. Levels of anti-ADAMTS13 antibodies in plasma were measured using Technozym inhibitor ELISA (Technoclone, Vienna, Austria) according to the instructions provided by the manufacturer. Values of > 15 U/mL were considered to reflect the presence of anti-ADAMTS13 antibodies in accordance with the instructions provided by the manufacturer. The ADAMTS13 antibody assay has produced very high values in occasional individuals without TTP or severe ADAMTS13 deficiency. To confirm the presence of antibodies to ADAMTS13, we assessed the binding of patient-derived antibodies to a panel of recombinant ADAMTS13 fragments, essentially as described previously.

Figure 1. Plasma concentrations of thrombocytes and immunoglobulins, and ADAMTS13 activity. The course of the number of circulating thrombocytes (open circles), plasma immunoglobulin concentration (filled squares), and activity of ADAMTS13 (open triangles) in time (days after first plasma exchange).
Our patient presented with subacute loss of vision due to bilateral serous retinal detachment. This is a rare ocular complication of TTP, which can develop, mostly late, in the course of the disease. Retinal detachment is thought to occur due to localized ischaemic injury to the retinal pigment epithelium, caused by microthrombi in the small choroidal vessels, which inhibits adequate transport of fluid from the subretinal space. Our patient regained complete vision by treatment with plasma exchange therapy.

We found that ADAMTS13 activity was undetectable in plasma. Anti-ADAMTS13 antibody levels were high (1632 U/mL). Because our patient had a history of a benign monoclonal gammopathy, we hypothesized that the M-protein might be directed against ADAMTS13. It is known that M-proteins can possess antibody activity, but M-proteins directed against ADAMTS13 have never been described, although the possibility has been postulated in 1991, when the pathophysiology of TTP was not yet elucidated. We isolated the M-protein to determine whether this immunoglobulin was involved in the pathogenesis of the TTP.

In most patients with acquired TTP, circulating antibodies are present that neutralize ADAMTS13 activity. Recently, it was demonstrated that in all presently characterized patients with anti-ADAMTS13 antibodies, these antibodies are directed against the cysteine-rich/spacer domains. To confirm the presence of anti-ADAMTS13 antibodies we determined the binding of patient derived IgG with a series of recombinant ADAMTS13 fragments. These experiments revealed predominant binding of antibodies to the carboxy-terminal CUB1-2 domains. In addition, less prominent, but significant binding to a fragment containing the disintegrin/TSR1/cysteine-rich/spacer domains was observed (Figure 3). Antibodies directed towards the CUB domains have previously been observed by Klaus et al. The CUB domains are dispensable for VWF cleavage activity in vitro (2,14), although in vivo the CUB-1 domain may serve as the docking site for ADAMTS13 to bind unusually large VWF under flow. Alternatively, antibodies directed towards the CUB domains of ADAMTS13 contribute to the pathogenesis of acquired TTP.

In summary, we report a patient with MGUS, with bilateral serous retinal detachment as the first presenting sign of TTP, and we studied the possible relationship between these conditions. The study emphasizes that ocular symptoms may be the first sign of TTP and that early diagnosis and treatment can induce complete recovery of vision. In addition, we demonstrated that MGUS and TTP can co-exist in one patient as distinct pathophysiological entities.

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**Figure 2.** Binding of IgG to ADAMTS13. Binding of the isolated M-protein (open squares), total IgG purified from plasma of the patient (open triangles), and IgG purified from NHP (open circles), to immobilized ADAMTS13.

**Figure 3.** Epitope-mapping of anti-ADAMTS13 antibodies by immunoprecipitation. Antibodies present in 30 µL of plasma of the patient (p) were immobilized on protein G sepharose and incubated with recombinant ADAMTS13 protein fragments. Antibodies present in 30 µL of NHP were included as negative control (-), as a positive control a monoclonal antibody directed against the carboxy-terminal V5-tag on the recombinant fragments was included (+). ADAMTS13 fragments that bound specifically to the immobilized antibodies were detected on immunoblot.
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Author contribution: NPR, NC, and MvD were treating physicians of the patient, initiated further in vitro studies, and wrote the paper.  
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References