Acquired TTP: ADAMTS13 meets the immune system
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Summary

Autoantibodies directed against ADAMTS13 prohibit the processing of VWF multimers, initiating a rare and life-threatening disorder called acquired thrombotic thrombocytopenic purpura (TTP). At present it is not clear why previously healthy individuals develop anti-ADAMTS13 antibodies. The studies described in this thesis aim to increase our understanding of the molecular mechanisms involved in the generation of anti-ADAMTS13 antibodies in patients with acquired TTP.

In Chapter 1 we summarize the current knowledge on the biology and function of ADAMTS13. We focus on the risk factors for inhibitor formation in patients with acquired TTP and highlight the basic features of a humoral auto-immune response. In Chapter 2 we explored the epitope specificity and immunoglobulin class and subclass distribution of anti-ADAMTS13 antibodies in plasma of acquired TTP patients. Antibody profiling revealed that the majority of anti-ADAMTS13 antibodies were composed of IgG1 and IgG4, followed by IgM and IgA1. Subsequently, we assessed whether residues Arg658 and Phe592, present in two surface loops in close proximity to the previously described antibody epitope comprising residues Arg660, Tyr661 and Tyr665, contribute to the binding of anti-ADAMTS13 antibodies. Introduction of multiple alanine substitutions at Arg658, Phe592, Arg660, Tyr661 and Tyr665 abolished binding of polyclonal antibodies present in plasma of a large cohort of TTP patients to the spacer domain. In this study we also show the presence of anti-TSR2-8 and CUB1-2 domain antibodies in respectively 17% and 35% of the patient samples analyzed.

The next chapters focus on the mechanisms involved in development of anti-ADAMTS13 antibodies. This process requires immune recognition, endocytosis and subsequent processing of ADAMTS13 into peptides that are presented on MHC class II molecules to ADAMTS13 specific CD4+ T cells. In Chapter 3 we investigated endocytosis of recombinant ADAMTS13 by immature monocyte-derived dendritic cells (iDCs). Endocytosis of ADAMTS13 was blocked upon addition of EGTA and mannan. We subsequently explored the involvement of C-type lectin receptors (CLRs) in the uptake of ADAMTS13 using specific blocking antibodies and siRNA silencing. We found that ADAMTS13 endocytosis was significantly decreased in cells treated with a monoclonal antibody directed towards the macrophage mannose receptor (MR) or after siRNA silencing. These data show that ADAMTS13 is internalized by iDCs in a MR-dependent manner. In vitro binding studies confirmed that ADAMTS13 interacts with the carbohydrate recognition domains of MR.

In Chapter 4, we identified ADAMTS13 derived peptides presented on MHC class II alleles from a panel of both HLA-DRB1*11 positive and negative donors by mass
spectrometry. Interestingly, at low antigen concentrations a limited number of CUB2 derived peptides were presented by HLA-DRB1*11 or DRB1*03 positive donors. Pulsing of dendritic cells with higher concentrations of ADAMTS13 resulted in presentation of larger number of peptides on both HLA-DRB1*11 positive and negative donors. Although the presented peptides were derived from several ADAMTS13 domains, inspection of the peptide-profiles revealed that CUB2 domain-derived peptides were presented with a higher efficiency when compared to other peptides. Interestingly, dendritic cells from HLA-DRB1*11 donors only presented a specific CUB2 domain-derived peptide. Our data suggests that functional presentation of the CUB2 domain-derived peptide by HLA-DRB1*11 patients contributes to the onset of acquired TTP by stimulating low affinity self-reactive CD4\(^+\) T cells that have escaped negative selection in the thymus.

Our data indicate that sugar moieties on ADAMTS13 are important for binding to the macrophage mannose receptor on DCs thereby promoting its endocytosis and the subsequent presentation of ADAMTS13-derived peptides on MHC class II. Furthermore, several studies have shown that aberrant glycosylation can play an important role in the pathogenesis of autoimmune diseases. We therefore analyzed in Chapter 5 the glycosylation profile of plasma ADAMTS13. Nine N-linked sites were identified in or near the metalloproteinase, spacer, thrombospondin type 1 repeat (TSR) and the CUB domain of plasma ADAMTS13. Moreover, six O-fucosylated sites were identified in the TSR domains of plasma ADAMTS13. In addition to N- and O-linked modifications, two novel C-mannosylation sites were identified within the TSR1 and TSR4 domains of ADAMTS13.

In Chapter 6 we studied the endocytosis of ADAMTS13 by macrophages. Internalization of ADAMTS13 was reduced upon addition of mannan and EDTA suggesting a possible role of C-type lectin receptors (CLRs). We have demonstrated that the macrophage mannose receptor (MR) is involved in endocytosis of ADAMTS13 by human monocyte derived dendritic cells (Chapter 2). However, uptake of ADAMTS13 by monocyte derived macrophages was not affected by a blocking monoclonal antibody directed towards the macrophage mannose receptor. Interestingly, a robust inhibition of ADAMTS13 uptake was observed upon incubation with polyanions such as dextran sulphate and fucoidan, suggesting a role for class A scavenger receptors. Taken together our data suggest that internalization of ADAMTS13 by macrophages proceeds via a mechanism that is dissimilar from the previously defined mechanism in dendritic cells.

In Chapter 7 we discuss the major findings of this thesis and speculate on the mechanisms underlying the initiation of autoimmune reactivity towards ADAMTS13 in patients with acquired TTP. Potential implications of our findings and perspectives for future studies on the etiology of inhibitor formation in acquired TTP are discussed.