The RGS handoff in platelets. RGS proteins serve to limit platelet activation by limiting the duration of G protein–dependent signaling, illustrated here for Gq-mediated activation of phospholipase C leading to the production of IP$_3$ and diacylglycerol from phosphatidylinositol-4,5-bisphosphate (PIP$_2$), the second messengers that increase the Ca$^{2+}$ concentration in platelets and activate protein kinase C. This model suggests that the amount of free RGS18 (and by implication RGS10) in resting platelets is determined in part by binding to spinophilin and by protein kinase A (PKA)– or protein kinase G (PKG, not shown)–mediated phosphorylation of Ser216. In activated platelets, release of RGS18 from spinophilin/SHP-1 complex promotes inhibition of signaling, but subsequent phosphorylation of Ser49 and Ser218 allows sequestration of the RGS protein by a 14-3-3 unless Ser216 phosphorylation is present. Not all aspects of this model have been established, but it provides testable hypotheses for tightly regulating the availability of free RGS proteins in platelets. AC indicates adenylyl cyclase; NO, nitric oxide; PGI$_2$, prostaglandin I$_2$; SFK, Src family kinase; PKC, protein kinase C; and TxA$_2$, thromboxane A$_2$. The authors thank Dr Timothy J. Stalker (Perelman School of Medicine, University of Pennsylvania) for assistance with the figure.

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COMMENT ON SORVILLO ET AL, PAGE 3828

ADAMTS13 meets MR, then what?

X. Long Zheng  CHILDREN’S HOSPITAL OF PHILADELPHIA

In this issue of Blood, Sorvillo and colleagues demonstrate that a macrophage mannose receptor (MR) on antigen-presenting cells facilitates uptake of ADAMTS13 antigen. The findings provide the first hint on how ADAMTS13 antigen may be endocyotically processed, and presented to immune T cells.
Most cases of idiopathic thrombotic thrombocytopenic purpura (TTP) in adults are caused by acquired immunoglobulin G (IgG) autoantibodies against the metalloprotease ADAMTS13. The mechanism underlying the formation of autoantibodies is poorly understood. TTP, a potentially fatal syndrome, is characterized by profound thrombocytopenia and microangiopathic hemolytic anemia with various symptoms and signs of organ dysfunction. Nearly all adult idiopathic TTP patients harbor IgG autoantibodies that inhibit plasma ADAMTS13 activity, thus resulting in compromised proteolytic processing of ultra large von Willebrand factor (VWF) multimers anchored on endothelial cells and in circulating blood. The ultra large VWF multimers are hyperactive, interacting with platelets in flowing blood and leading to unwanted thromboses in small arteries and capillaries. Epitope mapping demonstrates that almost all patients’ IgG autoantibodies recognize the ADAMTS13 spacer domain, particularly exosite 3 and its surrounding residues. These residues have been shown to play an essential role in recognition and cleavage of VWF. Whether the spacer domain of ADAMTS13 is particularly immunogenic or resembles epitopes found in microbial pathogens is not known.

The association between HLA-DRB1*11 and acquired idiopathic TTP indicates that antigen-specific CD4+ T cells may contribute to formation of autoantibodies against ADAMTS13 in these patients. Sorvillo et al convincingly demonstrate that a fluorescein-labeled ADAMTS13 protein is readily taken up by immature monocyte-derived dendritic cells (iDCs) in culture. This process can be blocked by EGTA and mannan, a polymer of mannose. The results suggest an involvement of a calcium- and mannan-sensitive C-type lectin receptor in the endocytosis of ADAMTS13. Further experiments with a monoclonal antibody against an MR or small interfering RNA silencing the MR gene confirm that the MR mediates the endocytosis of ADAMTS13 by iDCs. Other C-type lectin receptors on iDCs, such as the dendritic cell specific ICAM3 grabbing nonintegrin receptor (DC-SIGN), do not appear to be involved in ADAMTS13 endocytosis under these conditions. Binding experiments show that ADAMTS13 interacts with 4 to 7 C-type lectin-like carbohydrate recognition domains (CTLDs) of the MR (see figure). A removal of N-linked glycans from ADAMTS13 protein dramatically reduces ADAMTS13 endocytosis, but the removal of O-linked oligosaccharides has no effect. Together, these results suggest that the MR plays an important role in facilitating endocytosis of N-glycosylated ADAMTS13 by dendritic cells.

What is the fate of endocytosed ADAMTS13 in dendritic cells? Sorvillo and colleagues show that a significant amount of the endocytosed ADAMTS13 is detected in the early endosome as indicated by early endosomal antigen-1 (EEA-1). This observation is consistent with the localization of endocytosed ovalbumin through the MR pathway. The endocytosed ovalbumin is excluded from late endosome or lysosome marked by Rab7 or lysosomal-associated membrane protein-1 (LAMP-1). It has been well documented in the literature that the endocytosed antigens through the MR...
pathway into early endosomes associate with the MHC class I molecules and are presented to CD8+ T cells for cross-presentation, a pathway to activate cytolytic CD8+ T cells (see figure). Whether the MR pathway plays a major role in the antigen presentation and processing by the MHC class II molecules that activate CD4+ T cells remains controversial. The MR involvement in the antigen presentation through the MHC class II molecules is supported by the delivery of lipoglycan antigens to the late endosome and lysosome for presentation to the CD4+ T cells by CD1b molecules and by the generation of an isotype-switching antibody in response to immunization with anti-MR monoclonal antibody in vivo. Furthermore, the MR expression on the inflammatory macrophages is increased in response to inflammatory cytokines, such as IL-4, IL-13, and IL-10. In cytokine-treated cells, the MR is detected in the late endosome, suggesting that antigen-derived peptides can be loaded on the MHC class II molecules for presentation. The MHC class I and/or MHC class II presentation may depend on the activation status and maturation stage of the dendritic cells. These findings may help us understand how bacterial or viral infections may trigger the formation of autoantibody against ADAMTS13, thereby resulting in an acute burst of TTP episodes.

In conclusion, the discovery of the role of mannose receptor in facilitating ADAMTS13 endocytosis by the antigen-presenting cells may promote further research on immune biology of ADAMTS13. The results of these investigations may shed new light on the pathogenesis of acquired autoimmune TTP.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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Thrombosis & Hemostasis

Comment on Jian et al, page 3836

Improving on nature: redesigning ADAMTS13

Johanna A. Kremer Hovinga and Jan Voorberg

In this issue of Blood, Jian and coworkers report on a gain-of-function variant of ADAMTS13 that is resistant to the autoantibodies responsible for acquired thrombotic thrombocytopenic purpura (TTP). Auto-antibodies directed toward ADAMTS13 prohibit cleavage of von Willebrand factor (VWF) resulting in systemic platelet aggregation in the microcirculation. Our knowledge on the etiology of the misregulated immune response at the onset of acquired idiopathic TTP is still very limited. A genetic predisposition is suggested by the observation of severe autoantibody-mediated ADAMTS13 deficiency leading to acute TTP episodes in identical twin sisters and the over-representation of the HLA-DRB1*11 allele in TTP patients. A number of different bacterial and viral infections preceding a first acute episode or relapse have been reported (reviewed by Pos et al), although specific triggers have not been identified yet, molecular mimicry as documented in other autoimmune disorders cannot be excluded. Moreover, a recent study suggests that ADAMTS13 is efficiently internalized by antigen-presenting cells, thereby potentially contributing to initiation of CD4+ T-cell responses to ADAMTS13 in previously healthy individuals.

Over the past years we have learned that a major binding site for antibodies resides in the spacer domain (reviewed in Pos et al). Detailed mutagenesis studies pointed at an exposed surface area in the spacer domain composed of Arg660, Tyr661, and Tyr665 as being a crucial part of the epitope. Examination of a large panel of plasma from patients with acquired TTP revealed that Arg568 and Phe592 also contribute to the binding of anti-ADAMTS13 antibodies. Progressive replacement of residues Arg568, Phe592, Arg660, Tyr661, and Tyr665 for Ala reduced antibody binding to the spacer domain (see figure). Building on these results, Jian and colleagues made conservative changes within these 5 residues. Unexpectedly, the resulting “M5-variant” exhibited a 10- to 12-fold increase in activity. In excellent agreement with earlier results from Pos and coworkers, the M5-variant was resistant to inhibition of a panel of autoantibodies from acquired idiopathic TTP patients. The gain-of-function and autoantibody-resistant ADAMTS13 variant provides perspective of a novel therapeutic avenue for treatment of patients with acquired TTP.

The mainstay of treatment of acquired idiopathic TTP is daily plasma exchange with replacement of plasma until normalization of platelet count and lactate dehydrogenase levels as well as stabilization of clinical symptoms is achieved. Suppression of anti-ADAMTS13 autoantibody formation is attempted by the addition of corticosteroids and more and more by the administration of the monoclonal anti-CD20 antibody rituximab. During the past...
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