

definitive role for early rituximab administration in nonsplenectomized ITP patients. RCTs are particularly important in ITP because spontaneous remissions are known to occur⁶ and because formal evaluations of treatment efficacy that include clinically important end points—such as bleeding and quality-of-life—are needed.⁷

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REFERENCES

1. Arnold DM, Dentali F, Crowther MA, et al. Systematic review: efficacy and safety of rituximab for adults with idiopathic thrombocytopenic purpura. *Ann Intern Med.* 2007;146:25-33.

2. Fleming TR. One-sample multiple testing procedure for phase II clinical trials. *Biometrics.* 1982;38:143-151.
 3. Cooper N, Stasi R, Cunningham-Rundles S, et al. The efficacy and safety of B-cell depletion with anti-CD20 monoclonal antibody in adults with chronic immune thrombocytopenic purpura. *Br J Haematol.* 2004;125:232-239.
 4. Stasi R, Del Poeta G, Stipa E, et al. Response to B-cell depleting therapy with rituximab reverts the abnormalities of T-cell subsets in patients with idiopathic thrombocytopenic purpura. *Blood.* 2007;110:2924-2930.
 5. Cooper N, Evangelista ML, Amadori S, Stasi R. Should rituximab be used before or after splenectomy in patients with immune thrombocytopenic purpura? *Curr Opin Hematol.* 2007;14:642-646.
 6. Simanek R, Panzer S, Lechner K, Pabinger I. Late spontaneous remissions in severe adult autoimmune thrombocytopenia. *Ann Hematol.* 2007;86:705-710.
 7. Rodeghiero F. First-line therapies for immune thrombocytopenic purpura: re-evaluating the need to treat. *Eur J Haematol.* 2008;80(suppl. 69):19-26.

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Comment on Gblin et al, page 957

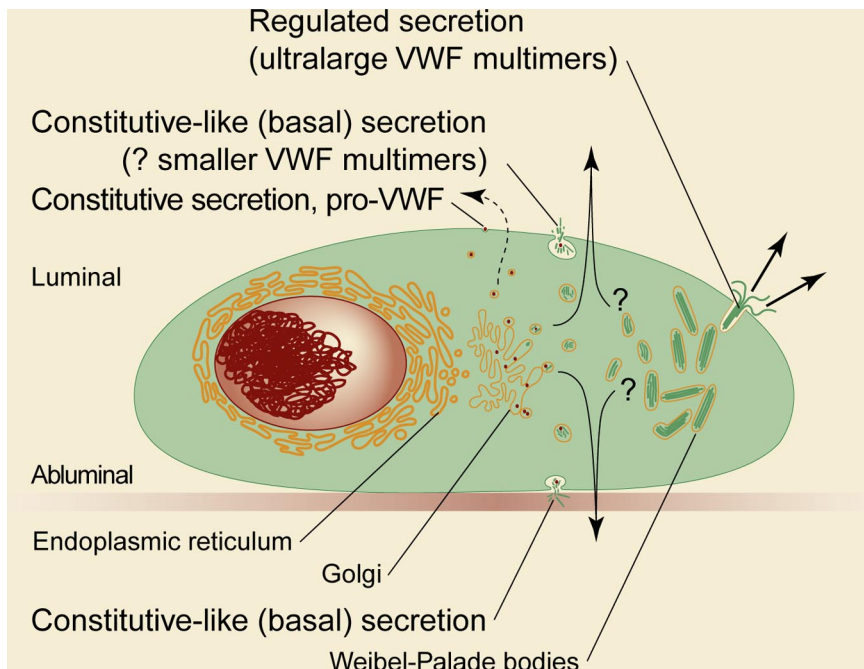
VWF secretion: what's in a name?

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In this issue of *Blood*, Gblin and colleagues report that the continuous spontaneous release of VWF from endothelial cells is largely due to secretion of a stored form of the mature protein and not to its continuous transit out of the cell by classic constitutive secretion.

von Willebrand Factor (VWF) is secreted by 2 pathways, one continuous and requiring no cellular stimulation, and the other regulated

and responsive to secretagogues. The distinction between regulated and constitutive protein secretion pathways at first blush appears



Depicted are the various pathways and modes of VWF secretion from endothelial cells. See text. Professional illustration by Paulette Dennis.

obvious. Regulated secretion requires a stimulus to provoke protein release from storage granules, whereas constitutive secretion is continuous, requires no stimulus, and is thought to occur by uninterrupted synthesis and exocytosis of proteins after their processing in the Golgi. However, a third pathway, which the authors call basal secretion (also called constitutive-like secretion), involves elements of both, with proteins targeted to storage granules after Golgi processing (as in regulated secretion) but continuously secreted from this storage pool without provocation (similar to constitutive secretion).

The continuous unstimulated release of VWF has been termed “constitutive” secretion. Its mechanism is unknown, but it differs from regulated secretion in human umbilical vein endothelial cells (HUVECs) in that it produces smaller VWF multimers on average, is sensitive to inhibitors of protein synthesis, does not require microtubules, and releases VWF to both the luminal and abluminal cell surfaces (regulated secretion is only toward the luminal surface).¹

Gblin and colleagues bring a fresh perspective to VWF secretion by integrating older studies of VWF trafficking with new evidence in HUVECs to conclude that continuously secreted VWF derives primarily from post-Golgi secretory organelles and not from uninterrupted protein passage from the Golgi to the plasma membrane (see figure). They show that most VWF is held in the cell before release, and that spontaneously secreted VWF originates from a storage pool. Unstimulated HUVECs also continuously secrete a small amount of pro-VWF (VWF containing the propeptide), which has the hallmarks of being secreted by the conventional constitutive pathway. One of the defining characteristics of constitutive VWF release is that it can be inhibited by blocking protein synthesis.² In the article by Gblin and coauthors, an experiment with carefully timed blockade of protein synthesis showed that VWF that would otherwise be spontaneously secreted is retained in the cell, consistent with inhibition of release from secretory granules.

This work raises many interesting questions. Previous observations have suggested that VWF multimer size depends on the route of secretion, with larger, more hemostatically active multimers secreted after stimulation.¹ If Weibel-Palade bodies are the main secretory organelles for both continuous release and regulated secretion, then they may be heterogeneous based on the multimeric composition of their cargo. Some Weibel-Palade bodies have been shown to move continuously but stochastically in unstimulated HUVECs³; could these be the source of continuous VWF secretion? Understanding these pathways has clinical implications. If endothelial cells

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in vivo secrete VWF by the constitutive-like route, this could account for a significant portion of circulating plasma VWF. Disruption of VWF secretion has been implicated in Type 2A von Willebrand disease, while targeted disruption of one (or both) pathways could be useful in some prothrombotic states, such as thrombotic thrombocytopenic purpura. Whether continuous VWF secretion is called constitutive, constitutive-like, or basal, the insightful work of Gblin and colleagues raises questions that warrant further investigation.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

REFERENCES

1. Wagner DD. Cell biology of von Willebrand factor. *Annu Rev Cell Biol.* 1990;6:217-246.
2. Loesberg C, Gonsalves MD, Zandbergen J, et al. The effect of calcium on the secretion of factor VIII-related antigen by cultured human endothelial cells. *Biochim Biophys Acta.* 1983;763:160-168.
3. Romani de Wit T, Rondaij MG, Hordijk PL, Voorberg J, van Mourik JA. Real-time imaging of the dynamics and secretory behavior of Weibel-Palade bodies. *Arterioscler Thromb Vasc Biol.* 2003;23:755-761.
4. Tsai HM, Nagel RL, Hatcher VB, Seaton AC, Sussman IL. The high molecular weight form of endothelial cell von Willebrand factor is released by the regulated pathway. *Br J Haematol.* 1991;79:239-245.

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Comment on Olsson et al, page 1078; Stasi et al, page 1147; and Yu et al, page 1325

ITP three R's: regulation, routing, rituximab

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Tregs create a controlling milieu that promotes immune suppression and autoimmune tolerance. Their versatility and adaptability makes them the “multitalented masters of immune regulation.”¹ In this issue of *Blood*, 3 studies have shown this concept to be true in patients with chronic ITP. Collectively, the studies show that functional deficiencies of Tregs are responsible for the immune etiology of ITP and that targeting them may alleviate the disease.

Our immune system has elegantly evolved to arm us with many mechanisms that destroy invading microorganisms or stop the spread of tumors. Our system also has extensive built-in mechanisms for preventing attack on healthy self tissues.¹ This “self-tolerance” mechanism involves the elimination of self-reactive T and B lymphocytes during selection in the thymus and bone marrow, respectively. However, since these central mechanisms are not absolute, we have evolved peripheral mechanisms to deal with immune cells that “escape” central tolerance. Over the past 50 years, immunologists have postulated the existence of suppressor T cells that police the peripheral immune system to stop unwanted self immune responses. But this postulated entity was cast into doubt because of hard-to-reproduce model systems, each with complexities and idiosyncrasies.¹ With an explosion of biochemical and molecular advances, however, the field of immune suppression has been resurrected Phoenixlike, and the notion of T regulatory cells (Tregs), marked by specific cell surface molecules (eg, CD4⁺ CD25⁺ Foxp3⁺), arose.¹ Tregs appear to be a

relatively rare CD4⁺ T-cell subset that comprise many subpopulations, including IL-10-producing “Tr1” cells, TGF- β -producing T helper type 3 cells, CD8⁺ T suppressor cells, natural killer T cells, CD4-CD8-T cells and $\gamma\delta$ T cells.²⁻⁵ Some of these cells originate in the thymus during ontogeny and are referred to as “natural” Tregs. Some Tregs can also be induced from naive T cells in the periphery.⁶ These Tregs appear to be the natural immune “magic bullets” that keep all of our normal and abnormal immune responses in check. They are critical to our survival, and absence of them can lead to either autoimmunity or inflammatory disorders, with fatal consequences in both mice and humans.¹⁻⁵ Although defects of these cell types have previously been described in immune thrombocytopenic purpura (ITP),⁷⁻⁹ 3 papers in this issue collectively suggest that a deficient Treg compartment allows enhanced T-cell and B-cell autoreactivity in ITP, and that therapies like rituximab actually correct the deficiency and reverse autoimmune platelet reactivity, thus leading to increased platelet counts.

The first study in this series was based on the authors' previous observations that rituximab (Rituxan) is an efficacious therapy for patients with ITP and that this is due to normalizing abnormal autoreactive T-cell responses in ITP.^{10,11} Stasi and colleagues have extended these intriguing results to show that rituximab primarily reverses the Treg deficiency in patients with ITP. The authors studied 26 adult patients with chronic ITP (a different cohort from their last paper) who were treated with rituximab; they examined Tregs by flow cytometry and assessed their regulatory function by cell proliferation assays. Compared with control individuals, pretreatment patients with ITP had a significantly reduced number and defective suppressive capacity of Tregs. In addition, the Tregs in the patients with ITP showed a polyclonal spectratype. In contrast, upon treatment with rituximab, patients, particularly responders, showed restored numbers of Tregs as well as restored regulatory functions. The authors suggested that patients with active ITP have a defective Treg compartment, which can be significantly modulated by a B-cell targeted therapy.

In the second paper, Yu and colleagues studied 17 patients with chronic ITP and tested the frequency and functional capabilities of CD4⁺ CD25⁺ Foxp3⁺ Tregs in peripheral blood. Although they found a similar frequency of Tregs in controls and patients, the ability to functionally suppress in vitro T proliferation was significantly reduced in ITP patients. These data further support the notion that functional defects in Tregs probably contribute to a breakdown in self-tolerance in chronic ITP.

In the third paper, Olsson and colleagues studied the bone marrow and peripheral blood of 26 patients with chronic ITP and found, particularly in the bone marrow, increased numbers of infiltrating activated CD3⁺ T cells with elevated surface expression of VLA-4 and CX3CR1. Compared with controls, the increased T-cell number in the bone marrow was also associated with significantly lower numbers of bone marrow Tregs. They suggest that chronic ITP is a disease of increased activated T cell due to a Treg defect within the bone marrow and that this may contribute to suppressed megakaryocyte production in ITP.¹²

The 3 studies collectively shed light on how Tregs may initiate and/or mediate the autoimmunity of ITP. It appears that a central deficiency of Tregs breaks tolerance and allows unchecked activation of autoreactive Th1