domains contributes to the binding and proteolytic processing of VWF under physiologic conditions.

Previous studies have shown that the metalloprotease domain of ADAMTS13 alone is ineffective in cleaving VWF,[6,7] but if the various noncatalytic domains are incrementally added back, proteolytic activity is gradually restored.[6,7] These results suggest a linear relationship between the domains of ADAMTS13 and VWF proteolysis. Gao et al[8] have identified several potential sites on the VWF-A2 domain that may make direct contacts with various proximal noncatalytic domains of ADAMTS13 under static conditions. This result is in agreement with that reported previously by Ai et al,[6] in which ADAMTS13 variants truncated after the spacer domain with an additional internal deletion of either disintegrin domain or disintegrin plus TSP1-1 repeat have markedly reduced proteolytic activity toward VWF fragment and exhibit no proteolytic activity toward full-length VWF. Collectively, these data support a hypothesis that all the proximal noncatalytic domains of ADAMTS13 are required for productive engagement with VWF-A2 domain at least under static/denaturing conditions.

In this issue of *Blood*, de Groot et al[9] focus on the involvement of the disintegrin domain of ADAMTS13 in VWF processing in more detail. They use molecular modeling (panel B in the figure) and site-directed mutagenesis to identify the amino acid residues within this domain that are essential for successful cleavage of VWF. They show that 3 out of 8 ADAMTS13 disintegrin mutants they have produced exhibit dramatically reduced activity toward VWF fragment, namely VWF115 (amino acid residues 1554-1668 of VWF), and full-length VWF polymers under static/denaturing conditions.[9] Kinetic analyses show a 5- to 20-fold reduction in the catalytic efficiency in cleavage of VWF115 by these mutants.[9] Further experiments have identified that the positively charged Arg349 on ADAMTS13 appears to directly interact with the negatively charged Asp1614 on the VWF-A2 domain (figure panel B).[9] The authors hypothesize that this seemingly weak interaction between the disintegrin and VWF-A2 appears to be essential for efficient catalysis of VWF under static/denaturing conditions. This task may be achieved in collaboration with other proximal noncatalytic domains. Indeed, the first TSP1 repeat, the Cys-rich domain, and the spacer domains bind VWF-A2 fragment with higher affinity than the disintegrin domain.[6] These results

suggest that binding of all the proximal noncatalytic domains of ADAMTS13 to VWF is necessary to position the active site of ADAMTS13 to the scissile bond (Tyr^{1605}-Met^{1606}) on VWF, resulting in productive cleavage.

It remains to be seen how this domain functions in concert with the other domains of ADAMTS13 in the presence of shear stress that alters VWF conformation in a more physiologic way. Could it be that the other domains of ADAMTS13 are more important than the disintegrin domain in binding VWF in order to align it with the scissile bond for cleavage in vivo? For instance, the recent report by Zhang et al [10] suggests a role of the middle and distal parts of the noncatalytic region in participating in binding and proteolytic processing of VWF under fluid shear stress. Therefore, further investigation of the precise interactions between each of ADAMTS13 domains and VWF may shed light on understanding the pathogenesis of thrombotic thrombocytopenic purpura, a potentially fatal illness caused primarily by the absence of plasma ADAMTS13 proteolytic activity, as a result of ADAMTS13 mutations or acquired autoantibodies against ADAMTS13 enzyme.

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

REFERENCES

1. Levy GG, Nichols WC, Lian EC, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. Nature. 2001;413:488-494.

2. Zheng XL, Chung D, Takayama TK, et al. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. J Biol Chem. 2001;276:41059-41063.

3. Soejima K, Nakamura H, Hirashima M, et al. Analysis on the molecular species and concentration of circulating ADAMTS13 in blood. J Biochem (Tokyo). 2006;139:147-154.

4. Zheng XL, Sadler JE. Pathogenesis of thrombotic microangiopathies. Annu Rev Path Mech Dis. 2008;3:249-277.

5. Chauhan AK, Goerge T, Schneider SW, Wagner DD. Formation of platelet strings and microthrombi in the presence of ADAMTS-13 inhibitor does not require P-selectin or beta3 integrin. J Thromb Haemost. 2007;5:583-589.

6. Ai J, Smith P, Wang S, et al. The proximal carboxyl-terminal domains of ADAMTS13 determine substrate specificity and are all required for cleavage of von Willebrand factor. J Biol Chem. 2005;280:29428-29434.

7. Gao W, Anderson PJ, Majerus EM, et al. Exosite interactions contribute to tension-induced cleavage of von Willebrand factor by the antithrombotic ADAMTS13 metalloprotease. Proc Natl Acad Sci U S A. 2006;103:19099-19104.

8. Gao W, Anderson PJ, Sadler JE. Extensive contacts between ADAMTS13 exosites and von Willebrand factor domain A2 contribute to substrate specificity. Blood. 2008;112:1713-1719.

9. de Groot R, Bardhan A, Ramroop N, Lane DA, Crawley JTB. Essential role of the disintegrin-like domain in ADAMTS13 function. Blood. 2009;113:5609-5616.

10. Zhang P, Pan W, Rux AH, et al. The cooperative activity between the carboxyl-terminal TSP-1 repeats and the CUB domains of ADAMTS13 is crucial for recognition of von Willebrand factor under flow. Blood. 2007;110:1887-1894.

● ● ● **TRANSPLANTATION** ▬▬▬▬▬▬

Comment on Opiela et al, page 5635

RTEs: lazy T-cell teenagers


Pamela J. Fink UNIVERSITY OF WASHINGTON

In this issue of *Blood*, Opiela and colleagues analyze the phenotype and function of the lymphoid periphery's youngest T cells, RTEs.

Recent thymic emigrants (RTEs) are T cells that have just exited from the thymus, having completed an approximately 2-week journey that takes them from stem cell to committed T cell. Only 1% to 5% of thymocytes survive this complex maturation process that begins with T-cell receptor gene rearrangement and ends with a select population of lineage committed T cells that are both self-major histocompatibility complex (MHC)–restricted and self-tolerant.[1] Throughout the lifetime of the individual, RTEs are essential for the maintenance of a diverse population of naive peripheral T cells, ready to further differentiate into appropriate effector T cells upon encounter with foreign antigen.

It has long been of interest to identify and analyze RTEs as a population distinct from the bulk of peripheral T cells, in order to quantify thymic output and to assess whether T-cell maturation continues after thymic egress. Understanding RTE biology is of particular importance for predicting recovery of the immune system following lymphoablative therapy or viral infection, and for the study of immunity in neonates (in which the bulk of the lymphoid periphery consists of RTEs) and in aged individuals (in which RTEs represent a small minority of peripheral T cells). Previous methods for tagging RTEs have included intrathymic injection of fluorochromes, transplantation of congenically marked thymus
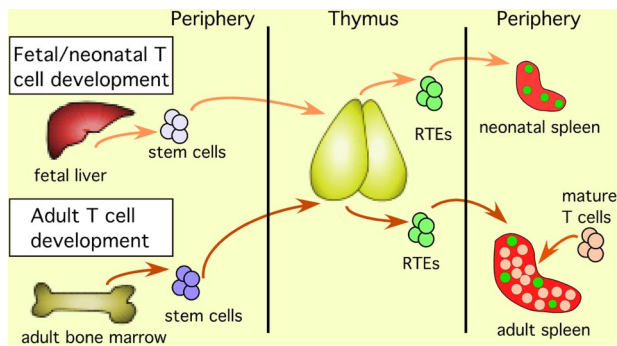
T-cell development in the fetus and the neonate begins with immigration of fetal liver–derived stem cells into the thymus. After intrathymic T-cell maturation is complete, RTEs exit the thymus and enter the lymphoid periphery. RTEs are marked by green fluorescence in mice carrying a transgene-encoding green fluorescent protein driven by the RAG2 promoter. RTEs in the neonate enter a lymphopenic periphery and constitute the majority of peripheral T cells. In the adult, stem cells arise from the bone marrow, and after completing intrathymic maturation, the resulting RTEs exit the thymus and enter a lymphoreplete periphery, where they are surrounded by a majority of mature T cells.

grafts, and analysis of T cell–receptor rearrangement excision circles. Each of these methods is problematic: they either result in cell death, thereby precluding functional analyses, or involve surgical manipulation that can alter the very parameters being measured.

Recent advances have highlighted new means of identifying RTEs in both unmanipulated mice and humans, allowing the isolation of live RTEs for functional and phenotypic analyses. In humans, CD4^{+} RTEs are specifically marked by expression of PTK7, a protein tyrosine kinase of unknown function in T cells.[2] In the mouse, RTEs can be identified as fluorescent peripheral T cells in mice expressing a transgene encoding green fluorescent protein under the control of the RAG2 promoter.[3] We have learned from these studies that thymic output (as a function of the size of the generative compartment) is relatively constant throughout life, that many more CD4^{+} RTEs emigrate than can ultimately be incorporated into the peripheral T-cell pool, and that in both mice[3-5] and humans,[2] RTEs are phenotypically immature and functionally defective, compared with their mature counterparts.

Opiela et al have now contributed to this body of work by comparing the function and phenotype of RTEs in the neonatal and adult mouse.[6] Such a comparison is clearly warranted, given the known differences in neonatal and adult T-cell biology. In the neonate, RTEs differentiate from fetal liver stem cells to enter a lymphopenic peripheral environment in which they constitute the majority of peripheral T cells while in healthy adults, RTEs comprise a minority of T cells in a lym-

phoreplete periphery. According to this latest study, stimulated neonatal RTEs secrete higher levels of effector cytokines (IL–2, IL–4 and IFNγ) than do adult RTEs.[1] Furthermore, RTEs from neonates, but not from adults, proliferate in response to the key homeostatic cytokine IL-7 in the absence of T cell–receptor stimulation. CD4^{+} RTEs in humans also show increased IL-7–driven proliferation, although no comparative analysis of neonatal and adult RTEs was presented in this study.[2] The heightened response of murine neonatal RTEs to IL-7 is accompanied by differences in the kinetics of IL-7Rα down-regulation and downstream transcription factor activation. Analysis of radiation chimeras has indicated that the key differences between neonatal and adult RTEs do not result solely from their distinct stem cell source. Instead, the availability of IL-7 and the density and identity of the cellular neighbors of RTEs may contribute to the distinct properties of adult and neonatal RTEs.

Now that the "hows" are beginning to be understood, attention is likely to turn to the "whys" of continued postthymic maturation of T cells and the functional and phenotypic differences between neonatal and adult RTEs. Does the 2- to 3-week transition period of postthymic T-cell maturation provide a means for further selecting the T-cell population for "best fit"? Do

the functional defects allow the individual to purge self-reactive T-cells by permitting new emigrants to scan the periphery for tissue-specific antigens without the danger of eliciting autoimmunity? Neonates must uniquely cope with lymphopenia and the absence of mature peripheral T cells. Given that T cells undergoing homeostatic proliferation adopt a memory cell phenotype and heightened function,[7] the IL-7– driven proliferation of neonatal RTEs may both help fill up empty space and provide a population of memorylike T cells. Clearly, much remains to be learned about how the youngest peripheral T cells cope with their adolescence and successfully transition into adulthood.

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REFERENCES

1. von Boehmer H. Selection of the T cell repertoire: receptor-controlled checkpoints in T cell development. Adv Immunol. 2004;84:201-238.

2. Haines CJ, Giffon TD, Lu L-S, et al. Human CD4+ T cell recent thymic emigrants are identified by protein tyrosine kinase 7 and have reduced immune function. J Exp Med. 2009;206:275-285.

3. Boursalian TE, Golub J, Soper DN, et al. Continued maturation of thymic emigrants in the periphery. Nat Immunol. 2004;5:418-425.

4. Hale JS, Boursalian TE, Turk GL, et al. Thymic output in aged mice. Proc Natl Acad Sci U S A. 2006;103:8446-8452.

5. Makaroff LE, Hendricks DW, Niec RE, et al. Postthymic maturation influences the CD8 T cell response to antigen. Proc Natl Acad Sci U S A. 2009;106:4799-4804.

6. Opiela SJ, Koru-Sengul T, Adkins B. Murine neonatal recent thymic emigrants are phenotypically and functionally distinct from adult recent thymic emigrants. Blood. 2009;113:5635-5643.

7. Tanchot C, Le Campion A, Leaument S, et al. Naïve CD4+ lymphocytes convert to anergic or memory-like cells in T cell deprived recipients. Eur J Immunol. 2001;31:2256-2265.

● ● ● **VASCULAR BIOLOGY**

Dll1 and Dll4: similar, but not the same

Manfred Gessler WUERZBURG UNIVERSITY

The VEGF-Dll4-Notch1 signaling cascade has taken center stage in angiogenesis, but it now appears that Dll1 ligands have precedence in arteries and even seem to control VEGF signaling.

Arteries and veins are structurally and functionally different types of vessels, and they display independent molecular signatures. A genetic program imposes arterial or

venous fate onto embryonic precursors prior to formation of patent vessels and onset of blood circulation. This has been shown most elegantly in zebrafish and seems to hold true