Comment on Nurden et al, page 2587

Impaired megakaryocytopoiesis in type 2B von Willebrand disease

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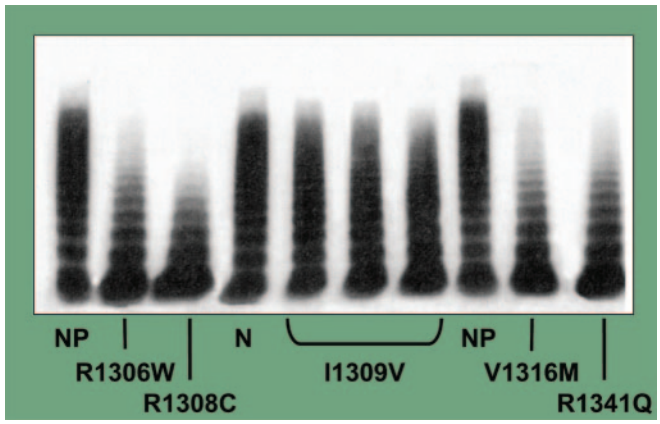
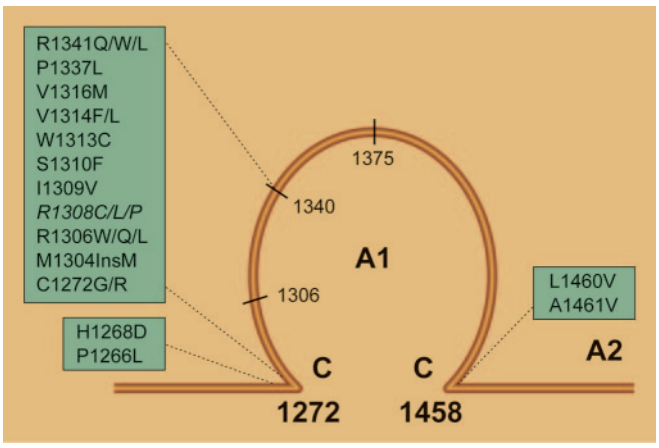
In this issue, Nurden and colleagues provide evidence that the severe thrombocytopenia found in a family with type 2B von Willebrand disease (VWD) is due also to impaired megakaryocytopoiesis, as shown both by changes in proteins involved in platelet calcium homeostasis and by electron microscopy (EM).



Multimeric structure of plasma VWF in selected patients with type 2B VWD and different VWF mutations as obtained by low-resolution SDS agarose gel electrophoresis. Two normal samples (NP) are shown for comparison. The cathode is at the top of the figure, and the direction of electrophoretic migration is from top to the bottom. Adapted from Federici et al.[3]

Type 2B von Willebrand disease (VWD) is an inherited bleeding disorder characterized by enhanced ristocetin-induced platelet agglutination (RIPA) in a patient's platelet-rich plasma[1,2] and is due to a group of mutations clustered within von Willebrand factor (VWF) A1 domain (see figure at bottom left). The mutant type 2B VWF displays increased affinity to its platelet receptor, the glycoprotein Ibα (GPIbα). The gain of function in the VWF A1 domain creates 3 conditions that explain bleeding at the sites of injury: (1) the large, hemostatically most effective VWF multimers are decreased in plasma; (2) platelet count may be reduced; and (3) part of the GPIbα receptors may be occupied by soluble VWF and unable to interact with VWF immobilized on the tissues exposed at the sites of injury.[2] The plasma VWF multimeric pattern and the degree of thrombocytopenia can vary significantly in patients with type 2B VWD.[2,3] A wide degree of heterogeneity has been found in the VWF multimeric patterns, ranging from the complete loss of the high-intermediate multimers to the partial preservation of the larger VWF forms (see figure at top right). Mild to severe thrombocytopenia especially following physiologic or pathologic stress conditions such as pregnancy, infections, surgery, or use of desmopressin can be observed, while cases with severe thrombocytopenia with platelet agglutinates at baseline are very rare.[2] Severe thrombocytopenia with giant platelets has been also reported.[4]

Nurden and colleagues report for the first time a family with type 2B VWD in which severe thrombocytopenia is also due to abnormal megakaryocytopoiesis. The affected members of this family carry a heterozygous mutation in the VWF A1, resulting in R1308P substitution. The net result is in vivo platelet agglutination and severe thrombocytopenia. Electron microscopy (EM) demonstrated that agglutinates were composed of rounded platelets with distinct zones of contact confirming a major role of the enhanced type 2B VWF–GPIb binding. Abnormal megakaryocytopoiesis was also involved, because plasma membrane Ca^{2+} ATPase and inositol triphosphate receptor were selectively increased. Moreover, findings typical of immature megakaryocytes (MKs), cultured from peripheral blood CD34 cells in the presence of TPO, were observed. Immunolocalization showed VWF not only associated with platelets, but already on the MK surface and within internal channels. Specific amino acid substitutions in the VWF A1 domain are critical, since other affected VWD patients with R1308L showed normal platelet count and no significant loss of the largest multimers.[5] These studies open novel research approaches to explore basic mechanisms on type 2B VWD and on megakaryocytopoiesis. However, since platelet counts can be normal in 2B VWD, more 2B cases with thrombocytopenia should be investigated to better understand if these findings are the exception or the rule. The relevance of VWF A1–GPIb interactions influencing megakaryocytopoiesis is a novel observation likely to produce new insights into platelet production.



Summary of the main mutations associated with type 2B VWD. Note that they are all included within the VWF A1 domain. Illustration by Frank Forney.

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NEOPLASIA

Comment on Chng et al, page 2755

Gene expression relates WM to CLL

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Waldenström macroglobulinemia (WM) shares traits with both chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). Chng and colleagues now demonstrate that by gene expression WM appears more closely related to CLL than MM.

Waldenström macroglobulinemia (WM) is a rare hematologic malignancy characterized by an IgM monoclonal gammopathy and bone marrow infiltration by small lymphocytes that can undergo plasmacytoid and plasma cell differentiation.[1] It has long been recognized that WM shows features suggestive of an intermediate process between chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). WM cells express B-cell markers but not CD5 or CD23 in contradistinction to CLL. WM cells appear to derive from a postgerminal B cell that has undergone somatic hypermutation but not switch recombination. The clonal cells reside primarily in the bone marrow similar to MM but secrete an IgM paraprotein that causes many of the clinical sequelae. Effective therapeutic agents in WM are by and large the same as in CLL (see figure).

		CLL	WM	MM
Biologic and clinical characteristics	Clonal cell	Small, mature lymphocyte	Small & plasmacytoid lymphocyte, plasma cell	Plasma cell
	Immunoglobulin	Surface IgM	Surface & cytoplasmic IgM	Cytoplasmic IgG, IgA,
	VH gene mutation	Mutated & unmutated	Mutated	Mutated
	Isotype switching	No	No	Yes
	Immunophenotype	Pos: CD5, CD19, dim CD20, CD22, CD23	Pos: CD19, CD20, CD22 neg: CD5, CD23, CD138	Pos: CD38, CD138 neg: CD19, CD20
	Paraprotein	Rare, low level	IgM	IgG, IgA, light chain
	Clinical Characteristics	Lymphocytosis, autoimmunity	Hyperviscosity, peripheral neuropathy	Bone & renal disease
	Lymphadenopathy & splenomegaly	Common	Possible/common	No
	Preferred/active agents	Fludarabine, rituximab, chlorambucil	Fludarabine, rituximab, chlorambucil	Steroids, melphalan, bortezomib
Chng et al	Source of cells	Blood	Marrow	Marrow
	Purification of malignant cells	CD19	CD19 & CD138	CD138
	Gene Expression	SYK, CD52, CD79a, PAX5, CD19, VAV	SYK, CD52, CD79a, PAX5, CD19, VAV	DKK1, HGF, FRZB, MAF, IL-6-R
	Pathogenic role of IL-6 ?	No	Autocrine	(Paracrine)*

Summary of characteristics of WM, CLL, and MM and of methods and results from the study by Chng et al. See the complete figure in the article beginning on page 2755.

Gene expression profiling can establish molecular diagnoses and uncover or better define distinct diseases.[2] This approach has been especially successful when the clinical material was relatively homogeneous, for instance lymph node biopsies or purified leukemic cells. Chng and colleagues used genomic-scale gene expression analysis to investigate the relationship between WM, CLL, and MM. Such a comparison is no easy undertaking due to distinct expression of cell-surface markers, differences in anatomic distribution, and the variable degree of blood or marrow involvement. The malignant cells studied here were obtained from peripheral blood (CLL) or bone marrow (WM, MM) and purified for CD19 (CLL), CD138 (MM), and combined CD19 and CD138 (WM) expression. The nature of the diseases and the approach chosen introduced confounding factors, notably differences in gene expression apparently contributed by contaminating cells, and made the use of statistical filters necessary. Nevertheless, the authors made several interesting observations (see figure).

Gene expression among the 23 WM cases appeared homogeneous, supporting the concept of a common biology despite the morphologic variability of the clonal cells. WM shared expression of typical B-cell markers with CLL and by hierarchic clustering analysis most WM samples aligned with the CLL samples. A small set of genes that interestingly included IL-6 and CD1c were overexpressed in WM. A role for IL-6 in WM has been previously suggested by the observation of IL-6–induced differentiation of the clonal B cells into plasma cells and the secretion of IL-6 by WM cells in vitro.[3] The current study thus provides a valuable confirmation of IL-6's potential pathogenic role in WM. The CD1 family of MHC-like glycoproteins presents glycolipids to antigen-specific T cells and plays important roles in innate immunity and autoimmunity.[4] Because WM cells often produce IgM paraproteins reactive with phospholipids, it is tempting to speculate that phospholipid antigens could contribute to the pathogenesis of WM.

In summary, this study adds further evidence that WM constitutes more a B-cell than a plasma cell malignancy and that WM is more closely related to CLL than MM. The unique gene expression profile of WM can provide a framework for further studies and focus attention on the possible pathogenic role of IL-6 production by the malignant clone. ■