Radiation-Induced von Willebrand Factor Release

To the Editor:

von Willebrand factor (vWF) is a large multimeric adhesive glycoprotein with a molecular weight ranging from 0.4×10^6 to over 20×10^6. It is synthesized by megakaryocytes and endothelial cells, and serves a major role in the initial step of platelet adhesion at sites of vessel injury by forming a bridge between the platelet and constituents of the subendothelium. Ionizing radiation has been shown to increase vWF release from cultured human endothelial cells within 24 hours after exposure to doses above 10 Gy.[1,2] However, the mechanism of this radiation-induced vWF release remains unclear. In a recent report, Jahroudi et al[3] addressed this issue and demonstrated that radiation induced an increase in vWF mRNA accumulation due to enhanced transcriptional activity. Furthermore, the radiation inducible promoter region of the vWF gene was identified. These observations provide important insights into the molecular mechanisms that control endothelial vWF release upon irradiation.

There are two distinct pathways of endothelial vWF release. The majority of the newly synthesized protein is constitutively secreted, while a small portion is first stored in specific organelles, the Weibel-Palade bodies, and rapidly released upon stimulation (the regulated pathway).[4] VWF secreted by the constitutive pathway consists of predominantly small multimers, while the vWF stored in Weibel-Palade bodies contains the largest and biologically most active multimers.[5,6] Although it is generally accepted that vWF is secreted to both the luminal and abluminal side of the cell, the polarity of the two secretory pathways remains a matter of debate. The findings by Jahroudi et al suggest an important role for the constitutive secretory pathway in this process.

We have pursued a different approach to investigate the secretory mechanism of radiation-induced vWF release. By making use of a three-compartment culture model with a loose collagen gel as artificial subendothelial support, we were able to quantify vWF and analyze the multimeric composition of the protein in both the luminal, abluminal, and cellular compartment. We have previously shown in this model that the basal, constitutive release of vWF occurs predominantly at the abluminal side, whereas regulated vWF release is polarized toward the luminal compartment.[7,8] After irradiation, most of the vWF was released into the abluminal compartment, with no significant changes in cellular vWF content.[2] Moreover, immunofluorescence studies showed no discernible depletion of the Weibel-Palade bodies after irradiation, in contrast to what was observed after stimulation with phorbol myristate acetate (PMA). These observations favor a role for the constitutive pathway in radiation-induced vWF release. Recent unpublished observations from our laboratory on the multimeric composition of vWF provide additional evidence to support this notion. Samples from the luminal, cell-associated, and abluminal compartments of cultured human umbilical vein endothelial cells were analyzed for the multimeric composition by Western blotting (Fig 1). At 48 hours after 20 Gy

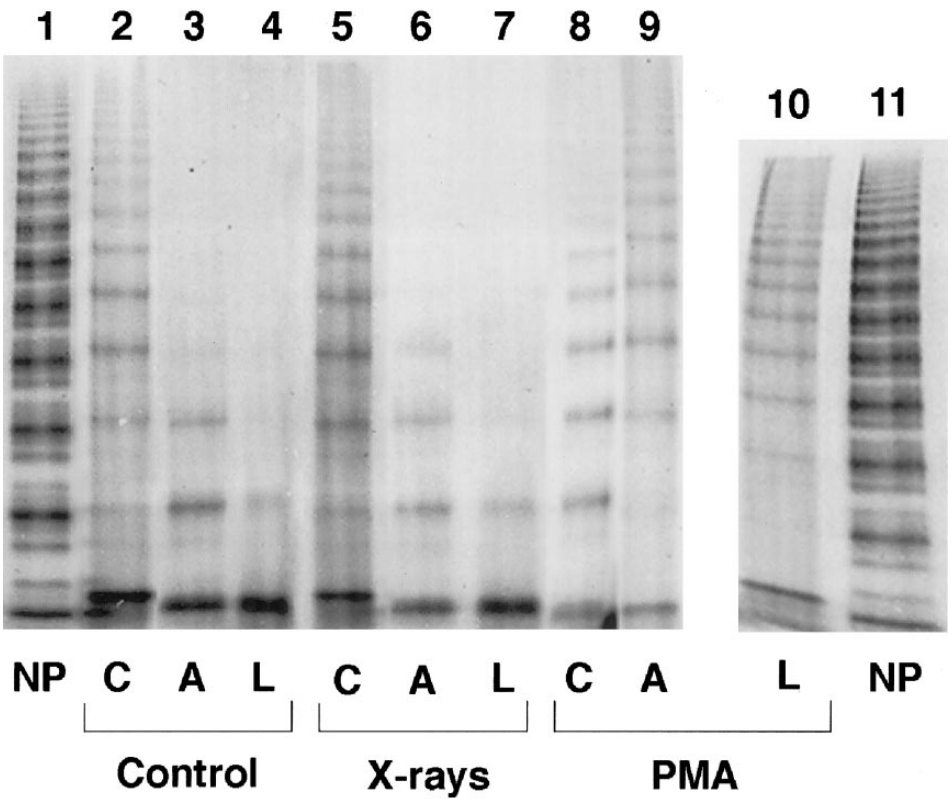

Fig 1. Multimeric composition of vWF, analyzed by electrophoresis under nonreducing conditions on a 2.5% sodium dodecyl sulfate-agarose gel. At 48 hours samples were taken from the cellular (C), abluminal (A), and luminal (L) compartment of quiescent cells (lanes 2 through 4), 20 Gy single-dose irradiated cells (lanes 5 through 7), and PMA-stimulated cells (lanes 8 through 10). Lanes 1 and 11, normal plasma; lanes 10 and 11 were derived from the same experiment, run on a separate gel.

single-dose irradiation, vWF multimers of predominantly low molecular weight were found in both the luminal and abluminal compartment. No changes in the multimeric pattern of intracellular vWF were observed. A similar distribution of vWF multimers was found in the respective compartments of unstimulated endothelial cells. In contrast, after stimulation with PMA, which causes the release of vWF from the Weibel-Palade bodies, a significant increase in the amount of high-molecular-weight multimers was observed in both the luminal and the abluminal compartment. This was accompanied by a selective decrease of the large multimers in the cell-associated fraction.

Both the data on secretion polarity and multimeric composition indicate that ionizing radiation enhances vWF release mainly through the constitutive pathway of secretion, and are in agreement with the data of Jahroudi et al.

Marcel Verheij
Luc Dewit
Department of Radiotherapy
The Netherlands Cancer Institute (Antoni van Leeuwenhoek Huis)
Amsterdam, The Netherlands
Jan A. van Mourik
Department of Blood Coagulation
Central Laboratory of the Netherlands Red Cross Blood Transfusion Service
Amsterdam, The Netherlands

REFERENCES

1. Sporn LA, Rubin P, Marder VJ, Wagner DD: Irradiation induces release of von Willebrand protein from endothelial cells in culture. Blood 64:567, 1984
2. Verheij M, Dewit LGH, Boomgaard MN, Brinkman HJM, van Mourik JA: Ionizing radiation enhances platelet adhesion to the extracellular matrix of human endothelial cells by an increase in the release of von Willebrand factor. Radiat Res 137:202, 1994
3. Jahroudi N, Ardekani AM, Greenberger JS: Ionizing radiation increases transcription of the von Willebrand factor gene in endothelial cells. Blood 88:3801, 1996
4. Handin RI, Wagner DD: Molecular and cellular biology of von Willebrand factor, in Coller BS (ed): Progress in Hemostasis and Thrombosis. Philadelphia, PA, Saunders, 1989, p 233
5. Tannenbaum SH, Rick ME, Shafer B, Gralnick HR: Subendothelium matrix of cultured endothelial cells contains fully processed high molecular weight von Willebrand factor. J Lab Clin Med 113:372, 1989
6. Sporn LA, Marder VJ, Wagner DD: von Willebrand factor released from Weibel-Palade bodies binds more avidly to extracellular matrix than that secreted constitutively. Blood 69:1531, 1987
7. Van Buul-Wortelboer MF, Brinkman HJM, Reinders JH, van Aken WG, van Mourik JA: Polar secretion of von Willebrand factor by endothelial cells. Biochim Biophys Acta 1011:129, 1989
8. Hakkert BC, Rentenaar JM, van Mourik JA: Monocytes enhance endothelial von Willebrand factor release and prostacyclin production with different kinetics and dependency on intercellular contact between these two cell types. Br J Haematol 80:495, 1992

Identity Between Hairy B-Cell Lymphoproliferative Disorder and Persistent Polyclonal B Lymphocytosis?

To the Editor:

Hairy B-cell lymphoproliferative disorder (HBLD) was recently identified as a distinct entity: Machii et al[1] described four adult patients with the presence of chronic, moderate, and polyclonal lymphocytosis. A few binucleated lymphocytes and atypical lymphoid cells with long microvilli and prominent membranous ruffles on their surfaces were detected on peripheral blood smears. The B cells expressed the CD19, CD11c, and CD22 antigens and were not stained by the anti-CD5, anti-CD24, or anti-CD25 anibodies. Furtheremore, Southern blot analysis and polymerase chain reaction (PCR) amplification of the CDR3 region failed to show a clonal rearrangement of immunoglobulin heavy chain genes.

In adults, persistent lymphocytosis is usually related to B-chronic lymphoproliferative disorders, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), splenic lymphoma with villous lymphocytes (SLVL), hairy cell leukemia (HCL) or its variant form (HCL-V), and the leukemic phase of follicular lymphoma. These disorders are characterized by a progressive expansion of a clone of neoplastic B lymphocytes, and all cells in the clone contain the same unique Ig gene rearrangement.

In contrast, a polyclonal lymphocytosis is observed in hyposplenic state, nodular lymphocyte predominance Hodgkin's disease, rheumatoid arthritis, persistent polyclonal B lymphocytosis (PPBL),[2,3] and more recently in HCL Japanese variant. We studied 19 patients (17 women and 2 men), median age 40 years (range, 33 to 50) with PPBL. Six of the 19 patients have been previously reported.[3] Three patients presented splenomegaly and no patient had hepatomegaly or adenopathy. All patients presented an absolute lymphocytosis: the majority of lymphocytes were large with abondant faintly, basophilic cytoplasm (Fig 1, see page 2111) and in all patients a characteristic nuclei demonstrating a rounded or more commonly irregular form, with variable (2% to 9%) numbers of binucleated cells (Fig 2, see page 2111). Despite chronic lymphocytosis and morphology of the lymphoid cells similar to that of the lymphoid B cells of HBLD, a close relationship was excluded between both entities. Environmental (tobacco) factors have been implicated in the etiology of PPBL but we observed one female patient who never smoked. In a recent review based on 38 patients with PPBL, 24 of 27 patients with PPBL were HLA-DR7-positive[4]: 3 patients expressed DR4 in our series. The CD11c antigen was expressed in 3 of 13 cases and 5 of 17 patients had a high serum level of IgG. Cytogenetic analysis was performed in 18 of 19 patients: 16 patients showed the presence of an extra chromosome 3 long arm i(3q)[5] (Fig 3). Premature chromosome condensation (PCC) was also observed in 15 patients. The morphology of the PCC indicated condensation of G1 and G2 cells, exhibiting single and double chromatids, respectively. Thirteen patients presented both abnormalities. In contrast, 100 metaphases each from 5 cases of B-CLL and 5 healthy donors failed to show either PCC or i(3q). The i(3q) was observed almost exclusively following culture

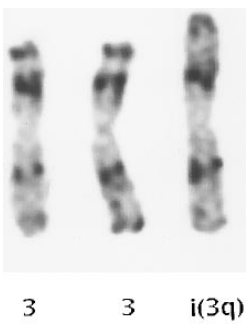

Fig 3. Partial karyotype showing i(3q).