Macrophagocyte with abnormal demarcation membranes in megakaryocytes and neutropenia with a complete lack of sialyl-Lewis-X antigen in leukocytes—a new syndrome?

Thiebaut-Noel Willig, Jeanine Breton-Gorius, Carole Elbim, Vincent Mignotte, Cecile Kaplan, Rosella Mollicone, Catherine Pasquier, Anne Filipe, Francoise Milot, Jean-Pierre Cartron, Marie-Anne Gougerot-Pocidalo, Najet Debili, Josette Guichard, Jean-Paul Dommergues, Narla Mohandas, and Gil Tchernia

A new macrothrombocytopenic syndrome with giant platelets in peripheral blood and severe thrombocytopenia was diagnosed in a 4-month-old boy. His clinical course included repeated hemorrhagic incidents leading to death at age 37 months. Bone marrow ultrastructural analysis revealed numerous dystrophic megakaryocytes with giant membrane complexes. Although these features were similar to those described for megakaryocytes in mice lacking the gene for transcription factor p45-NF-E2, no abnormalities in the p45-NF-E2 gene could be documented. Platelet membrane analysis showed a reduction in glycoprotein (GP) Ib, but normal content of GPIIb and GPIIIa. Analysis of genes encoding for GPIIb α and β, GPV, and GPIX ruled out the possibility that the observed platelet abnormality is a variant of Bernard-Soulier syndrome. A moderate neutropenia was associated with a complete lack of expression of sialyl-Lewis-X on the surface of polymorphonuclear neutrophils. A common defect in posttranslational modification of glycoproteins could account for the diverse cellular abnormalities.

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Ultrastructural studies
Aspirated bone marrow samples, platelets isolated from blood, and megakaryocytes cultured from CD34+ progenitors were processed for examination with an electron microscope as previously described.

Platelet membrane glycoprotein analysis
Platelets from the patient and a normal donor were analyzed using sodium dodecyl sulfate–polyacrylamide gel electrophoresis, periodic acid-Schiff staining, and Western blot analysis as previously described. Monoclonal antibodies GS 296, SZ 22, and XII F9 specific for GPIbα, GP IIb, and GP IIIa, respectively, were used in Western blot analysis.

Molecular studies
Polymerase chain reaction (PCR)-amplified NF-E2 complementary DNA (cDNA) and genomic DNA were sequenced using a fluorescent automated DNA sequencer. The genomic sequence of GPIbα, GPIV, GPIX, FUT4, and FUT7 genes was determined following PCR amplification using appropriate primers. We also assessed the genomic sequence of 2 sialyltransferase genes (ST3Gal4 and ST3Gal6) and 2 fucosyltransferase genes (FUT 4 and FUT 7).

PMN studies
Function of PMNs was assessed using a chemotaxis assay and the nitroblue tetrazolium (NBT) test, as previously described. Surface expression of adhesion molecules on resting PMNs and following activation by N-formyl-methionyl-leucyl-phenylalanine (fMLP) was quantitated by flow cytometry.

Sialyltransferase and fucosyltransferase assays
Global sialyltransferase activity of PMNs and platelets was quantitated as previously described. The α-2- and α-3-fucosyltransferases were assayed in plasma and in the extracts prepared from an isolated lymphocyte/monocyte fraction.

Sequences of the different primer sets used in the molecular studies and details of various experimental protocols are available on request.

Results and discussion
Morphologic examination of peripheral blood smears revealed hypogranular platelets and the presence of some giant platelets (diameter > that of lymphocytes). Bone marrow aspirates

Figure 1. Platelet and PMN counts. Evolution of platelet counts (top) and PMN counts (bottom) during a 3-year follow-up period.

Figure 2. Ultrastructural aspect of megakaryocytes. (Top) A large mononucleated megakaryocyte with large membrane complex (MBC). Only few demarcation membranes are free (arrows). Magnification × 4750. (Bottom) Lytic mature megakaryocyte. The nucleus has the typical aspect of apoptotic cells with clumps of chromatin. The cytoplasm contains numerous granules (arrows). The cell membrane and intracytoplasmic membrane are lysed. A granulocytic peroxidase is present at the periphery. Note the presence of a fibroblast (F) closely associated to the lytic megakaryocyte. Magnification × 5320.
showed normocellular marrow with megakaryocyte hyperplasia. Megakaryocyte morphologic abnormalities included numerous small mononuclear or hyposegmented megakaryocytes, vacuolated cells, and abnormal fragmentation of megakaryocyte cytoplasm into large platelet masses. This abnormal subpopulation coexisted with a population of apparently normal megakaryocytes. Electron microscopic studies showed that alpha granules were produced but appeared to exhibit abnormal cytoplasmic distribution due to the presence of giant membrane complexes with associated smooth endoplasmic reticulum and demarcation membranes (Figure 2). These membrane complexes appeared very early in megakaryocyte maturation and were also observed in cultured megakaryocytes. In addition, numerous large lytic megakaryocytes were seen, some exhibiting apoptotic nuclei (Figure 2). Analysis of platelet membrane proteins showed a markedly increased amount of GPIb but normal content of GPIIb and GPIIIa. Furthermore, GPIb exhibited a higher apparent molecular weight, suggesting altered glycosylation. Because the ultrastructural features of the patient’s megakaryocytes were similar to those described in NF-E2 knockout mice,17 the cDNA and genomic sequence of this gene was analyzed but failed to reveal any mutation. The genomic sequences of GPIb and β3-integrin adhesion molecules (CD11a/CD18, CD11b/CD18, and CD11c/CD18) and of L-selectin was normal on both unstimulated PMNs and following stimulation by formyl peptides. However, expression of sialyl Lα2 was undetectable on the patient’s PMNs (median fluorescence intensity 25 versus 1500-2500 for normal). There was also a 2-fold increase in FUT4 activity was noted in PMNs. Because the patient’s PMNs failed to express sialyl Lα2, we explored if mutations in fucosyltransferase (FUT4 and FUT7) genes or in sialyltransferase (ST3Gal4 and ST3Gal6) genes were responsible for the observed phenotype. Sequencing of these 4 genes in the patient failed to reveal any abnormality. However, we cannot rule out the possibility of mutations in noncoding regions or a chromosomal rearrangement involving these genes.

Our study provides the first description of a new dysmegakaryo-cytopenic syndrome that appears to be due to a defect in posttranslational modification of membrane glycoproteins. Although we were unsuccessful in our attempts to delineate the precise molecular defect responsible for the complex clinical phenotype, the heterogeneous features encountered in our patient could all be related to an abnormal defect in fucosylation or sialylation pathways.

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References


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