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**Impaired proplatelets formation by human megakaryocytes derived from variant
Bernard Soulier syndrome type Bolzano
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The variant Bernard Soulier syndrome (BSS) type Bolzano (1) is a rare congenital bleeding disorder due to a structural and functional abnormality of the glycoprotein GPIb-V-IX complex and characterized by thrombocytopenia and giant blood platelets. Since the pathogenic mechanisms are still poorly understood, in this work we have analyzed the megakaryocyte (MK) differentiation and proplatelet formation (PPF) (2) by haemopoietic progenitor cells derived from cord and peripheral blood of patients as compared to normal controls.

MKs were differentiated from cord blood derived CD34⁺ cells and peripheral blood derived mononuclear cells for 12 days. Mature MKs were plated onto glass coverslips coated with collagen I, fibrinogen (FNG), or von Willebrand Factor (VWF), or grown in suspension. MK differentiation and PPF were evaluated by phase contrast and fluorescence microscopy upon cell staining with anti-tubulin and CD41 antibodies. Controls were analyzed in parallel with each patient sample.

The time-dependent PPF by BSS cord blood derived MKs, in suspension, was detectable after 4 hours, and increased progressively to reach a plateau at 24 hours. This process involved a lower number of cells than control. Adhesion to FNG or VWF resulted in a similar impairment of PPF with respect to control. As in the control type I collagen totally inhibited PPF by BSS cord blood derived MKs.

PPF by MKs derived from the peripheral blood of 3 BSS patients was also analysed. As for the cord blood PPF by BSS peripheral blood derived MKs decreased with respect to control. Similar results were observed in adhesion experiments to FNG and VWF.

In morphologic analysis proplatelets derived from both cord and peripheral BSS blood revealed an altered structure of the tips that presented altered tubulin distribution and an enlarged diameter with respect to controls.

In conclusion in this work we report an altered extension and morphology of proplatelet formation by megakaryocytes derived from variant Bernard Soulier syndrome type Bolzano cord and peripheral blood. These results may enlighten some aspects of the pathogenetic mechanisms that result in the thrombocytopenia with giant blood platelets characteristic of this syndrome.

References

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