Modulation of pathological phenotype of red blood cells generated ex vivo from hematopoietic stem cells in patients with sickle cell disease

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Background: In sickle cell anemia (SA), synthesis of pathological hemoglobin S (HbS) induces sickling of red blood cells (RBC), their reduced deformation capacity and increased adherence to the endothelial wall. This results in vaso-occlusive events, responsible for much of the morbidity and mortality. Fetal hemoglobin (HbF) is known as the most potent modifier in disease severity, since it protects RBC from pathological HbS polymerization. The aim of the study was the modification of RBC pathology by the ex vivo generation of RBC, containing a high level of HbF, derived from hematopoietic stem cells (HSC) of SA patients.

Material and Methods: Peripheral blood (PB) CD34+ HSC from SA patients were cultured over 25 days in an in-vitro erythropoiesis assay. Generated RBC were compared with native RBC from the same patient for their level of HbF (HPLC, flow cytometry), the expression of adhesion molecules CD36, Integrin α4β1, CD239, ICAM-4, CD47, CD147 (flow cytometry), their deformation capacity (ektacytometry) and adhesion to human laminin (flow adhesion assay). Results: CD34+ HSC were found to be increased in PB of SA patients (~10-fold) and could be isolated in a sufficient way. The applied in-vitro erythropoiesis assay was able to generate 100% enucleated RBC with at least 16,500-fold amplification. Compared to native SA-RBC, ex vivo generated RBC showed an increased HbF level (~5-fold). Whereas native SA-RBC showed strong adhesion to laminin, this was reduced or absent in ex vivo generated RBC. In line with this, cultured RBC showed a modified expression of adhesion molecules. In contrast to the reduced deformation of native SA-RBC, the deformation capacity of ex vivo generated RBC was also normalized.

Conclusion: RBC generation under ex vivo conditions allows for the modulation of the pathological phenotype in SA. Besides an increased level of HbF, ex vivo generated cells show a normalized deformation capacity and reduced adhesion to elements of the endothelial wall. Further work will be carried out to identify underlying pathways. As the most interesting clinical application, such ex vivo generated RBC might be suitable as an...
autologous transfusion product, able to reduce the frequency of vaso-occlusive events and to circumvent storages of compatible RBC units.