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Transcriptome analyses unveils the unique requirement for human lipoproteins for optimal ex-vivo expansion of cultured red blood cells for transfusion.

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Cultured red blood cells (cRBCs) generated from discarded stem cell sources are being considered as alternative transfusion products. We developed a humanized media (HEMA^{def}) composed of clinical grade components and dexamethasone (Dex) that sustain amplifications of cRBCs from stem cells discarded from regular blood donations in numbers sufficient for transfusion [1]. These cells, however, have an altered morphology of their plasma membrane which suggests that may reduce their survival in vivo. Since ~50% of the cRBC mass is constituted by lipid, we hypothesized that the plasma membrane abnormalities were caused by insufficient lipid supply. cRBCs produce their plasma membranes starting from lipids supplemented by the media and through a dedicated biosynthetic pathway, well known to be affected by Dex. Comparison of the expression profiling of cRBCs generated with/without Dex [2] identified remarkable similarities in gene expression. The majority of the differences were however detected in genes involved in lipid metabolism. In particular genes involved in lipid synthesis (GPAM and PRKACB) and efflux/degradation (HMGCL and ABCA1) were respectively down and up-regulated in cRBCs obtained with Dex, suggesting that in cultures with Dex cRBCs are exquisitely dependent on exogenous lipids for their membrane biosynthesis. This hypothesis led us to optimize the lipid formulation of HEMA^{def} by replacing the synthetic liposomes with lipoproteins purified from human plasma which represents the natural carriers for delivering lipids to the cells. These experiments compared the levels of amplification/maturation of cRBCs in HEMA^{def} supplemented with either synthetic liposomes or the total lipoproteins contained in human plasma (TLP) or its high density (HDL), low density (LDL) and very low density (VLDL) lipoprotein fraction. Addition of LDL and VLDL both increased by 3-2-fold the numbers of cRBCs generated in HEMA^{def}. TPLF has modest effects on the number of cRBCs generated in HEMA^{def} but drastically reduced the frequency of cRBCs with membrane abnormalities. More importantly, addition of TLP increased both the number (by 2-3-fold) and the membrane quality of cRBCs generated in HEMA^{def}. These results confirm the importance of an appropriate lipid supply for correct generation of RBC and identify culture conditions which assure maximal expansion of morphologically normal cRBCs for transfusion.

References

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Keywords

Human plasma, lipoproteins, membrane biogenesis, human erythroid cells, blood farming

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