Towards a detailed understanding of the red blood cell storage lesion - and its consequences for in vivo survival following transfusion

Andreas Hult

Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av medicine doktorsexamen framläggs till offentligt förvar i Sal BiA201, Biologihuset, torsdagen den 11 juni, kl. 09:00.

Avhandlingen kommer att förvaras på svenska.

Fakultetsopponent: Professor Martin L. Olsson, Institutionen för transfusionsmedicin, medicinska fakulteten, Lunds Universitet, Sverige.
Towards a detailed understanding of the red blood cell storage lesion - and its consequences for \textit{in vivo} survival following transfusion

Abstract

Red blood cells (RBCs) are vital for oxygen delivery to tissues and constitute the vast majority of all cells in blood. After leaving the red bone marrow as mature cells, RBCs have a lifespan of approximately 120 days before they are removed from the circulation by macrophages, mainly in the spleen and liver. RBC transfusion is a common therapy in modern healthcare. Major surgery, numerous cancer treatments and other, often lifesaving, interventions would be unthinkable without available blood supply. For this reason, hospitals store donated RBCs in blood banks.

The metabolic and structural changes that occur during prolonged storage of RBCs ( \textit{the storage lesion} ) have been studied in detail \textit{in vitro} and include oxidative stress, a reduction in glycolysis, increased membrane rigidity and shedding of microparticles from the RBC membrane. Stored RBCs share several features of senescent RBCs, but also with RBCs undergoing an apoptotic-like process called eryptosis. A consequence of the storage lesion is the fact that as much as 25\% of stored RBCs could be rapidly removed from the circulation within 24 hours after transfusion. The mechanisms behind this rapid macrophage-mediated recognition and removal of stored RBCs, and its immunological consequences, remain largely unknown. Therefore, the aims of this thesis were to investigate if cryopreserved human RBCs induced an inflammatory response following autologous transfusion into healthy volunteers, and to further understand the mechanisms behind macrophage recognition of stored RBCs \textit{in vitro} and \textit{in vivo}.

Autologous transfusion of two units of cryopreserved RBCs into healthy human recipients was found to be associated with an increased extravascular RBC elimination already at 2 hours after transfusion. However, there were no signs of an increased production of any of the investigated pro-inflammatory cytokines, indicating that an increase in the destruction of RBCs \textit{per se} did not induce an inflammatory response.

Eryptosis is a form of induced RBC death associated with an increased cytoplasmic Ca\textsuperscript{2+} uptake. We found that a subset of human RBCs increased their Ca\textsuperscript{2+} permeability during prolonged storage at +4°C. Using a murine model, to further understand how RBCs with an increased Ca\textsuperscript{2+} permeability were eliminated by phagocytic cells in the spleen, it was found that such RBCs were taken up by marginal zone macrophages and dendritic cells (DCs) in a manner distinct from that of naturally senescent RBCs. The DC population particularly efficient in this process expressed CD207 and are known for their ability to promote immunological tolerance. Eryptotic cell uptake was not regulated by the phagocytosis-inhibitory protein CD47 on the RBCs.

To investigate how RBCs damaged during liquid storage are recognized and taken up by macrophages, a model to store and transfuse murine RBCs was developed. This storage model generated murine RBCs with several characteristics similar to that of stored human RBCs (i.e. loss of ATP, formation of RBC microparticles and rapid clearance of up to 35\% of the RBCs during the first 24 h after transfusion). \textit{In vitro} phagocytosis of human as well as murine stored RBCs was serum dependent and could be inhibited by blocking class A scavenger receptors using fucoidan or dextran sulphate.

In conclusion, the findings of this thesis contribute to further understanding how changes inflicted to RBCs during storage direct the fate of these cells in their interaction with cells of the immune system after transfusion. The observation of an increased Ca\textsuperscript{2+} permeability of stored RBCs, and the possible recognition of such cells by tolerance-promoting DCs, in combination with the findings that class A scavenger receptors and serum factors may mediate recognition of stored RBCs, may result in novel new directions of research within the field of transfusion medicine.

Keywords

Red blood cell, erythrocyte, transfusion, macrophage, phagocytosis, storage lesion, eryptosis, Class A scavenger receptor

Language  ISBN  ISSN  Number of pages

English  978-91-7601-288-8  0346-6612  58 + 3 papers