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The effect of pre-storage whole-blood leukocyte reduction on cytokines/chemokines levels in autologous CPDA-1 whole blood

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ABSTRACT

Background: In this study, we aimed to investigate the effectiveness of pre-storage leukocyte filtration of autologous blood (AB), especially focusing on the cytokines/chemokines accumulation on blood products.

Materials and methods: After approval of the ethics committee of the University of Tokyo, a total of 26 orthopedic patients, who donated AB prior to surgery after informed consent, were enrolled. The effects of filtration on blood cell counts were analyzed, and the accumulation of cytokines and chemokines were measured on pre- and post-leukoreduced (LR) samples, using the Luminex system. The time-dependent changes of the cytokines/chemokines and the effect of the filtration on their concentration were analyzed, and compared with the normal plasma levels reported in the literature.

Results: LR effectively reduced the number of leukocytes and platelets, without affecting that of red cells. The concentration of most of the cytokines/chemokines analyzed, except the EGF, sCD40-L and sFas-L, decreased time-dependently of storage or did not change in pre-LR samples. However, EGF, sCD40L and sFas-L were significantly reduced by LR. Some, such as IL-8 and RANTES, were also importantly decreased by LR, and others, such as IL-1β and TNF-α, were not significantly affected by LR.

Conclusions: Leukocyte filtration effectively removes platelets and leukocytes from AB, thus preventing the accumulation of cytokines/chemokines. Since adverse effects due to AB transfusion, although rare, are observed, there is need to consider the implementation of pre-storage leukocyte reduction (PSLR) for AB.

1. Introduction

The non-hemolytic transfusion reactions (NHTRs) are the most frequently observed post-tranfusional adverse effects. NHTRs range from mild reactions, such as fever [1,2], urticaria, and chilly, to more serious complications, such as the transfusion-related acute lung injury (TRALI), hypotension and anaphylaxis [3,4]. The most frequently implicated causative agents of these adverse reactions are the leukocytes and platelets, through the induction of alloimmune reactions, and the production of cytokines/chemokines (C/C) [1,2]. The C/C, such as interleukin (IL)-1β, IL-6, IL-8

Abbreviations: AB, autologous blood; CPDA-1, citrate-phosphate-dextrose-adenine-1; EGF, Epidermal growth factor; b-FGF, basic fibroblast growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HGF, hepatocyte growth factor; IFN, interferon; IL, interleukin. IP10, IFN-γ-inducible protein 10; MCP-1, monocyte chemotactic protein 1; MIG, monokine induced by IFN-γ; MIP, macrophage inflammatory protein; RANTES, Regulated upon Activation Normal T-cell Expressed and Secreted; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; IL2R, interleukin 2 receptor; G-CSF, granulocyte colony-stimulating factor; sCD40-L, soluble CD40 ligand; sFas-L, soluble Fas ligand; SDF-1, stromal cell-derived factor-1; ENA78, Epithelial neutrophil-activating protein 78; RBC, red blood cell.

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and TNF-α, which accumulate in the blood bags during the storage period, especially in platelets concentrates (PCs), seem to be implicated in the pathogenesis of the febrile NHTR (FNHTR) [1,2,5,6]. RANTES has been shown to be associated with allergic reactions [7–9]. The soluble CD40 ligand (sCD40L) induces synthesis of pro-inflammatory mediators, such as IL-6, IL-8 and MCP-1 [10] and it may be involved in the pathogenesis of TRALI [11]. Also, the Fas ligand (sFasL) and the TGF-β may cause transfusion-related immunomodulation (TRIM) [12–14], also by the transfusion of autologous blood (AB) [15].

The removal of leukocytes and platelets from the blood components prior to storage may prevent the alloimmune reactions [16], the C/C induced FNHTRs [17–19], and the clot formation in the blood bags [20], besides the transmission of infectious agents [21]. In Japan, the prestorage leukocyte reduction (PSLR) was gradually implemented by the Japanese Red Cross Blood Center (JRCBC), and since 2007, the universal leukoreduction was achieved. Also, the diversion of the initial blood flow during blood collection is performed to reduce the risk of bacterial contamination [22]. AB transfusion is a safer alternative and in our institution more than 1400 AB collections are performed yearly.

Taking into account the risk of blood shortage in Japan in a very near future, dependent on the decreasing birthrate and the increase in longevity [23], the government actively stimulates the use of AB [24]. AB is the safest blood for transfusion, considering that, theoretically, neither the risk of alloimmunization nor of blood-borne infection exists. However, since the PSLR is not implemented for AB, the risk of FNHTR due to infusion of C/C accumulated during storage exists. Although not frequent, adverse events during transfusion of AB, such as fever, are reported to our service. Also, the occasional aggregate formation in the blood bag, makes difficult or sometimes impossible to transfuse the AB. Thus, transfusion of AB may also be associated with adverse reactions, dependent on the C/C produced by leukocytes and platelets during the storage period. In the present study, we aimed to investigate the effect of PSLR on the accumulation of C/C in AB.

2. Materials and methods

2.1. Patients

Orthopedic patients receiving elective surgery at the University of Tokyo hospital, donating AB pre-operatively, and from whom informed consent was obtained, were enrolled. This study was approved by the ethics committee of the University of Tokyo. AB is collected in the appropriate bags containing CPDA-1 as the preservative, and all blood was preserved as whole blood at 4 °C, for a maximum of 35 days.

2.2. Leukocyte reduction of AB products and samples preparation

AB was collected in bags equipped with leukoreduction filters (Sepacell Integra CA, Asahikasei Kuraray Medical, Tokyo), which consists of two polyvinyl chloride bags connected by a segment tube, with a positive-charged polyester non-woven fabric leukoreduction filter between the bags, in a completely closed system. After the collection, AB was kept at 4 °C for 2 h, and then, the non-leukoreduced (non-LR) sample (approximately 10 ml) was sterilely transferred from the bag to the polyvinyl chloride segment tube, made of the same material as the LR bag. Then, the blood was subjected to leukoreduction, and stored until transfusion. Therefore, the non-LR samples were maintained under the same conditions, i.e., in containers made of the same material, and under the same conditions, as the leukoreduced blood in the bag. The LR sample was collected at the day of operation, by sterilely transferring approximately 10 ml of leukoreduced blood from the bag to the polyvinyl chloride segment tube, and examined together with the non-LR sample. The plasma sample for the analysis of C/C was obtained by centrifugation of the blood samples, and stored at −40 °C until analysis.

2.3. Blood cell counts of non-LR and LR samples

The blood cell counts were performed using an automated hematology analyzer (XE-5000, Sysmex, Japan). For the leukocyte counts, the body fluid mode, which is suitable for automated quantification of white blood cell until a detection limit of less than 10 cells/μL, was applied [25]. RBCs and platelet counts were performed by the normal mode of XE-5000.

2.4. Measurement of C/C concentration levels

C/C concentration was determined by the Luminex system using Procarta cytokine assay (Affymetrix, Santa Clara, CA, USA), and measured 34 different kinds of C/C, as shown in Fig. 1. The beads were analyzed in the Luminex 100 instrument (Applied Cytometry Systems, Dinnington, UK). C/C concentrations were calculated by the use of the respective standard curves.

The difference between the concentration of the respective C/C in non-LR and LR samples, which, theoretically, represents their concentrations in the absence of leukocytes/platelets, was plotted against the period of storage. The coefficient of correlation was obtained for all tested C/C, according to the period of storage. Also, the mean concentration of the C/C in non-LR samples stored for less than 15 days and those stored for 15 or more days was compared. The normal levels of C/C were referred to a literature assessment [26–36], because the normal values are not provided by the manufacturer.

2.5. Statistical analysis

The minimum detectable C/C concentration (0 pg/ml) was assigned to those lower than the limit of detection. Statistical analyses were performed using PASW statistics 18 for Windows. The correlation between the concentration of C/C and the period of storage was calculated by nonparametric Spearman rank correlations, and the difference at $P < 0.05$ was considered a significant association between the storage period and the C/C concentration.