Cytokine Generation in Whole Blood, Leukocyte-Depleted and Temporarily Warmed Red Blood Cell Concentrates

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Abstract

Background and Objectives: It has been suggested that inflammatory cytokines such as Interleukin (IL)-1β, IL-8, tumor necrosis factor-α (TNF-α) and IL-8 might be responsible for a large number of non-antibody-mediated adverse reactions to the transfusion of blood components, especially of platelet concentrates (PCs). The aim of this study was to compare the levels of proinflammatory cytokines in different blood components containing red cells such as buffy-coat-free packed red cells (RBCs), filtered RBCs and whole blood (WB) during storage under several conditions. Materials and Methods: WB (CPD-A1, n = 16) was stored for 35 days at 2–6°C; samples were taken on days 0, 21 and 35. Buffyc-coat-poor RBCs in additive solution PAGGS-M (n = 16) were divided into halves, one half was leukocyte (WBC)-depleted by filtration on day 0, both halves were stored for 49 days at 2–6°C (samples: days 0, 21, 49). Furthermore, buffy-coat-poor, unfiltered SAG-M RBCs (n = 16) were halved immediately after production and stored at 2–6°C until day 42 (samples: days 0, 21, 42). One half remained at room temperature for 24 h on day 3. Cytokine levels were determined with commercial enzyme-linked immunosorbert assays. Results: Levels of IL-1β and TNF-α rose during storage of WB and RBCs. IL-6 could be detected markedly above the detection threshold in WB only. At the end of storage, we detected IL-8 in 1 of 16 units of WB tested, in 10 of 16 standard PAGGS-M RBCs and in 15 of 16 temporarily warmed SAG-M RBCs. Prestorage filtration of RBCs prevented the accumulation of IL-1β and TNF-α. Temporally warming of RBCs for 24 h did not cause any substantial increase in cytokine levels other than IL-8. RBCs stored in different additive solutions (PAGGS-M versus SAG-M) showed similar cytokine concentrations during storage. The cytokine content of WB was very similar to that of buffy-coat-poor RBCs. Conclusion: Cytokine levels measured in WB and buffy-coat-poor RBCs result in levels which are unlikely to cause febrile reactions even in the case of massive transfusion. We conclude that, according to present knowledge, there is no reason for prestorage filtration of buffy-coat-poor RBCs or WB to avoid febrile transfusion reactions due to cytokine accumulation during storage.

Introduction

Febrile reactions rather occur after the transfusion of platelet concentrates prepared by the platelet-rich plasma method (PRP PCs) than after transfusion of PCs derived from buffy coats (BC PCs), apheresis PCs or units of packed red cells (RBCs) [1–5]. Recent evidence demonstrates a correlation between the frequency and severity of febrile reactions and contamination of blood components with white blood cells (WBCs), storage time and content of proinflammatory cytokines such as interleukin (IL)-1β, IL-
6, tumor necrosis factor-α (TNF-α) and IL-8, especially in PCs [6–13]. This proinflammatory subset of cytokines accumulates during storage and seems to be released from contaminating WBCs [11, 13, 14]. The observation that most febrile reactions are caused by plasma and not by the cellular fraction of PCs gives good evidence that proinflammatory cytokines might be the causative agents in febrile reactions [8]. IL-1β, IL-6 and TNF-α are pyrogens, IL-8 is a neutrophil-chemotactic and -activating factor which reaches the highest levels in vitro during platelet storage among the cytokines measured to date [15, 16]. There is less information about cytokine generation in RBCs compared to the wealth of work concerning PCs in this field. In all probability, contaminating WBCs in RBCs stored at 2–6°C might synthesize and secrete cytokines to a much lower extent than WBCs in PCs stored at room temperature. Stack et al. [17] reported the accumulation of IL-8 and IL-1β in the supernatants of RBCs suspended in additive solution despite cold-storage conditions. They described IL-8 levels of >1,000 pg/ml in some RBC units. They investigated RBCs with a mean (± SD) leukocyte contamination of 4,760 ± 3,780/μl. WBC filtration early in storage prevented the accumulation of IL-8 and IL-1β. They did not detect IL-6 in any unit and did not measure TNF-α levels.

The clinical significance of these findings in RBCs remains unclear. Since IL-8 has no direct pyrogenic activity and the levels of IL-1β detected were low, it seems unlikely that these substances found in RBCs could mediate acute reactions to RBC transfusions. But it was speculated that the passive administration of low levels of cytokines might have additive or synergistic effects on the cytokine network in critically ill patients and might contribute to the systemic inflammatory and metabolic response in trauma and infection [18]. The report by Stack et al. [17] did not deal with whole blood (WB) and left some clinically important questions concerning RBCs used in our department. Performing quality control measurements, we observed a considerably lower leukocyte contamination in Buffy-coat-poor RBCs produced in our department than Stack et al. did in their RBCs. Furthermore, in clinical practice, RBCs usually undergo phases of handling and transport. This might result in temporarily warming of RBCs to room temperature, giving leukocytes the opportunity to synthesize and secrete cytokines at higher rates than at 2–6°C. We tried to address this problem by modelling an extreme worst-case scenario warming one group of RBCs for 24 h at room temperature.

The use of WB in homologous transfusion practice is obsolete. However, in Germany the use of WB is common in preoperative autologous blood donation, especially in smaller hospitals due to the financial and technical demands of WB fractionation to RBCs and fresh frozen plasma [19]. As the Buffy coat is not removed, leukocyte contamination of WB is higher than in RBCs, which might result in an elevated accumulation of cytokines during storage of WB. Therefore, the aim of this study was (1) to compare the levels of proinflammatory cytokines in WB compared to unfiltered, Buffy-coat-poor RBCs; (2) to assess the effect of a temporary storage period at room temperature and the effect of leukocyte depletion by filtration on the accumulation of cytokines using pairs of identical RBCs, and (3) to evaluate the influence of different additive storage solutions on cytokine generation in RBCs. The assessment entailed the measurement of IL-1β, IL-6, TNF-α and IL-8 by enzyme-linked immunosorbent assays (ELISAs).

Materials and Methods

Preparation of Blood Products, Study Design and Sampling

Whole blood (450 ml) was collected from 48 volunteer donors selected from the donor pool of our blood bank according to current German and European guidelines [20, 21]. No donor had taken acetylsalicylic acid or other nonsteroidal anti-inflammatory agents within 10 days before donation.

Sixteen units were stored as WB in blood bags containing 63 ml CPD-A1 (CPD-A1: citric acid, 3.27 g/l; sodium citrate, 26.3 g/l; sodium dihydrogen phosphate, 2.51 g/l; glucose, 31.9 g/l; and adenine, 0.275 g/l; Biopack® No. 733 143, Biotrans, Dreieich, Germany) for 35 days at 2–6°C. We took samples for cytokine determination using a sterile technique at the following storage times: day 0, 21 and 35. The samples were removed through sampling site couplers (4C-2405, Baxter, Unterschleissheim, Germany) by using plastic syringes with 19-gauge needles. The samples were centrifuged at room temperature at 2,000 g for 15 min (Rotanta, Hettich, Tuttingen, Germany). The upper two-thirds of the supernatant were immediately transferred to 1.3-ml tubes and stored at –70°C in multiple small aliquots.

Sixteen units of Buffy-coat-poor RBCs suspended in 100 ml PAGGS-M solution (PAGGS-M: adenine, 0.194 g/l; guanosine, 0.408 g/l; sodium dihydrogen phosphate, 1.25 g/l; sodium monohydrogen phosphate, 1.432 g/l; glucose, 9.4 g/l; mannitol, 10.0 g/l, and sodium chloride, 4.21 g/l) were prepared using a three-bag plastic container system including a top and bottom outlet primary bag containing 63 ml CPD (CPD: citric acid, 3.27 g/l; sodium citrate, 26.3 g/l; sodium dihydrogen phosphate, 2.51 g/l; glucose, 25.5 g/l; Biopack® No. 733 965, Biotrans). PAGGS-M RBCs were divided into halves, one half was leukocyte depleted by filtration (RCS0, Pall, Dreieich, Germany) on day 0, both halves were stored for 49 days at 2–6°C in 150 ml blood bags composed of polyvinylchloride with a 2-diethylhexylphthalate (2-DEHP) plasticizer (Biopack® No. 733 295, Biotrans) (samples for cytokine measurement: days 0 before division into halves, 21, 49. For WB count: day 0 after filtration). Component preparation was performed automatically by means of the Biotrans device (type TA 0100, Biotrans) after centrifugation (4,000 g for 10 min at 20°C; Roto Silenta RP, Hettich).
Table 1. Initial hematocrit, platelet count and volume, and free plasma hemoglobin and hemolysis rate at the end of the storage time of RBCs and WB (mean ± SD)*

<table>
<thead>
<tr>
<th></th>
<th>RBCs (PAGGS-M)</th>
<th>RBCs (SAG-M)</th>
<th>WB</th>
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<tbody>
<tr>
<td></td>
<td>filtered unfiltered</td>
<td>unwarmed warmed</td>
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<tr>
<td>Hematocrit, l/l</td>
<td>58.2±3.7 61.7±3.6</td>
<td>60.9±3.1 60.9±3.1</td>
<td>39.2±3.0*b</td>
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<tr>
<td>Platelets, 10^9/l</td>
<td>3.4±5.1 5.9±10.4</td>
<td>6.4±6.0 6.4±6.0</td>
<td>198.6±39.2*b</td>
</tr>
<tr>
<td>WBC count, 10^9/l</td>
<td>&lt;0.1 1.9±1.2</td>
<td>2.3±1.4 2.3±1.4</td>
<td>4.5±1.1*b</td>
</tr>
<tr>
<td>Volume, ml</td>
<td>96.4±9.5 124.9±9.5</td>
<td>115.6±10.8 115.6±10.8</td>
<td>435.4±23.5*b</td>
</tr>
<tr>
<td>Free plasma</td>
<td>91.2±26.3 96.8±46.0</td>
<td>91.5±37.8 89.8±44.8</td>
<td>64.0±16.2</td>
</tr>
<tr>
<td>Hemoglobin, mg/l</td>
<td>0.2±0.1 0.2±0.1</td>
<td>0.2±0.1 0.2±0.1</td>
<td>0.4±0.1*b</td>
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</table>

* n = 16 for all groups of blood products.

Another 16 units of buffy coat-poor RBCs suspended in 100 ml SAG-M solution (SAG-M: sodium chloride, 8.77 g/l; adenine, 0.169 g/l; glucose, 8.18 g/l; mannitol, 5.25 g/l) were prepared using a three-bag plastic container system including a top and bottom outlet primary bag containing 63 ml CPD (CPD: citric acid, 2.99 g/l; sodium citrate, 26.3 g/l; sodium dihydrogen phosphate, 2.51 g/l; glucose, 23.2 g/l; Optipre® No. R 1693, Baxter). Those unfiltered SAG-M RBCs were halved as well immediately after production. One half remained at room temperature for 24 h on day 3, both parts were stored at 2–6°C until day 42 (samples: days 0 before division into halves, 21, 42).

Blood Count, Free Plasma Hemoglobin and Microbiologic Examination

At the time of initial sampling on day 0 (after filtration of PAGGS-M RBCs) we performed a blood count using an automated counter (Sysmex E-4500, TOA Medical, Cobe, Japan). Free plasma hemoglobin was measured by multi-wavelength spectrophotometry [22]. The hemolysis rate at the end of storage time was calculated according to the following formula: hemolysis rate (%) = (100 – hematocrit) · free plasma hemoglobin/Hb-value where all parameters were measured at the end of storage time.

We tested for bacterial contamination in all blood components used in this study according to standard operating procedures of our transfusion service. All units proved to be sterile.

Cytokine Assays

Plasma concentrations of IL-1β, IL-6, TNF-α and IL-8 were evaluated by sensitive and specific immunoassays (R&D Systems, Minneapolis, Minn., USA). Assays were done according to the manufacturer’s recommendations. The detection thresholds were 0.1 pg/ml for IL-1β, 0.1 pg/ml for IL-6, 0.18 pg/ml for TNF-α and 18.1 pg/ml for IL-8. Standards as well as cytokine levels were assayed in duplicate. Concentrations below the detection thresholds were set to zero, and thus the means of some parameters given in table 2 arithmetically become smaller than the detection threshold.

Statistical Methods

We compared data between groups of blood products with the Mann-Whitney U-Test using SPSS® for Windows® (release 6.1.3, SPSS Inc., Chicago, Ill., USA). Comparison of subsequent values of parameters were done using the Wilcoxon matched-pairs signed-ranks test. Statistical analysis was done using nonparametric methods because cytokine levels were not normally distributed. We considered differences to be significant when p<0.05. Data are shown as mean ± standard deviation (SD) in tabular form (tables 1, 2) for clarity of presentation. Linear associations between two variables were ascertainned with Pearson’s r test. p<0.05 were considered to be significant.

Results

Leukocyte Count, Hematocrit, Platelet Count, Volume and Hemolysis Rate in RBCs and WB

Table 1 shows the volume, hematocrit, platelet count and hemolysis rate of the four different groups of RBCs and WB. RBCs were very similar to each other in these parameters, filtered RBCs showed a volume loss of about 25 ml. The volume of RBC halves was low due to the study design. WB demonstrated a significant lower hematocrit and higher platelet count and hemolysis rate than buffy-coat-poor RBCs (p<0.0005). The leukocyte count in WB was significantly higher (p<0.0005) than in all types of RBCs tested (table 1). As expected, filtered RBCs in PAGGS-M showed a WBC contamination below the detection threshold of the automated counter (table 1). WBC counts in unfiltered RBCs in PAGGS-M and in RBCs in SAG-M were similar (table 1). Looking at all 80 different blood products as a whole, WBC contamination was correlated to the cytokine levels of IL-1β (r = 0.47; p<0.0005), TNF-α (r = 0.49; p<0.005) and IL-6 (r = 0.59; p<0.0005) but not IL-8 (r = 0.06; p = 0.594) at the end of storage. Only IL-8 levels at the end of storage were slightly negative correlated to the hemolysis rate (r = −0.24; p<0.04).
Table 2. Cytokine levels in WB and RBCs during storage (mean ± SD)

<table>
<thead>
<tr>
<th>Cytokines and blood products</th>
<th>Cytokine levels during storage, pg/ml</th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>day 0</td>
<td>day 21</td>
<td>end of storage</td>
<td></td>
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<tr>
<td><strong>IL-1β</strong></td>
<td></td>
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<tr>
<td>Whole blood (CPD-A1)</td>
<td>0.3±0.5</td>
<td>4.4±2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.4±1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>RBCs (PAGGS-M) filtered&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.1±0.2</td>
<td>&lt;0.1</td>
<td>1.3±2.8&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>RBCs (PAGGS-M) unfiltered&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.0±2.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.9±2.4&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>RBCs (SAG-M) unwarmed</td>
<td>&lt;0.1</td>
<td>4.6±2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.7±2.1&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>RBCs (SAG-M) warmed&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.5±2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.1±2.3&lt;sup&gt;b,c&lt;/sup&gt;</td>
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<tr>
<td><strong>IL-6</strong></td>
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<tr>
<td>Whole blood (CPD-A1)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.6±0.9</td>
<td>0.5±0.5</td>
<td>0.5±0.3</td>
<td></td>
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<tr>
<td>RBCs (PAGGS-M) filtered&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1±0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
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<tr>
<td>RBCs (PAGGS-M) unfiltered&lt;sup&gt;f&lt;/sup&gt;</td>
<td>&lt;0.1</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
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<tr>
<td>RBCs (SAG-M) unwarmed</td>
<td>&lt;0.1</td>
<td>0.1±0.1</td>
<td>&lt;0.1</td>
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<tr>
<td>RBCs (SAG-M) warmed&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.1±0.2</td>
<td>0.1±0.2</td>
<td>0.1±0.3</td>
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<tr>
<td><strong>TNF-α</strong></td>
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<tr>
<td>Whole blood (CPD-A1)</td>
<td>0.1±0.1</td>
<td>0.4±0.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.95±0.47&lt;sup&gt;b,e&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>RBCs (PAGGS-M) filtered&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.2±0.4</td>
<td>&lt;0.18</td>
<td>&lt;0.18</td>
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<tr>
<td>RBCs (PAGGS-M) unfiltered&lt;sup&gt;f&lt;/sup&gt;</td>
<td>&lt;0.18</td>
<td>0.6±0.5&lt;sup&gt;d,e&lt;/sup&gt;</td>
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<tr>
<td>RBCs (SAG-M) unwarmed</td>
<td>&lt;0.18</td>
<td>0.2±0.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.6±0.5&lt;sup&gt;b,e&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>RBCs (SAG-M) warmed&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.7±0.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.2±0.6&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><strong>IL-8</strong></td>
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<tr>
<td>Whole blood (CPD-A1)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>1.3±5.3</td>
<td>6.1±14.3</td>
<td>1.7±7.0</td>
<td></td>
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<tr>
<td>RBCs (PAGGS-M) filtered&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.5±18.1</td>
<td>2.9±8.0</td>
<td>50.1±116.6&lt;sup&gt;d,e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>RBCs (PAGGS-M) unfiltered&lt;sup&gt;f&lt;/sup&gt;</td>
<td>10.0±14.3</td>
<td>26.8±31.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>RBCs (SAG-M) unwarmed</td>
<td>1.6±6.5</td>
<td>19.2±17.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>23.4±23.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>RBCs (SAG-M) warmed&lt;sup&gt;i&lt;/sup&gt;</td>
<td>80.5±86.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>128.5±158.6&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a</sup> n = 16 for every group of blood products.
<sup>b</sup> p<0.001 compared to day 0 values of this preparation.
<sup>c</sup> p<0.0005 compared to RBCs (PAGGS-M) unfiltered at days 21 and 49.
<sup>d</sup> p<0.05 compared to day 0 values of this preparation.
<sup>e</sup> p<0.05 compared to day 21 values of this preparation.
<sup>f</sup> Day 0 measurements were made before splitting RBCs.
<sup>g</sup> p<0.0002 at any storage time. Statistics were done for the comparison of IL-6 levels between WB and either of the other 4 preparations.
<sup>h</sup> p<0.0002 compared to RBCs (PAGGS-M) unfiltered at day 49.
<sup>i</sup> p<0.04 compared to RBCs (SAG-M) unwarmed at days 21 and 42.

**Cytokines in WB**

IL-1β and TNF-α levels in WB rose significantly during storage, while IL-6 did not (table 2). The IL-8 level in 11 of 16 units of WB remained below the detection threshold of 18.1 pg/ml during the whole storage period. One unit had an IL-8 level of 21.2 pg/ml at day 0, 3 other units showed IL-8 levels of 23.5–50.6 pg/ml at day 21 and the IL-8 level of another unit was 27.8 pg/ml at the end of the storage time. IL-6 levels in WB at any storage time were significantly (p<0.0002) higher than in all RBCs tested, where IL-6 could not be detected markedly above the detection threshold (table 2). At the end of storage, IL-8 in WB was significantly lower (p<0.003) compared to all RBC preparations. The levels of IL-1β and TNF-α in WB showed no significant differences compared to unfiltered RBCs during storage.

**Cytokines in Filtered versus Unfiltered RBCs**

IL-1β and IL-8 rose statistically significantly (p<0.001) until day 49 in filtered RBCs in PAGGS-M (table 2). TNF-α and IL-6 could be detected only initially in some PAGGS-M units before division into halves (TNF-α detectable in 5 of 16 units, IL-6: 9 of 16 units). Filtered RBCs contained significantly less IL-1β at day 21 and 49 (p<0.0005) and TNF-α at day 49 (p<0.0005) than their unfiltered PAGGS-
M counterparts, whereas IL-6 which could be detected only in 5 units of unfiltered PAGGS-M RBCs at day 49 with a maximum concentration of 0.2 pg/ml and IL-8 were not influenced by prestorage leukocyte depletion (table 2). The variance of the IL-8 concentration at day 49 in filtered RBCs (detectable in 9 of 16 units) with a range of 22–94.1 pg/ml and one outlier with 475 pg/ml was rather high. Exclusion of this outlier from the analysis did not result in a significantly lower level of IL-8 in filtered compared to unfiltered units. At the end of the storage time, IL-8 could be detected in 10 of 16 standard PAGGS-M RBCs (22.5–99.3 pg/ml).

**Cytokines in RBCs Warmed to Room Temperature for 24 h**

RBCs in SAG-M temporarily stored at room temperature (24 h at day 3) showed slightly elevated levels of TNF-α and elevated IL-8 levels (p<0.04) compared to RBCs in SAG-M permanently kept at 2–6°C at days 21 and 42 while IL-1β and IL-6 remained unaffected (table 2). IL-8 was detectable in 15 of 16 temporarily warmed units (19.3–443.7 pg/ml) compared to 11 of 16 standard SAG-M RBCs (18.4–91.3 pg/ml) at the end of the storage time.

**Cytokines in PAGGS-M RBCs versus SAG-M RBCs**

PAGGS-M and SAG-M had similar effects on cytokine levels during storage of RBCs (table 2).

**Discussion**

In this study, we investigated WB and four different preparations of buffy-coat-poor RBCs with respect to cytokine content on different days of storage. IL-1β as well as TNF-α accumulated until the end of the storage period in WB and all types of RBCs, while IL-6 could be detected at very low concentrations in a small number of units only.

Leukocyte reduction of buffy-coat-poor RBCs by filtration prevented the accumulation of IL-1β until the end of storage. This is in accordance with the findings of Stack et al. [17]. We found a similar result for TNF-α, a parameter which was not investigated by Stack et al. Corresponding results have been reported by several groups who examined the effect of leukocyte depletion on cytokine production in PRP PCs [11–13]. We could not demonstrate any reduction of IL-8 levels by filtration in RBCs at the end of the storage period, which was regularly described for filtered RBCs as well as filtered PRP PCs [11–13, 17]. However, our findings are in line with the results of Stack et al. [17] because the IL-8 levels we observed in filtered RBCs were within the range described by Stack et al. for WBC-reduced RBCs, while the IL-8 concentration in unfiltered buffy-coat-poor RBCs in PAGGS-M was about 20 times lower than that described by these authors [17]. This might be due to the lower WBC count in our buffy-coat-poor PAGGS-M RBCs, i.e. 1.9±1.2×10⁹/l compared to 4.8±3.9×10⁹/l in AS-1 RBCs described by Stack et al. [17].

A possible explanation for this observation of a basal accumulation of IL-8 in RBCs which could not be prevented by WBC elimination might be that erythrocytes could be the origin of IL-8 in RBCs. Erythrocyte antigens Fyα and Fyβ are known receptors for IL-8 [23]. There are no data on the fate of IL-8 after binding to these receptors. One possibility is that previously bound IL-8 might be released from these receptors during storage of RBCs or WB. This would also explain why IL-8 accumulation could not be completely prevented by filtration of PCs. This hypothesis is supported by the findings of Darbonne et al. [23] that the binding of IL-8 to erythrocytes is rapidly reversible and does not result in receptor internalization.

Permanent storage of RBCs at 2–6°C cannot always be warranted under clinical routine conditions. We made an attempt to investigate whether temporary storage of RBCs at room temperature might influence the production of cytokines in RBCs and therefore might increase the risk of febrile transfusion reactions. RBCs (SAG-M) temporarily stored at room temperature for 24 h, a period that should never be reached under clinical conditions, an extreme worst-case scenario, nevertheless, showed unaffected IL-1β and IL-6 levels, whereas TNF-α was slightly elevated. Only IL-8 demonstrated an increase with peak levels up to 513 pg/ml. But all these concentrations we found in warmed RBCs are within the range of those earlier described in standard RBCs [17]. We assume, that there is no increased risk of febrile transfusion reactions due to the production of proinflammatory cytokines in RBCs which were warmed for periods much shorter than 24 h under clinical routine conditions. Of course, every longer-lasting warming of RBCs should be avoided to minimize the risk of bacterial growth.

We found no difference in cytokine kinetics between buffy-coat-poor RBCs stored in two different additive solutions. RBCs stored in PAGGS-M and SAG-M showed similar WBC counts and similar cytokine concentrations until the end of storage. The main difference was a 49-day storage period for PAGGS-M RBCs compared to 42 days for SAG-M RBCs. But the cytokine levels at day 21 which were directly comparable showed no advantage for either product.
The use of WB in homologous transfusion practice is obsolete. However, in Germany the use of WB is common in preoperative autologous blood donation, especially in smaller hospitals due to the financial and technical demands of WB fractionation into RBCs and fresh frozen plasma [19]. We therefore aimed to investigate whether there should be concerns about cytokine production in WB, where no WBC depletion during preparation occurs. We found only slight differences in IL-1β, IL-6 and TNF-α levels during storage of WB compared to standard RBCs despite a significantly higher WBC contamination. Surprisingly, no IL-8 could be detected in 11 of 16 units during the whole storage period, a result which is significant at the end of storage compared to unfiltered RBCs. We have no explanation for this surprisingly low cytokine generation of passenger WBC in WB. The observed amounts of cytokine accumulation in stored WB give no arguments against the usage of WB in autologous blood transfusion. The moderate but significant elevated hemolysis rate of WB compared to RBCs was completely within the maximum of 0.8% specific for RBCs in modern international guidelines [21].

It was criticized that the usage of ELISAs for cytokine measurement may have serious limitations [14]. The concentration of a cytokine as shown by immunoassays may not necessarily represent functional activity, since ELISAs can detect not only active molecules but also cytokine-receptor complexes, denatured or degraded material or precursor proteins with minimal or no biological activity [14]. It has been claimed that the biological potential of cytokine levels measured by ELISAs in stored blood products should be confirmed by means of bioassays. Since bioassays for cytokine measurement often show poor specificity and sensitivity and their handling is cumbersome, nearly all workers dealing with cytokine measurements in blood products avoided bioassays [2–9, 11–13, 15–18]. Nevertheless, levels of IL-1β, IL-6 and TNF-α we measured in WB and RBCs using ELISAs were 10–1,000 times lower compared to those described in PRP PCs and are comparable to levels found in filtered PRP PCs or in BC PCs [3–5, 7–14, 16]. Using ELISAs, we might have overestimated the biological activity of cytokines in RBCs and WB. In this case, the risk of an acute transfusion reaction would be even lower compared to PRP PCs, if administration of proinflammatory cytokines by transfusion contributes to such a reaction at all. We are not able to estimate the clinical significance of IL-8 levels of up to 513 pg/ml found in RBCs with an intercurrent 24-hour period of room temperature storage. We found no dose-response studies for the effects of IL-8 in man in the literature. IL-8 causes transient granulocytopenia followed by prolonged granulocytosis in nonhuman primates and lung injury in rats after repeated infusions [24, 25]. With transfusion of RBCs or WB, IL-1β levels will hardly reach concentrations of 1–10 ng/kg, which have been described to cause mild clinical symptoms, such as a low-grade fever, rigor or a transient increase in heart rate, or concentrations of 10–100 ng/kg, which have been reported to cause fever, insomnia, myalgias and arthralgias [26, 27]. Doses of 1,000 ng/m² TNF have been described to cause fever and rigor [28]. Such concentrations will hardly be reached by the transfusion of RBCs or WB with the TNF-α concentrations described above. At this time, we conclude that there is no reason for prestorage filtration of RBCs or WB to avoid cytokine accumulation. However, these low levels of IL-1β, IL-6 and TNF-α and IL-8, which might be negligible as monosubstances, might exert synergistic effects in conjunction with each other and could, besides the generation of nonhemolytic transfusion reactions, and possibly aggravate systemic inflammatory response after trauma or infection in critically ill patients, especially following massive transfusion. Clinical studies are necessary to evaluate this hypothetical risk associated with the administration of proinflammatory cytokines via blood component transfusion. Nevertheless, we conclude that, according to present knowledge, there is no reason for prestorage filtration of buffy-coat-poor RBCs or WB to avoid febrile transfusion reactions due to cytokine accumulation during storage. There might be other rationales for prestorage filtration of buffy-coat-poor RBCs, for example to avoid bedside filtration, which lacks every quality control and provides inadequate leukocyte reduction in many cases [29].
References


