Proteomic analysis of supernatant from pooled buffy-coat platelet concentrates throughout 7-day storage

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BACKGROUND: The platelet (PLT) storage lesion remains incompletely understood. To gain a greater insight into the PLT storage lesion, a proteomic analysis of supernatant from leukofiltered pooled buffy-coat PLT concentrates (PCs) was undertaken.

STUDY DESIGN AND METHODS: PCs were prepared in PLT additive solution and stored according to standard blood bank procedures. Supernatant samples were collected throughout 7 days of storage. Maps of supernatant proteins were generated by two-dimensional (2D) gel electrophoresis and mass spectrometry. Cytokine antibody microarrays and enzyme-linked immunosorbent assay were used to investigate bioactive molecules.

RESULTS: The 2D gel maps of PC supernatant proteins displayed many features of plasma protein maps. Several storage-induced protein changes were identified including modifications to major plasma proteins. PLT-derived proteins were also identified, including tremlike transcript 1 and integrin-linked kinase, which may influence PLT–endothelium interactions. Cytokine antibody microarrays revealed a number of bioactive proteins that have not been previously associated with PCs produced for transfusion, such as brain-derived neurotrophic factor (BDNF). The concentration of PLT-derived cytokines including BDNF, CXCL7, epidermal growth factor, PLT-derived growth factor (PDGF), and CCL5 significantly increased during storage of PCs. Extended storage from Day 5 to Day 7 caused significantly increased levels of BDNF, PDGF, and CCL5 in PC supernatant.

CONCLUSION: Proteomic techniques provide valuable new insight into the effects of storage on PCs and the contribution of soluble proteins to the development of the PLT storage lesion and recipient responses to PLT transfusion.

P latelet (PLT) transfusions are associated with a relatively high incidence of adverse transfusion reactions. Prestorage leukoreduction of PLT concentrates (PCs) has reduced the incidence of adverse transfusion reactions, but has not completely eradicated their occurrence, suggesting that factors other than white blood cells (WBCs) are involved. Removal of the supernatant from PCs before transfusion has been shown to significantly reduce adverse reactions. This suggests that soluble factors such as plasma proteins and bioactive molecules released from PLTs (and WBCs) during storage of PCs contribute to adverse transfusion reactions. The risk of an adverse transfusion reaction has also been correlated with the length of storage of the PC before transfusion. This is likely to be due in part to the progressive release of cytosolic and granular contents from degraded PLTs, which contributes to the “PLT storage lesion.” Depending on the procedure used for the preparation of a PC, the supernatant can be composed entirely of plasma or can be diluted with PLT additive solution (PAS) to yield a suspension medium containing 25 to

ABBREVIATIONS: 2D = two dimensional; BDNF = brain-derived neurotrophic factor; CTAP-III = connective tissue-activating peptide III; EGF = epidermal growth factor; ILK = integrin-linked kinase; NAP-2 = neutrophil-activating peptide (CXCL7); PAS = platelet additive solution; PC(s) = platelet concentrate(s); PDGF-BB = platelet-derived growth factor BB; pI = isoelectric point; TLT-1 = tremlike transcript 1; VEGF = vascular endothelial growth factor.

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30 percent plasma. A purported benefit of PAS is the reduced amount of donor plasma incidentally transfused with the PLTs, which may decrease adverse events associated with transfused plasma derived factors. Previous work to identify the soluble factors that may contribute to adverse PLT transfusion reactions and/or the PLT storage lesion have focused on a limited range of candidate molecules known, or expected, to be present in PCs.

In contrast to investigations of specific known proteins, proteomic techniques such as two-dimensional (2D) gel electrophoresis have the capacity to provide a global map of proteins present in PCs in a nontargeted manner. Consequently, proteomic techniques have the potential to identify proteins that have not been investigated in PCs or PLTs previously or to identify entirely novel proteins (D.W. Greening, K.M. Glenister, E.A. Kapp et al., submitted). Although several proteomic investigations of PLT proteins have been published, these have generally focused on the proteins present in lysates of PLTs or in the releasate from artificially activated PLTs. Few studies have used proteomic techniques to examine proteins present in PCs produced for transfusion. Two early studies applied 2D gel electrophoresis analysis of PCs to detect changes in the protein maps of PLTs after irradiation or storage. Significant technologic advancements in proteomics since these early studies now enables systematic identification of specific proteins that was not previously possible.

Although very useful, proteomic techniques can be restricted in their ability to detect low-abundance proteins, particularly in the presence of highly abundant proteins, which is typical in biologic samples. Cytokine antibody microarrays are a relatively new technique that have the potential to complement traditional 2D gel electrophoresis-based proteomic investigations because of their ability to detect dozens of low-molecular-weight and low-abundance proteins simultaneously.

The aim of this study was to construct maps of the proteins present in the supernatant of stored pooled random-donor leukoreduced buffy-coat PCs, by use of 2D electrophoresis and mass spectrometry. PCs prepared by the buffy-coat method, according to standard procedures. Briefly, whole blood was centrifuged (4200 x g, 10 min, 22°C) and the buffy coat was obtained by an automated blood component separator (Optipress II, Baxter). Buffcoats were rested at room temperature for between 3 and 16 hours before pooling. Buffcoats from five ABO-matched donors were pooled and mixed with 300 mL of PAS (PAS-II, T-sol, Baxter) and centrifuged (445 x g, 7.5 min, 22°C). The PLT-rich supernatant was leukoreduced by filtration (OptiPure PC PLX-5, Baxter) and collected into gas-permeable 1.3-L storage bags (PL2410, Baxter). PCs were stored on a horizontal reciprocating agitator set at 50 to 70 oscillations per minute (linear PLT reciprocator, Melco, Glendale, CA) at 22°C and sampled aseptically on Days 1, 3, 5, and 7 (n = 2) or Day 1 only (n = 5), Day 6 only (n = 10), or Day 7 only (n = 6).

Residual WBC counts in the PCs on Day 1 were determined by a flow cytometric absolute counting assay with test tubes (TruCOUNT, BD Biosciences, San Jose, CA). At each sampling time pH was determined (22°C, PHM210, Radiometer, Brønshøj, Denmark), and blood cell counts were performed with an automated hematology analyzer (CellDyn 3200, Abbott, Santa Clara, CA). The mean PLT count was 3.1 x 10¹¹ ± 0.2 x 10¹¹ per unit and WBC counts were less than 1 x 10⁶ per unit. The pH was maintained between 6.8 and 7.2 from Days 1 through 6 of storage, and only 1 PC unit displayed a pH outside this range on Day 7 (pH 6.7). Except for this latter PC all PCs met quality acceptance criteria, as defined by the Council of Europe guidelines for blood components. Samples of PCs were centrifuged (1000 x g, 15 min, 22°C) to separate supernatant and cellular fractions. The upper portion of supernatant was collected to avoid contamination with PLTs and stored in aliquots at –80°C. Supernatant total protein content was determined by a Bradford protein assay with bovine serum albumin as a standard (Bio-Rad, Hercules, CA). Microbial screening was performed on repeatedly sampled PCs at the end of storage and all PCs tested were found to be negative for microbial contamination.

### 2D gel electrophoresis

Supernatant samples from PCs were precipitated with ice-cold methanol (10× volume at –80°C for ≥16 hr). The precipitated protein pellets were air-dried and prepared for 2D gel electrophoresis as previously described. Equivalent amounts of total protein (1.5 mg) of each PC supernatant sample were loaded onto each gel. Broad-range 2D gel electrophoresis (isoelectric point [pI] 3-10, 10-100 kDa) was used to construct maps of the proteins present in the PC supernatant throughout storage, and narrower-range electrophoresis (pI 4-7, 10-100 kDa) was performed to achieve greater resolution of the acidic to neutral region. The pH 3 to 10 and 4 to 7 immobilized pH gradient strips were isoelectrically focused for 25,000
Mass spectrometry and protein identification

Mass spectrometry was performed as described previously. Proteins were identified with a search engine (Mascot, Matrix Science, Boston, MA) and database (NCBInr, Version 29_9_04, containing 2,052,411 sequences, 688,443,072 residues). The criteria applied for protein identification included matches limited to human proteins, tandem mass spectrometry scores greater than 50, and at least three unique peptides.

Cytokine antibody microarray

The cytokine antibody microarray, preloaded with 79 different cytokine antibodies (human cytokine array V) was purchased from RayBiotech (Atlanta, GA). Supernatant samples (1 mL, diluted 1:10 in blocking buffer) from Day 1 and Day 5 PCs were incubated with the microarray membranes, and the bound cytokines were detected according to the manufacturer’s instructions. Cytokine spots that differed in intensity between the Day 1 and Day 5 samples, and those spots that appeared to be saturated were selected for quantification by ELISA.

ELISA

The concentration of cytokines in the supernatant of PCs (n ≥ 6) were determined in duplicate by sandwich ELISA according to manufacturer’s instructions (R&D Systems, Minneapolis, MN). Stated assay ranges were angiogenin, 7.8 to 500 pg per mL; brain-derived neurotrophic factor (BDNF), 39.1 to 2500 pg per mL; epidermal growth factor (EGF), 3.9 to 250 pg per mL; interleukin-6 (IL-6), 9.4 to 600 pg per mL; interleukin-8 (IL-8 or CXCL8), 31.3 to 2000 pg to mL; interleukin-10 (IL-10), 62.5 to 4000 pg/mL; neutrophil-activating peptide (NAP-2 or CXCL7), 15.6 to 1000 pg per mL; pulmonary and activation-regulated chemokine (or CCL18), 7.8 to 500 pg per mL; PTLD-derived growth factor BB (PDGF-BB), 31.3 to 2000 pg/mL; regulated upon activation, normal T-cell expressed and presumably secreted (RANTES or CCL5), 15.6 to 1000 pg per mL; tumor necrosis factor-α (TNF-α), 15.6 to 1000 pg per mL; and vascular endothelial growth factor (VEGF), 31.25 to 2000 pg per mL. The mean normal plasma level of these cytokines was estimated from segments of fresh whole blood–derived plasma units prepared from healthy volunteer blood donors (n ≥ 16).

Statistical analysis

Results are shown as the mean ± standard error. Between-group comparisons were determined by unpaired t tests, rank sum U tests, or one way analysis of variance as appropriate (Sigma-Stat, Version 3.0, Systat, Richmond, CA). Significance was defined as p < 0.05.

RESULTS

Protein content and 2D gel analysis

The total protein content of the PC supernatants remained constant throughout the storage period (15.8 ± 0.5 mg/mL on Day 1 and 15.5 ± 0.5 mg/mL on Day 7) and is in agreement with the estimated plasma content of approximately 22 to 26 percent of the total supernatant volume. The contribution of plasma proteins was evident by the 2D gel maps of PC supernatant (Fig. 1), which showed strong similarity to previously published 2D gel maps of human plasma.

The number of protein spots resolved by the broad range (pH 3-10) 2D gels increased gradually throughout storage but did not reach significance (66 ± 14 on Day 1 and 87 ± 12 on Day 7). The enhanced resolution of proteins by the acidic range (pH 4-7) 2D maps (Fig. 1B compared to Fig. 1A) was evident by the increased number of protein spots observed (for example, 118 ± 9 protein spots were resolved by acidic range 2D gels of Day 1 supernatant compared to 66 ± 14 protein spots resolved by broad range 2D gels; p = 0.03). The number of protein spots resolved by the acidic-range 2D gels, however, did not change significantly across the storage period (118 ± 9 on Day 1 and 104 ± 14 on Day 7).

Protein identification

Mass spectrometry was used to identify selected proteins present in PC supernatant resolved by 2D gel electrophoresis. In particular, proteins that changed in abundance during storage were selected for identification by mass spectrometry, along with distinctive proteins that served as landmark proteins including haptoglobin and albumin. A number of proteins, such as clusterin (Fig. 1B Spots 6, 33, and 34) were present as “trains,” indicated by incremental shifts in pI. Several of these proteins trains displayed storage associated changes, often characterized by one member of the train increasing in intensity, while other members decreased. In these instances, one member of the train was selected for identification. As expected, many of the proteins identified were high-abundance plasma proteins such as albumin, haptoglobin, and lipoproteins (Table 1). Many fragments of albumin were identified, with molecular weights ranging from approximately 20 to 66 kDa and pIs ranging from approximately pI 5.5 to pI 8.5 (Table 1; Fig. 1). Based on
peptide sequence data, the albumin fragments were derived from both the C-terminus and the N-terminus regions of the albumin precursor protein. PLT-derived proteins identified by 2D gel analyses included clusterin (Spots 6, 33, and 34), connective tissue–activating peptide III (CTAP-III; Spot 11), tremlike transcript-1 (TLT-1; Spot 36), and integrin-linked kinase (ILK; Spot 37) (Table 1; Fig. 1). Hemoglobin (Spots 12 and 20), presumably from hemolyzed passenger red blood cells (RBCs), was also identified (Table 1; Fig. 1).

Cytokines
To augment the 2D gel–mass spectrometry approach, cytokine antibody microarrays and ELISA were applied to identify bioactive proteins in the supernatant of stored PCs, which if present, would likely be in low abundance and may not be readily detected by 2D gel analysis. Figure 2 shows a representative cytokine antibody microarray analysis of Day 1 PC supernatant. It is important to note that due to the wide differences in sensitivity of detection of individual cytokines by the cytokine antibody microarray, the intensity of different cytokines cannot be compared and does not correlate with relative abundance. Numerous cytokines were identified in the supernatant of stored PCs, including cytokines that have been reported previously (e.g., CCL5, CXCL7/β-thromboglobulin, TNF-α, transforming growth factor [TGF]-β, as reviewed by Heddle21), along with other cytokines that have been less extensively investigated in stored PCs (e.g., BDNF, EGF, PDGF-BB, CCL18, angiogenin). Cytokines that exhibited increases in spot intensity by microarray screening from Day 1 to Day 5 of storage or showed saturated signals were quantitated by ELISA (Table 2).

Quantitation by ELISA of PLT-derived cytokines, EGF, CXCL7, PDGF-BB, and CCL5 showed significant accumulation in the supernatant from Day 1 to Day 6 (p < 0.0001, p = 0.0012, p < 0.001, and p = 0.0008, respectively; Table 2). PCs stored for an additional 1 to 2 days (i.e., to Day 7) showed further significant increases in the amount of BDNF, PDGF-BB, and CCL5 compared to levels on Day 6 (p < 0.05). VEGF was detectable by ELISA but was not detectable by the cytokine antibody microarray. The concentration of VEGF in the supernatant did not significantly change during storage of PCs. Similarly, the concentration of WBC-derived cytokines IL-6, CXCL8, IL-10, and TNF-α did not significantly change during storage of PCs (Table 2). An exception was the significant increase in the concentration of TNF-α in the supernatant of PCs on Day 7 of storage (p = 0.038).

Compared with normal plasma, all of the PLT-derived cytokines quantitated in this study (BDNF, EGF, PDGF-BB, CCL5, VEGF) showed significantly increased levels on Day 7 of storage (i.e., 41- to 404-fold increase, p < 0.05; Table 2). In contrast, the WBC-derived cytokines (IL-6, IL-8/CXCL8, IL-10, TNF-α) and plasma-derived cytokines (angiogenin and pulmonary and activation-regulated chemokine/CCL18) showed much smaller fold change compared to normal plasma, and for some cytokines (i.e., IL-6, angiogenin, and CCL18), the concentration was
| Identification number* | Protein identity | gi and Swiss-Prot numbers† | Unique peptides/total peptides‡ | MS/MS score§ | Mass (kDa)||| Isoelectric point||| Predominant source | Comment |
|------------------------|------------------|-----------------------------|-------------------------------|--------------|------------|--|--|------------|-----------|----------------------|---------|
| 1, 10                  | Proapoliprotein   | gi178775 P02647             | 15-20/23-34                  | 548-743      | 28.1       | 5.27 | Plasma      | Transport/binding protein |
| 2, 13, 14, 15, 19, 25, 31 | Albumin and fragments | gi4389275 P02768 | 7-17/10-33                  | 349-2842     | 66.5       | 5.67 | Plasma      | Hemostatic protein |
| 3                      | Haptoglobin       | gi223976 P02768             | 6/15                          | 320          | 43.3       | 6.13 | Plasma      | Scavenge protein |
| 4                      | Apolipoprotein D  | gi619383 P05090             | 6/21                          | 282          | 19.3       | 5.20 | Plasma, synthesized by liver | Transport/binding protein |
| 5, 16, 18, 26          | Haptoglobin Hp2   | gi223976 P02768             | 4-5/7-11                      | 160-690      | 41.7       | 6.13 | Plasma      | Scavenge protein |
| 6, 33, 34              | Apolipoprotein J, clusterin, SP40, 40 | gi178855 P10909 | 7-9/9-24                    | 335-394      | 48.8-50.1  | 5.89 | PLTs        | Complement lysis inhibitor |
| 7, 17                  | Haptoglobin precursor | gi67586 P00737 | 5-12/8-22                    | 151-508      | 38.4-43.3  | 6.13 | Plasma      | Scavenge protein |
| 8, 9                   | Fibrinogen γ      | gi223170 P02679             | 13-20/16-32                   | 452-947      | 48.5       | 5.24 | Plasma      | Coagulation |
| 11                     | CTAP-III          | gi208127 P02775             | 7/15                          | 350          | 9.9        | 7.84 | PLTs        | Chemokine, neutrophil activator |
| 12, 20                 | Hemoglobin β      | gi4504349 P68871            | 3-14/3-27                     | 128-691      | 15.9-16.0  | 6.81 | Red cells   | Transport/binding protein |
| 21, 23                 | β-2 Glycoprotein 1 (apolipoprotein H) | gi6573461 P02749 | 12-19/15-27                  | 515-724      | 36.3       | 8.37 | Plasma, synthesized by liver | Inhibits coagulation cascade |
| 22, 24                 | β-Fibrinogen      | gi182430 P02675             | 19-28/24-36                   | 958-1457     | 50.8       | 7.95 | Plasma      | Coagulation factor |
| 27, 28                 | Transthyretin      | gi899653 P02766             | 5-15/11-54                    | 218-1222     | 13.8       | 5.35 | Primarily liver | Transport/binding protein |
| 29, 30                 | Lipoprotein C-III | gi224917 P02656             | 5-8/12                        | 329-338      | 8.8        | 4.72 | Plasma      | Transport/binding protein |
| 32                     | α1-Antitrypsin    | gi1942629 P01009            | 30/66                         | 1637         | 44.3       | 5.37 | Plasma      | Secreted, binding protein. Serpin |
| 35                     | Thioredoxin       | gi1827674 P10959            | 5/10                          | 222          | 11.6       | 4.82 | Ubiquitous  | Oxidoreductase enzyme |
| 36                     | TLT-1             | gi28261401 Q86YW5           | 3/5                           | 215          | 32.9       | 5.88 | PLTs/ megakaryocytes | Regulation of α granule dispersal |
| 37                     | Integrin-linked kinase | gi7828031 P57043 | 11/40                        | 626          | 51.4       | 8.30 | PLTs, plasma microparticles | Serine threonine kinase |

* The identification numbers correspond to the numbers shown in Fig. 1.
† The gi (genInfo) number is a unique identifier for an exact protein sequence. Swiss-Prot number: http://www.expasy.org/tools/.‡ Multiple forms of each peptide (e.g., glycosylated, oxidized, alkylated, or nonmodified) were counted independently (shown as number of total peptides) or collectively (shown as unique peptides) for each protein.
§ The MS/MS (tandem mass spectrometry) score is the probability that the match between the query and the entry in the database is nonrandom. A score of more than 50 was considered to indicate a good candidate.
|| Information is for the intact protein and therefore may not correspond to the location of the spots shown in Fig. 1.
Fig. 2. A representative cytokine antibody microarray incubated with supernatant from a PC sampled on Day 1 of storage. The key shows the position, identity, and intensity of reaction (by visual analysis) of the cytokines present on the array.

### TABLE 2. Levels of cytokines present in the supernatant of PCs during storage

<table>
<thead>
<tr>
<th>Cytokine/chemokine</th>
<th>Cytokine concentration (ng/mL)</th>
<th>Fold increase of Day 7 PC over plasma levels</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PLT-derived:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF</td>
<td>Day 1 PC (n = 6) 20.2 ± 0.2</td>
<td>Day 6 PC (n = 10) 27.0 ± 0.7</td>
</tr>
<tr>
<td>EGFR</td>
<td>0.15 ± 0.02</td>
<td>0.77 ± 0.06*</td>
</tr>
<tr>
<td>CXCL7</td>
<td>3.200 ± 600</td>
<td>10,200 ± 900*</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>2.2 ± 0.3</td>
<td>4.1 ± 0.3*</td>
</tr>
<tr>
<td>CCL5</td>
<td>124.2 ± 19.4</td>
<td>261.7 ± 23.8*</td>
</tr>
<tr>
<td>VEGF</td>
<td>0.09 ± 0.02</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td><strong>WBC-derived:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>0.01 ± 0.00</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>CXCL8</td>
<td>0.04 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.29 ± 0.03</td>
<td>0.39 ± 0.02*</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.25 ± 0.13</td>
<td>0.38 ± 0.12</td>
</tr>
<tr>
<td><strong>Plasma-derived:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiogenin</td>
<td>27.9 ± 16.2</td>
<td>75.3 ± 12.9*</td>
</tr>
<tr>
<td>CCL18</td>
<td>12.9 ± 1.0</td>
<td>12.1 ± 0.9</td>
</tr>
</tbody>
</table>

* Significant increase compared to Day 1, p < 0.05.
† Significant increase from Day 6 to Day 7, p < 0.05.
‡ Significant increase from plasma to Day 7 PC, p < 0.05.
lower than normal plasma, consistent with plasma dilution by PAS.

**DISCUSSION**

In this study 2D gel-based proteomics was used to survey the proteins present in the supernatant of leukoreduced pooled buffy-coat PCs stored in PAS for up to 7 days. The use of PAS reduces the plasma content of buffy-coat PCs to approximately 22 to 26 percent volume:volume; however, the 2D gel maps of PC supernatant showed strong resemblance to plasma maps throughout storage. 2D gel analysis revealed storage-induced changes that were evident within the clusters or trains of highly abundant plasma proteins including albumin, α1-antitrypsin, lipoprotein C-III, and haptoglobin. These storage associated changes in protein trains may reflect altered metabolism or oxidation. Fragmentation of albumin, as noted in our earlier studies of RBC product supernatant, was also observed during storage of PCs. The biological significance of these modifications and fragmentation of proteins during the storage of PCs is not known.

Several PLT-derived proteins were identified by mass spectrometry including clusterin, TLT-1, and ILK. Clusterin (also known as apolipoprotein J or SP40, 40) is a complement lysis inhibitor released from α-granules and transferred to the extracellular PLT membrane. TLT-1 is stored within the α-granules of resting PLTs and upon activation translocates to the cell surface where it may have a role (in conjunction with CD62P) in the adhesion of PLTs to endothelium. ILK interacts with the β subunit of the PLT integrin αβ1 and may influence PLT aggregation and adhesion to damaged endothelium. The biologic significance of the release of these PLT membrane-associated proteins into the supernatant during storage of PCs is not known.

The PLT-derived chemokine CXCL7 was also identified from the 2D gel maps. CXCL7 is a series of four progressively truncated forms of PLT basic protein (PBP; CTAP-III, β-thromboglobulin, and NAP-2). These proteins are very similar in size and amino acid composition and are therefore difficult to distinguish by biochemical or immunologic methods. Mass spectrometry was unable to distinguish which form(s) were present in the PC supernatant. Additionally, the CXCL7/NAP-2 ELISA used in this study almost certainly detects PLT basic protein and its truncated derivatives. As each of these proteins displays distinct and diverse biologic activity, it would be valuable to identify which form(s) accumulate in stored PCs. For example, NAP-2 is the only form of CXCL7 that possesses neutrophil-activating and chemotactic effects.

The usefulness of the cytokine antibody microarray as a screening tool was demonstrated in this study. Several of the PLT-derived cytokines that have previously been reported in PCs were identified by the cytokine antibody microarray, including CCL5, CXCL7, and TGF-β, in addition to other bioactive proteins that have not been as widely investigated in stored PCs and may be of potential biological relevance, including BDNF, EGF, and PDGF-BB.

The concentration of all the PLT-derived factors quantitated in this study (i.e., BDNF, EGF, PDGF-BB, CCL5, and CXCL7) increased significantly during 7-day storage and were present at levels significantly higher than normal plasma. These PLT-derived factors have a diverse range of potent biologic modulating potential, including effects on immune responses, cell chemotaxis, and proliferation. Of the PLT-derived cytokines quantitated, the concentration of BDNF and CCL5 showed the greatest fold increase compared to normal plasma levels. Both of these cytokines have been implicated in allergic responses. Only recently have PLTs been identified as a major source of BDNF. To our knowledge this is the first study to report on the levels of BDNF in the supernatant of stored PLT transfusion products. Further studies are required to understand the clinical significance of BDNF in PLT transfusion. The levels of CCL5 in the supernatant of our PCs stored in PAS-II were comparable to those reported for PAS-III–stored PCs and are sufficient to elicit proinflammatory responses, although were not as high as those reported for PCs implicated in allergic reactions. Other PLT-derived factors, such as EGF, VEGF, and PDGF, which were found to accumulate in the supernatant during storage of our PCs, are potent growth factors, particularly of nonhematopoietic tissues. This attribute of PLTs has prompted other investigators to consider PCs as a novel therapeutic for wound repair and tissue regeneration.

Extension of the storage period by 24 to 48 hours (i.e., to Day 7) resulted in significantly increased levels of BDNF, CCL5, and PDGF-BB in the PC supernatant, suggestive of progressive leakage from or degradation of PLTs. These findings are relevant in light of the current discussions around the safety and efficacy of extending the shelf life of PCs to 7 days instead of the present 5-day expiry, which primarily have focused on concerns such as the increased risk of bacterial growth.

The concentrations of the WBC-derived cytokines in the supernatant did not significantly change during storage of the PCs, which is consistent with findings reported previously for prestorage-leukofiltered, pooled random-donor PCs. Compared to normal plasma, the concentrations on Day 7 of the proinflammatory cytokines CXCL8 and TNF-α and the immunosuppressive cytokine IL-10 were significantly increased, although whether clinically significant is unclear. These increased levels of WBC-derived cytokines may be due to the buffy-coat manufacturing method, which requires the buffy coats to be rested for 3 to 16 hours before the final steps of pooling, fractionation, and leukofiltration of the PLTs.

In conclusion, with an integrated proteomics approach numerous qualitative and quantitative changes...
were seen in the proteins present in the supernatant of PCs during storage. These findings provide further insight into the effects of storage on PLTs and the mechanisms of the PLT storage lesion. Of note was the progressive accumulation of PLT-derived bioactive mediators in the supernatant after storage of PCs for an extended period to 7 days compared to the current 5-day shelf life. The implications for transfusion recipients receiving PCs that contain significant amounts of storage-induced modified proteins and soluble bioactive molecules requires further investigation and may provide an explanation for the continuing occurrence of adverse transfusion reactions seen with prestorage-leukoreduced PCs.

REFERENCES


