In vitro affinity reduction of biologic response modifiers from production buffy coat platelets exposed to recombinant protein receptors

Per Sandgren,[1] Jenny Rönnmark,[3] and Jonas Axelsson[2,3]

BACKGROUND: Recent studies link biologic response modifiers found in donor platelet (PLT) concentrates to transfusion reactions. We tested a novel method to deplete BRMs from PLT concentrates using apheresis.

STUDY DESIGN AND METHODS: Whole blood from 25 donors was treated to yield PLTs for in vitro measurements on Days 2, 5, and 7. On Day 7, PLTs were filtrated through columns with either antibody-coated agarose or rh-megalin bound to antibody-coated agarose. In addition, we also tested the naked matrix (agarose) and another apheresis surface containing rh-cubilin bound to agarose. Megalin and cubilin are parts of the protein complex mediating BRM endocytosis in the human kidney.

RESULTS: Compared to before filtration ($951 \times 10^9 \pm 41 \times 10^9$ cells/L), PLT numbers decreased slightly after filtration over both naked ($859 \times 10^9 \pm 38 \times 10^9$) and antibody-coated ($848 \times 10^9 \pm 41 \times 10^9$) matrices (both $p < 0.001$ vs. before). Concentrations of interleukin (IL)-1β, IL-12 (p40), IL-12 (p70), and IL-7 all decreased by approximately 40% even in the absence of a recombinant surface. After filtration over rh-cubilin, but not rh-megalin, concentrations of IFN-γ, IL-1β, tumor necrosis factor-α, IL-12, and IL-7 all further decreased by 30% to 50%.

CONCLUSION: In a pilot study of in vitro apheresis to deplete BRMs, we found that cell numbers and function remained largely unaffected by filtration. Significant reductions in BRMs occurred already with agarose. However, apheresis with the multiligand receptor rh-cubilin was able to further decrease concentrations.

It is well known that currently used protocols for the preparation and storage of platelet (PLT) concentrates from donor blood induce the release of biologic response modifiers (BRMs).[1-3] Consisting of both cytokines and other biomolecules, these BRMs are thought to activate autocrine and paracrine pathways to influence key cellular functions of both donor and recipient cells.[4-8] Of importance, recent data link BRMs to adverse transfusion reactions after human PLT concentrate therapy.[4,7,9-12] Register studies suggest that transfusion reactions occur in up to 5% of PLT treatments,[13,14] with a higher incidence in high-risk populations such as pediatric patients.[15] Most of these are likely to resolve relatively quickly, but their occurrence is associated with both adverse outcomes[4,16,17] and longer hospital stays.[18,19]

In this study, we hypothesized that a reduction or removal of PLT concentrate BRMs could be achieved

ABBREVIATIONS: BRM(s) = biologic response modifier(s); Cub matrix = cubilin-coupled Sepharose; ESC = extent of shape change; HBS = HEPES-buffered saline; HSR = hypotonic shock response; Meg-Ab matrix = megalin-coupled agarose; RANTES = regulated upon activation of normal t-cells expressed and secreted; sCD40L = soluble cluster of differentiation 40 ligand; WB = whole blood.

From the [1]Department of Clinical Immunology and Transfusion Medicine and the [2]Department of Medical Biochemistry and Biophysics, Karolinska University Hospital and Karolinska Institutet; and the [3]JJK Medical Development AB/South End Advisory AB, Stockholm, Sweden

Address reprint requests to: Dr Per Sandgren, Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital and Karolinska Institutet, 141 86 Stockholm, Sweden; e-mail: per.sandgren@karolinska.se.

Received for publication November 4, 2014; revision received January 16, 2015; and accepted January 16, 2015.

doi:10.1111/trf.13051

TRANSFUSION 2015;55;1919–1926

using a novel apheresis matrix based on recombinant versions of one of two receptors responsible for the removal of cytokines from the primary urine in vivo.[20] In a pilot study, we aimed to demonstrate that such a technology to remove cytokines would not adversely affect cell quality or remove a large number of PLTs as has been reported with current filtration techniques.[21]

MATERIALS AND METHODS

Preparation, storage, and sampling of PLTs

PLTs were collected (Day 0) from ambulatory blood donors who met standard donation criteria and who had given prior informed consent according to institutional guidelines. The local ethical review committee approved the study before initiation. To remove the possibility of identification using the assigned 13-digit international bottling number (ISBT128; indicating the country, blood center, and a serial number) this was removed from study units immediately after negative virus testing and replaced with an anonymous study number. Initially, a total of 450 mL of whole blood (WB) was drawn into either a CPD and SAG-M quadruple-bag blood container system (Fenwal, La Châtre, France) or another blood bag system (NPT 6280LE, MacoPharma, Mouvaux, France). After storage at room temperature for 2 to 6 hours, WB units were centrifuged (2700 × *g*) for 10 minutes at 22°C. Automatic equipment was used for the preparation of blood components (Optipress, Fenwal; or Macopress Smart, MacoPharma), including the buffy coat (BC) of 25 blood units.

All BC units were mixed in pools of 5 (BC pool volume range, 216 ± 3 mL) and prepared on Day 1 by using a BC PLT processing system (OrbiSac, TerumoBCT, Zaventem, Belgium)[22] to yield leukoreduced PLT units stored in approximately 30% plasma and 70% SSP+ (MacoPharma). The maximum *g*-force generated by the OrbiSac system is 1196 × *g*. To avoid disintegration and adverse negative effects on the PLTs,[23] the air and foam were excluded from all units immediately after preparation. All units were then stored on a flatbed agitator in a temperature-controlled incubator at 22 ± 2°C (Model PC900i, Helmer, Noblesville, IN). A (1 × 10)-mL PLT concentrate sample was collected from each unit on Days 2 and 5. On Day 7, a (2 × 10)-mL PLT concentrate sample was collected from each unit.

Preparation of apheresis columns

Apheresis columns were prepared at JJK Medical Development AB (Stockholm, Sweden). Briefly, a synthetic gene encoding human megalin or human cubilin was inserted into the expression vector pJ602-zeo by DNA2.0 (Menlo Park, CA). Only nonmembrane association parts of either protein were included, which are Amino Acids 26 to 4423 in megalin and Amino Acids 132 to 3623 in cubilin. In addition, the native leader sequence was replaced by the murine Ig κ-chain leader sequence to direct the recombinant proteins to the cultivation media. An affinity His_6-tag was inserted at the N-terminal end (megalin) or C-terminal end (cubilin), while the AVI-tag (Avidity LLC, Wilmington, DE) was inserted at the opposite end, before incorporation into the vector. The resulting sequence was transiently expressed in suspension adapted HEK293 (FreeStyle 293-F cells, Life Technologies, Bleiswijk, the Netherlands) during cultivation in medium (CDfortiCHO, Life Technologies) at 37°C for 5 (megalin) or 7 days (cubilin). After centrifugation of harvested media, megalin- or cubilin-containing supernatants were stored at −80°C.

Recombinant human megalin was directly captured on agarose with anti-AVI-tag antibody (Avidity LLC) by adding the agarose directly to filtered supernatant. After incubation at room temperature for 2 hours, the megalin-coupled agarose (Meg-Ab matrix) was collected in empty PD10 columns (GE Healthcare, Uppsala, Sweden), washed with HEPES-buffered saline (HBS) plus $CaCl_2$ (20 mmol/L HEPES, 150 mmol/L NaCl, 2 mmol/L $CaCl_2$, pH 7.3), and finally resuspended in HBS plus $CaCl_2$. Recombinant human cubilin was purified by AIEX and SEC before immobilization onto CNBr-activated Sepharose (GE Healthcare), according to the supplier's recommendations. The cubilin-coupled Sepharose (Cub matrix) was washed with and stored in HBS plus $CaCl_2$. After these steps, a total of three PD10 columns of respective matrix were filled with 1.5 mL (Meg-Ab matrix) or 1 mL (Cub matrix) of settled matrix. In parallel, three identical columns were prepared with antibody-coated agarose (1.5 mL of settled Ab matrix) or deactivated CNBr Sepharose (1 mL of settled naked matrix) for use as reference columns. The contents of reference and test apheresis columns are summarized in Table 1.

PLT filtration

On Day 7, packed columns were washed by gravity with wash buffer: 20 mmol/L HEPES, 150 mmol/L NaCl, 2 mmol/L $CaCl_2$, pH 7.3 (provided by JJK Medical Development AB). Thereafter, aliquots containing PLT sample were filtrated through the different columns preceding analysis while one aliquot remained untreated; 10 mL of PLT was used with the Ab and Ab-Meg columns and 6.7 mL of PLTs with the naked matrix and Cub columns. Filtration was performed by gravity and during a maximum of 10 minutes.

Analysis of cellular, metabolic, in vitro functional and phenotypic markers

Immediately after filtration, cellular in vitro variables including measurements of PLT counts ($\times 10^9$/L) and mean PLT volume (MPV) using hematology equipment

TABLE 1. Summary of apheresis columns used for filtrating PLT concentrates

Apheresis column	Abbreviation	Matrix composition
Naked matrix	N	Deactivated CNBr-Sepharose
Ab matrix	A	Anti-AVI-tag antibody coupled onto CNBr-Sepharose
Meg-Ab matrix	M	Megalin captured on Ab matrix using AVI-tag
Cub matrix	C	Cubilin coupled directly onto CNBr-Sepharose

(CA 620 Cellguard, Boule Medical, Stockholm, Sweden) were analyzed in representative samples from each unit. The extracellular metabolic environment was studied by use of routine blood gas equipment (ABL 800, Radiometer, Copenhagen, Denmark) including pH (37°C), pCO_2, pO_2 (kPa at 37°C), glucose (mmol/L), and lactate (mmol/L). Bicarbonate (mmol/L) was calculated based on the other measured variables. The pH of all samples was measured at 37°C. Rosenthal's factor of 0.0147 unit/1°C was used to correct pH to the temperature of sampling (22°C). This factor gives an approximation to the change in pH of the sample per degrees Celsius when it is warmed anaerobically from the collecting temperature 22 to 37°C.

According to Bertolini and Murphy,[24] swirling was scored as 0, 1, or 2 in all tested units. The white blood cell count on Day 1 was determined with a Nageotte chamber and a microscope (standard, Zeiss, Chester, VA).[25] Hypotonic shock response (HSR) reactivity as well as extent of shape change (ESC) measurements were performed using a dedicated microprocessor-based instrument (SPA 2000, Chronolog, Havertown, PA) modified as described by VandenBroeke and colleauges.[26] The total adenosine triphosphate (ATP) concentration (μmol/10^{11} PLTs) was determined with a luminometer (Orion microplate luminometer, Berthold Detection Systems GmbH, Pforzheim, Germany) on the basis of principles described by Lundin and coworkers.[27] The extracellular lactate dehydrogenase (LDH) activity (% of total), a marker for cell disintegration, was measured with a spectrophotometric method (Kit 063K6003, Sigma Aldrich, St Louis, MO; spectrophotometer 6500, Jenway, Staffordshire, UK).[28] The expression of PAC-1, Clone BALB/c (a marker of cellular responsiveness toward ADP), CD62P, Clone CLB-Thromb/6 (a marker of activation), CD42b, Clone SZ2 (a marker of adhesive capability), and PECAM-1, Clone WM-59 (PLT endothelial cell adhesion molecule 1) were measured using flow cytometry (on a FC500 from Beckman-Coulter, Villepinte, France). Computer software packages (MLP Acquisition and MLP Analysis, Beckman-Coulter) were used for data acquisition and analysis, respectively. All methods, including staining, were performed as described in recent publications.[23,29,30]

Secretion markers and cytokine analysis

Samples were collected in prechilled citrate-theophylline-adenosine-dipyridamole tubes and centrifuged at 2500 × *g* (5810R, Eppendorf, Hauppage, NY) for 30 minutes at +10°C, with the resulting supernatants stored at −70°C pending analysis. All cytokine concentrations were determined with a commercial human cytokine/chemokine magnetic bead panel (MILLIPLEX MAP, HCYTOMAG-60K, Merck-Millipore, Danvers, MA). In each case, we determined concentrations of soluble cluster of differentiation 40 ligand (sCD40L), regulated upon activation of normal t-cells expressed and secreted (RANTES), interferon (IFN)-γ, interleukin (IL)-1β, transforming growth factor-α, tumor necrosis factor (TNF)-α, vascular endothelial growth factor, IL-12 (p40), IL-12 (p70), and IL-7 in accordance with the manufacturer's recommendations. All measurements were performed in duplicate on a multiplexing platform (Magpix, Merck-Millipore) and analyses carried out using computer software (xPonent, Merck-Millipore). The chosen cytokines represent a selection of commonly assessed cytokines with known ligands and functions.

Bacterial detection

In all tested PLT units, bacterial contamination was assessed on Day 7 using an enhanced bacterial detection system (eBDS, Pall Corp., Port Washington, NY). This system indicates the presence of bacteria through a decrease in oxygen tension, as measured in PLT samples after incubation for 24 hours at 35°C.[31]

Statistical analyses

The mean values and standard deviations (n = 5 per group) are given unless otherwise indicated. Repeated-measures analysis of variance on log-transformed values were performed to determine differences between groups. The p value represents these at specific time points (days) and was considered significant at p values of less than 0.01 (*). The analyses were carried out using computer software (JMP, Version 9, SPSS, Chicago, IL; together with Prism 5.0, GraphPad, San Diego, CA).

RESULTS

Effect of storage, postrecovery filtration, and activated matrix

To test the hypothesis that unit BRMs would increase during storage, we assessed representative PLT samples on Days 2, 5, and 7. Not surprisingly, we saw an accumulation

TABLE 2. Impact on cell metabolism, morphology, and function of in vitro filtration on stored PLT concentrates from 25 donors*

		Day 7	
Variable	PLTs/L	Mean PLT volume (fL)	pH (22°C)
Reference unit	951 ± 41*	9.5 ± 0.6	7.338 ± 0.063
A (post filtration on Day 7)	859 ± 38	9.3 ± 0.6	7.341 ± 0.043
M (post filtration on Day 7)	848 ± 38	9.4 ± 0.4	7.333 ± 0.043
Variable	HSR (%)	ESC (%)	LDH (%)
Reference unit	53.7 ± 8.4	11.8 ± 3.2	3.5 ± 0.9
A (post filtration on Day 7)	45.2 ± 5.5	11.9 ± 2.5	3.5 ± 1.5
M (post filtration on Day 7)	54.2 ± 4.5	11.0 ± 4.4	3.0 ± 2.0
Variable	Glucose (mmol/L)	Lactate (mmol/L)	pCO_2 (kPa at 37°C)
Reference unit	2.1 ± 0.7	12.0 ± 1.1*	1.94 ± 0.5*
A (postfiltration on Day 7)	1.6 ± 0.5	9.8 ± 1.0	1.45 ± 0.1
M (postfiltration on Day 7)	1.6 ± 0.5	9.7 ± 0.9	1.50 ± 0.1
Variable	pO_2 (kPa at 37°C)	Bicarbonate (mmol/L)	ATP (μmol/10^{11} PLTs)
Reference unit	21.8 ± 1.2	4.5 ± 0.9*	7.67 ± 0.81
A (postfiltration on Day 7)	20.7 ± 1.2	3.4 ± 0.4	7.98 ± 0.59
M (postfiltration on Day 7)	20.4 ± 1.1	3.4 ± 0.3	7.90 ± 0.64
Variable	CD62P (%)	PAC-1 (%)	CD42b (%)
Reference unit	25.27 ± 0.46	21.43 ± 3.90	98.01 ± 0.87
A (postfiltration on Day 7)	24.02 ± 1.28	17.24 ± 3.61	97.40 ± 0.67
M (postfiltration on Day 7)	23.41 ± 1.60	18.24 ± 2.77	97.89 ± 0.97
Variable	PECAM-1 (%)	PECAM-1 (MFI)	
Reference unit	99.55 ± 0.11	15.8 ± 2.6	
A (postfiltration on Day 7)	99.38 ± 0.22	14.3 ± 2.4	
M (postfiltration on Day 7)	99.39 ± 0.20	15.4 ± 2.9	

* Values are reported as mean ± SD. The p value represents the differences versus filtered units and was considered significant at *$p < 0.01$. Abbreviations are listed in Table 1.

of many of the measured BRMs over time. In parallel, we also saw a slight but significant decrease in many commonly used markers of PLT quality (data not shown).

Furthermore, the degree of PLT depletion and decrease in quality after exposure to the matrix was tested by comparing values from immediately before filtration to the same values after filtration over either an Ab matrix or Meg-Ab matrix (Table 2). There was a small but significant decline in cell numbers from $951 \times 10^9 \pm 41 \times 10^9 \times 10^9$ to $859 \pm 38 \times 10^9$ (Ab matrix) and $848 \times 10^9 \pm 41 \times 10^9$ (Meg-Ab matrix) PLTs/L ($p < 0.001$) after filtration (Table 2 and Fig. 1). However, compared to before filtration, both columns were associated with minor changes in the extracellular metabolic environment while the pH in all filtered fractions at approximately 7 was well above the Council of Europe recommendations ($pH > 6.4$). This along with continued O_2 consumption and the ability to generate an equivalent

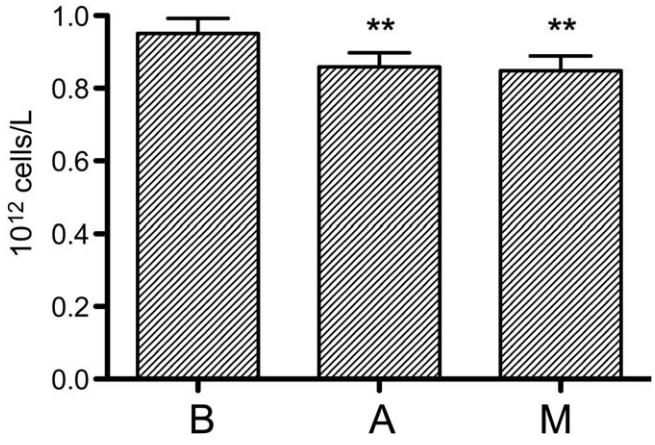

Fig. 1. The small but significant loss of cells occurring through filtration of 10-mL PLT concentrates over 1.5 mL of matrix. **$p < 0.01$. Bars show, respectively, before (B), after Ab matrix (A), and after Meg-Ab matrix (M).

concentration of ATP[32] indicates that oxidative phosphorylation was likely maintained in these PLTs. Additionally, filtration did not affect the ability of the PLTs to respond to agonists (ESC and PAC-1), indicating that cellular responsiveness was maintained relative to their activation state. Furthermore, all HSR scores were well above the level for which poor in vivo viability has been predicted to occur.[33] Also the expression level of the GPIb-IX-V complex[34,35] and maintenance of the PECAM-1 (CD31) levels of filtered PLTs[36,37] strengthens the conclusion that filtration does not adversely affect PLT quality. Moreover, our results on the filtered PLTs seem to agree with the functional integrity of the OrbiSac-produced PLTs proven earlier in a series of in vitro assays[2,22,23,29,30,37] and more than 10 years of clinical experience including more than 1.5 million units produced worldwide.

Effect of filtration on measured concentrations of BRMs

To quantify the nonspecific binding known to occur with many polyglucose matrices,[21,38] we measured the concentrations of BRMs immediately before and after filtration over either a naked agarose matrix or an antibody-coupled agarose matrix (Fig. 2). Briefly, compared to before filtration, IL-1β, IL-12 (p40), IL-12, (p70) and IL-7 all decreased by approximately 40%.

Effect of filtration over recombinantly coated matrices

Using the same set-up as previously, we next assessed the additive effect of coating matrix with either rh-megalin or rh-cubilin. As shown in Fig. 2, rh-cubilin but not rh-megalin was able to further decrease the concentrations of several BRMs. Specifically, concentrations of IFN-γ, IL-1β, TNF-α, IL-12, and IL-7 all decreased by an additional 30% to 50%. There was a trend toward higher RANTES in the rh-cubilin group but this difference did not reach significance.

DISCUSSION

In a pilot study evaluating the feasibility of in vitro plateletpheresis for the specific depletion of BRMs such as IFN-γ, IL-1β, and TNF we found that filtration over a negatively charged column already resulted in significant reductions of some BRMs. However, coating the matrix with the multiligand receptor rh-cubilin, thought to be responsible for endocytosis and metabolism of blood cytokines in the human kidney,[20] was able to augment the removal rate and extend removal to several other tested BRMs. Several markers of PLT quality and function remained unaffected by the filtration. Potential storage effects after filtration were not evaluated. Finally, compared to a previous report on the feasibility of in vitro BRM apheresis,[21] the loss of cells over the column was small, and—as indicated by consistently low LDH levels—likely not due to lysis. This study was not designed to evaluate the specificity or capacity of the matrices, the strength of binding, or the relative importance of depleted and remaining BRMs.

While leukoreduction has significantly reduced the rate of adverse febrile reactions after PLT transfusion,[13] anaphylactic and other adverse transfusion reactions remain an important concern.[4,15-17] Even after implementation of pathogen inactivation in PLT components, adverse reactions still occur.[39] Furthermore, compared to red blood cells, storage times for PLTs are currently short. This is mainly due to a perceived deterioration in cell quality and homeostatic capacity over time and concurrent with what has been termed lesion effects,[40,41] while the increased risk of bacterial contamination also contributes.[42] In this context, it is interesting to note that several BRMs linked to transfusion reactions also increase during storage.[36] Speculatively, the production or release of these BRMs may both be a result of the lesion effect and a driver of further alterations in thrombocyte homeostasis and survival. Supporting such a hypothesis, several BRMs have recently been linked to transfusion reactions,[1,5,7,8,12,43] while concentrations of several cytokines increase in the bag with storage times, suggesting de novo production or release from intracellular sites.[1-3,44] Thus it is possible that the depletion of BRMs may contribute to prolong PLTs shelf life and/or reduce transfusion reactions. Such an effect remains to be demonstrated, but with the technology described in this study it is feasible.

So far, the lack of an appropriate technology to remove a large number of heterogeneous BRMs has limited the clinical usefulness of an in vitro apheresis approach. However, Tanaka and coworkers[21] recently reported on their use of a positively charged polymer matrix to significantly reduce RANTES and sCD40L in 5-day-old PLTs. As in this study, they reported no significant effects on PLT activation, aggregation, lysis, or HSR. However, the matrix used was associated with a PLT recovery rate of approximately 60% of the starting value. In contrast, our uncharged matrix allowed for more than 90% PLT recovery and exhibited a completely different affinity profile. The recovery rate thus approaches what can be achieved with a coated-bead set-up such as the one used here, because approximately 5% of the PLTs are trapped in the void volume of the column (30% of the settled matrix volume). While many factors may contribute to increase PLT recovery after apheresis, it is interesting to note that PLT surface glycoprotein Ib contains a negatively charged region that binds to the positively charged von Willebrand factor on the vascular endothelium.[45] Indeed, we speculate that this well-described electrostatic interaction contributes to the low recovery seen with a negatively charged matrix and that it may also explain the removal of BRMs also observed with the Ab and naked matrices.

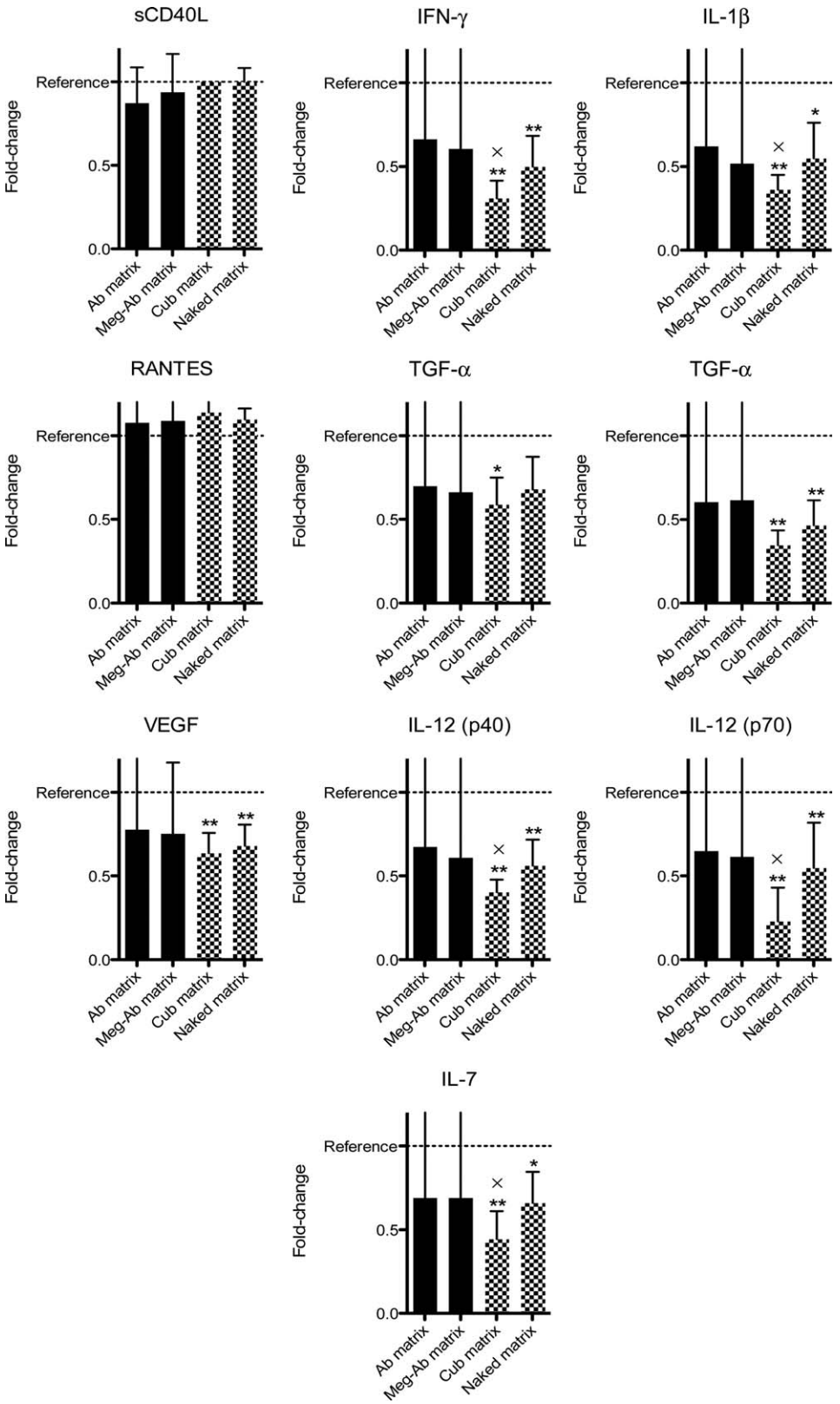

Fig. 2. Relative reduction in selected BRMs after filtration of stored PLTs (reference) over the four tested matrices. Abbreviations are listed in Table 1. *$p < 0.05$ and **$p < 0.01$ compared to values before filtration (reference). $^{x}p < 0.05$ compared to naked matrix. TGF-α = transforming growth factor-α.

The approach used in this study differs from previously published methods in that it is based on the use of a recombinant protein receptor. In this, our in vitro affinity surface more resembles novel apheresis columns for in vivo use rather than more traditional filtration columns.[46] Furthermore, while the use of antibody-coated surfaces to isolate specific epitope-carrying peptides is standard practice in the laboratory, to the best of our knowledge, this is the first application to harness the multiligand characteristics of the renal tubular proteins megalin and cubilin. Although the mechanisms and ligands of both remain poorly understood, each likely has affinity for a very broad range of small- to medium-sized proteins found in the circulation but not in the urine,[20] an ideal profile for the removal of a broad range of incompletely elucidated cytokines from PLTs. Despite the likely broad specificity of both receptors (who also act in concert for endocytosis),[20] our pilot experiment showed almost no removal over the rh-megalin column. Also, neither receptor removed RANTES or sCD40L under trial conditions. Interestingly, both of these molecules are lipophilic molecules associated with the cell membrane[47,48] and were the BRMs that exhibited significant depletion rates in the study by Tanaka and colleagues employing a positively charged matrix. In contrast, most cytokines are hydrophilic,[49] presumably to be able to travel rapidly between cells.

In summary, we demonstrate the feasibility of using recombinant human cubilin receptors to deplete significant amounts of several BRMs from PLT concentrates without triggering excessive PLT loss, activation, or dysfunction. The described approach may be useful to further assess the importance of BRMs for the PLT storage lesions and their putative role in transfusion reactions.

ACKNOWLEDGMENTS

JA acknowledges support from Marianne & Marcus Wallenbergs' stiftelse, the Swedish Heart and Lung Foundation, and the Swedish Research Council.

CONFLICT OF INTEREST

PS has no conflict of interest. JR is a JJK Medical Development AB/South End Advisory AB employee and as such involved in the commercial development of the described affinity surface. JA holds shares in JJK Medical Ltd., which owns the intellectual property rights to the affinity surface and provided the recombinant proteins used in the current study free of charge.

REFERENCES

1. Cognasse F, Boussoulade F, Chavarin P, et al. Release of potential immunomodulatory factors during platelet storage. Transfusion 2006;46:1184-9.
2. Sandgren P, Meinke S, Eckert E, et al. Random aggregates in newly produced platelet units are associated with platelet activation and release of the immunomodulatory factors sCD40L and RANTES. Transfusion 2014;54:602-12.
3. Stack G, Snyder EL. Cytokine generation in stored platelet concentrates. Transfusion 1994;34:20-5.
4. Nguyen KA, Hamzeh-Cognasse H, Sebban M, et al. A computerized prediction model of hazardous inflammatory platelet transfusion outcomes. PloS One 2014;9: e97082.
5. Cognasse F, Hamzeh-Cognasse H, Lafarge S, et al. Donor platelets stored for at least 3 days can elicit activation marker expression by the recipient's blood mononuclear cells: an in vitro study. Transfusion 2009;49:91-8.
6. Garraud O, Berthet J, Hamzeh-Cognasse H, et al. Pathogen sensing, subsequent signalling, and signalosome in human platelets. Thromb Res 2011;127:283-6.
7. Hamzeh-Cognasse H, Damien P, Nguyen KA, et al. Immunereactive soluble OX40 ligand, soluble CD40 ligand, and interleukin-27 are simultaneously oversecreted in platelet components associated with acute transfusion reactions. Transfusion 2014;54:613-25.
8. Urner M, Herrmann IK, Buddeberg F, et al. Effects of blood products on inflammatory response in endothelial cells in vitro. PloS One 2012;7:e33403.
9. Klüter H, Bubel S, Kirchner H, et al. Febrile and allergic transfusion reactions after the transfusion of white cell-poor platelet preparations. Transfusion 1999;39:1179-84.
10. Muylle L, Wouters E, Peetermans ME. Febrile reactions to platelet transfusion: the effect of increased interleukin 6 levels in concentrates prepared by the platelet-rich plasma method. Transfusion 1996;36:886-90.
11. Wang RR, Triulzi DJ, Qu L. Effects of prestorage vs poststorage leukoreduction on the rate of febrile nonhemolytic transfusion reactions to platelets. Am J Clin Pathol 2012;138: 255-9.
12. Cognasse F, Payrat JM, Corash L, et al. Platelet components associated with acute transfusion reactions: the role of platelet-derived soluble CD40 ligand. Blood 2008;112: 4779-80.
13. Paglino JC, Gregory J, Pomper GS, et al. Reduction of febrile but not allergic reactions to RBC and platelets after conversion to universal prestorage filtration. Transfusion 2004;44: 16-44.
14. Tobian AA, Fuller AK, Uglik K, et al. The impact of platelet additive solution apheresis platelets on allergic transfusion reactions and corrected count increment (CME). Transfusion 2013;54:1523-9.
15. Li N, Williams L, Zhou Z, et al. Incidence of acute transfusion reactions to platelets in hospitalized pediatric patients based on the US hemovigilance reporting system. Transfusion 2014;54:1666-72.
16. Bjursten H, Al-Rashidi F, Dardashti A, et al. Risks associated with the transfusion of various blood products in aortic valve replacement. Ann Thorac Surg 2013;96:494-9.

17. Quillen K. Hemolysis from platelet transfusion: call to action for an underreported reaction. Transfusion 2012;52:2072-4.
18. Karim F, Moiz B, Shamsuddin N, et al. Root cause analysis of non-infectious transfusion complications and the lessons learnt. Transfus Apher Sci 2014;50:111-7.
19. Fitzmaurice GJ, Parissis HD. Platelet transfusion associated with acute lung injury after coronary artery bypass grafting. Ann Thorac Surg 2011;91:1977-9.
20. Christensen EI, Birn H, Storm T, et al. Endocytic receptors in the renal proximal tubule. Physiology 2012;27:223-36.
21. Tanaka S, Hayashi T, Tani Y, et al. Removal of biological response modifiers associated with platelet transfusion reactions by columns containing adsorption beads. Transfusion 2014;54:1790-7.
22. Larsson S, Sandgren P, Sjödin A, et al. Automated preparation of platelet concentrates from pooled buffy coats: in vitro studies and experiences with the OrbiSac system. Transfusion 2005;45:743-51.
23. Sandgren P, Saeed K. Storage of buffy-coat-derived platelets in additive solution: in vitro effects on platelets of the air bubbles and foam included in the final unit. Blood Transfus 2011;9:182-8.
24. Bertolini F, Murphy S. A multicenter evaluation of reproducibility of swirling in platelet concentrates. Biomedical Excellence for Safer Transfusion (BEST) Working Party of the International Society of Blood Transfusion. Transfusion 1994;34:796-801.
25. Moroff G, Eich J, Dabay M. Validation of use of the Nageotte hemocytometer to count low levels of white cells in white cell-reduced platelet components. Transfusion 1994;34:35-8.
26. VandenBroeke T, Dumont LJ, Hunter S, et al. Platelet storage solution affects on the accuracy of laboratory tests for platelet function: a multi-laboratory study. Vox Sang 2004;86:183-8.
27. Lundin A, Rondahl H, Walum E, et al. Expression and intracellular localization of leptin receptor long isoform-GFP chimera. Biochim Biophys Acta 2000;1499:130-8.
28. Vanderlinde RE. Measurement of total lactate dehydrogenase activity. Ann Clin Lab Sci 1985;1:13-31.
29. Sandgren P, Stjepanovic A. High-yield platelet units revealed immediate pH decline and delayed mitochondrial dysfunction during storage in 100% plasma as compared with storage in SSP+. Vox Sang 2012;103:55-63.
30. Sandgren P, Tolksdorf F, Struff WG, et al. In vitro effects on platelets irradiated with short-wave ultraviolet light without any additional photoactive reagent using the THERAFLEX UV-Platelets method. Vox Sang 2011;101:35-43.
31. Ortolano GA, Freundlich LF, Holme S, et al. Detection of bacteria in WBC-reduced PLT concentrates using percent oxygen as a marker for bacteria growth. Transfusion 2003;43:1276-85.
32. Holme S, Heaton WA, Courtright M. Platelet storage lesion in second-generation containers: correlation with platelet ATP levels. Vox Sang 1987;53:214-20.
33. Holme S. Storage and quality assessment of platelets. Vox Sang 1998;74 Suppl 2:207-16.
34. Berndt MC, Shen Y, Dopheide SM, et al. The vascular biology of the glycoprotein Ib-IX-V complex. Thromb Haemost 2001;1:178-88.
35. Henrita van Zanten G, Saelman EU, Schut-Hese KM, et al. Platelet adhesion to collagen type IV under flow conditions. Blood 1996;88:3862-71.
36. Falati S, Patil S, Gross PL, et al. Platelet PECAM-1 inhibits thrombus formation in vivo. Blood 2006;2:535-41.
37. Sandgren P, Larsson S, Wai-San P, et al. The effects of pneumatic tube transport on fresh and stored platelets in additive solution. Blood Transfus 2014;1:85-90.
38. Axelsson J, Ferreira M, Adolfsson L, et al. Cytokines in blood from septic patients interact with surface-immobilized heparin. ASAIO J 2010;56:48-51.
39. Osselaer J, Messe N, Hervig T, et al. A prospective observational cohort safety study of 5106 platelet transfusions with components prepared with photochemical pathogen inactivation treatment. Transfusion 2008;48:1061-71.
40. Seghatchian J, Krailadsiri P. Platelet storage lesion and apoptosis: are they related? Transfus Apher Sci 2001;24:103-5.
41. Paglia G, Sigurjónsson ÓE, Rolfsson Ó, et al. Comprehensive metabolomic study of platelets reveals the expression of discrete metabolic phenotypes during storage. Transfusion 2014;54:2911-23.
42. Blajchman MA, Goldman M, Baeza F. Improving the bacteriological safety of platelet transfusions. Transfus Med Rev 2004;18:11-24.
43. Muylle L, Joos M, Wouters E, et al. Increased tumor necrosis factor alpha (TNF alpha), interleukin 1, and interleukin 6 (IL-6) levels in the plasma of stored platelet concentrates: relationship between TNF alpha and IL-6 levels and febrile transfusion reactions. Transfusion 1993;33:195-9.
44. Khan SY, Kelher MR, Heal JM, et al. Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury. Blood 2006;108:2455-62.
45. Murata M, Ware J, Ruggeri ZM. Site-directed mutagenesis of a soluble recombinant fragment of platelet glycoprotein Ib alpha demonstrating negatively charged residues involved in von Willebrand factor binding. J Biol Chem 1991;266:15474-80.
46. Winters JL. American Society for Apheresis guidelines on the use of apheresis in clinical practice: practical, concise, evidence-based recommendations for the apheresis practitioner. J Clin Apher 2014;29:191-3.
47. Wang X, Watson C, Sharp JS, et al. Oligomeric structure of the chemokine CCL5/RANTES from NMR, MS, and SAXS data. Structure 2011;19:1138-48.
48. Furman MI, Krueger LA, Linden MD, et al. Release of soluble CD40L from platelets is regulated by glycoprotein IIb/IIIa and actin polymerization. J Am Coll Cardiol 2004;43:2319-25.
49. Whicher JT, Evans SW. Cytokines in disease. Clin Chem 1990;36:1269-81.