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Postoperative Inflammatory Response after Autologous and Allogeneic Blood Transfusion

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Background: **Allogeneic blood transfusions cause immunosuppression. The aim of this study was to determine whether complement anaphylatoxins, cytokines, or both are released in the recipient, after blood transfusions in general, and after autologous blood transfusions in particular.**

Methods: **Thirty-one patients having total hip joint replacement surgery were randomized to receive either allogeneic red blood cells (n = 15) or predeposited autologous whole blood transfusion (n = 16). Plasma concentrations of the anaphylatoxins C3a and C5a, the terminal C5b-9 complement complex, and cytokines IL-6 and IL-8 in the recipients were repeatedly analyzed before, during, and after surgery.**

Results: **Significantly increased concentrations of IL-6 and IL-8 appeared in both groups, with a significantly greater increase in the autologous blood group. Patients in both groups developed a moderate but significant increase of C3a without a significant difference between them. C5a and terminal C5b-9 complement complex were not greatly changed.**

Conclusions: **The study showed a greater increase in cytokine concentration after autologous blood transfusion than after allogeneic blood transfusion. The lower response in the latter may result from transfusion-induced suppression of cellular immunity. (Key words: Anaphylatoxin. Blood transfusion, autologous: erythrocyte concentrate; whole blood. Complement, terminal: complement complex. Cytokines.)**

LARGE intra- or postoperative blood loss is usually replaced by allogeneic red blood cells. However, preoperative autologous blood collection (weekly phlebotomy 4 or 5 weeks before the scheduled operation) is becoming more common. The risks of allogeneic blood transfusions, such as virus transmission and immunologic reactions, are now common knowledge,[1,2] and patients increasingly demand the greater safety that autologous blood transfusion appears to offer. Allogeneic blood causes immunosuppression, evidenced by improved survival of renal allografts in previously transfused patients.[3-5] Complement is activated during storage of whole blood and plasma.[6,7] Intraoperative blood salvage is associated with activation of complement, release of polymorphonuclear elastase and cytokines in the shed blood, and elevated plasma levels of cytokines after retransfusion.[8-10] It has been suggested that cytokines play a central role in the development of hemolytic transfusion reactions with fever, capillary leakage, and vascular smooth muscle relaxation, leading to hypotension and shock.[11,12] In addition, the formation of anaphylatoxins (C3a, C4a, and C5a) may cause release of vasoactive amines, smooth muscle contraction, increased vascular permeability, and release of lysosomal enzymes.[13]

Autologous blood transfusions are associated with fewer postoperative infections than allogeneic blood transfusions.[14-16] This might be explained by the fact that cell immunity is less compromised.[17] Cytokine production is one of the immune functions depressed by allogeneic blood transfusion.[18] The effect of autologous blood transfusion on postoperative complement or cytokine response is not known.

The aim of the present study was to determine whether there are differences in immunologic response, assessed by the release of complement anaphylatoxins, proinflammatory cytokines, or both, after transfusion of allogeneic or autologous blood.

Materials and Methods

Fifty-six consecutive patients undergoing elective hip joint replacement surgery were randomized to receive

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autologous or allogeneic blood transfusions. Each patient in the autologous group deposited three units of whole blood in advance of the surgery. We included in the autologous group patients receiving one to three units of autologous blood; in the allogeneic group patients were included who receive one to three units of allogeneic blood. We excluded patients not receiving blood at all, patients receiving more than three units of allogeneic blood, or patients receiving autologous and allogeneic blood. Thirty-one patients were followed, and 25 were excluded. Spinal anesthesia with 15–20 mg bupivacaine and 0.2 mg morphine was used for surgery. All patients studied required blood transfusions during and after operation. In both groups, transfusion was started when the hemoglobin value decreased to less than 85 g/l or if the patient showed clinical signs of hypovolemia. Most transfusions were given during operation, and all were given within 24 h of surgery. In both groups, a 200-μm filter was used during transfusion. Intra- or postoperative blood salvage was not used. The study was approved by the ethics committee of Sahlgrenska University Hospital, Gothenburg, Sweden.

Allogeneic Blood Group

Fifteen patients (median age, 60 yr; range, 42–87 yr) received one to three units (median volume, 550 ml) of allogeneic, buffy coat–depleted erythrocytes during or after operation. The erythrocytes were stored in CPD-SAGMAN bags (Baxter, Deerfield, IL). Blood samples were taken from the patients 1 day before operation, 1 h after operation, on day 1 after operation, on day 4 or 5 after operation, and 5 weeks after operation. Analyses comprised plasma concentrations of the interleukins (IL) IL-6 and IL-8, the complement anaphylatoxins C3a and C5a, and the terminal C5b-9 complex.

Autologous Blood Group

Sixteen patients (median age, 57 yr; range, 39–75 yr) in the autologous blood group gave one unit of their blood 4, 3, and 2 weeks before operation (median volume, 460 ml). They received 2 mg Fe^{2+}/kg by mouth daily for 4 weeks. The blood was stored at 4°C in citrate-phosphate-dextrose (CPD) adenine bags containing 327 mg citrate, 251 mg phosphate, and 27.5 mg adenine per 100 ml (Baxter).

The patients were transfused during or after surgery with one to three units of autologous whole blood. Blood samples taken and analyzed were the same as in the allogeneic blood group, except that in the autologous blood group they were also taken 4 weeks before operation.

C3a, C5a, Interleukin-6, Interleukin-8, and Terminal C5b-9 Complex Determinations

All samples were drawn into tubes containing 0.054 ml ethylenediamine tetraacetic acid per 4.5 ml blood. The tubes were immediately centrifuged at room temperature (20–22°C) to remove the cells and the samples were frozen within 30 min and stored at -80°C, until the variables could be determined. All determinations were done twice. The samples were stored in separate tubes for the different analyses. After thawing, no samples were refrozen. All the variables were determined with commercially available systems: IL-6 and IL-8 with Biotrak cytokines human EIA systems (Amersham, Buckinghamshire, UK); terminal C5b-9 complex using a Quidel enzyme-linked immunosorbent assay (San Diego, CA); C3a-desArg with Progen Biotechnik (Heidelberg, Germany); and C5a by Behring enzyme immunoassay (enzygnost C5a micro; Behring, Marburg, Germany).

Statistical Methods

Assessment of changes within groups during the period from the last preoperative value to the value measured 4 or 5 days after operation was performed by Friedman's test, which can be considered a nonparametric method of analysis of variance for repeated measures. If significant changes were found, comparisons were also performed for the change to 1 h after operation, on day 1, and so forth by the two-tailed Wilcoxon test for pair comparisons. Between the allogeneic blood group and the autologous blood group, comparisons were first performed with respect to the area under the curve calculated by the trapezoidal method. If significant differences were obtained for area under the curve, comparisons were also performed for separate periods (1 h after operation, on day 1, and so on). The values 5 weeks after operation were not included in Friedman's test or in the calculation of area under the curve because a substantial number of values were missing. The Mann-Whitney test, two-tailed, was used for all comparisons between the groups. Differences were considered significant at $P < 0.05$.

Results

Age, sex, diagnosis, fixation technique, intra- and postoperative bleeding, blood volume transfused, total

Table 1. Clinical Data

	I. Allogeneic Blood (included)	II. Autologous Blood (included)	III. Allogeneic Blood (excluded)	IV. Autologous Blood (excluded)
No. of patients	15	16	13	12
Age (yr)	60 (42–87)	57 (39–75)	66 (42–87)	55 (23–80)
Sex (male/female)	2/13	9/7*	4/9	3/9
Diagnosis				
Osteoarthritis	9	13	8	12
Rheumatoid arthritis	6	3	5	0
Fixation technique				
Cemented	10	8	11	7
Uncemented	5	8	2	5
Intraoperative and postoperative bleeding (ml)	1,390 (1,000–1,770)	1,480 (600–2,420)		
Blood volume transfused (ml)	550 (275–825)	1,360† (480–1,872)		
Total red blood cell volume transfused (ml)	357 (171–572)	444 (170–537)		
Blood storage time (days)	13 (5–30)	19 (4–31)		
Operation time (min)	141 (110–170)	150 (105–180)		
Cause of Exclusion				
>3 units of blood transfused			10	7
No blood transfusion			2	0
Patient unwillingness			1	2
Donated <3 units of blood			—	3

Median values and ranges are given.

* $P < 0.05$, group I *versus* group II.

† $P < 0.001$, group I *versus* group II.

erythrocyte volume transfused, storage time, operation time, and, when relevant, cause of exclusion are given in table 1. Figure 1 shows C3a, C5a, terminal C5b-9 complex, IL-8, and IL-6 variables 4 weeks before operation, on day 1 before operation, 1 h after operation, on day 1 after operation, on day 4 or 5 after operation, and 5 weeks after operation.

Interleukin-6 increased in both groups after blood transfusion. In the allogeneic blood group, the plasma concentrations 1 h after operation ($P < 0.001$), on day 1 after operation ($P < 0.001$), and on day 4 or 5 after operation ($P < 0.05$) were significantly higher than the preoperative levels. At 5 weeks, levels had returned to normal. In the autologous blood group, the plasma levels 1 h after operation and on day 1 and day 4 or 5 after operation were all significantly higher ($P < 0.001$) than the preoperative levels. No increased levels of IL-6 were found 4 weeks before or 5 weeks after surgery. There were significantly higher levels in the autologous group than in the allogeneic group on day 1 ($P < 0.01$) and day 4 or 5 after operation ($P < 0.05$).

Interleukin-8 levels were not significantly changed in the allogeneic blood group. In the autologous blood group, significant increases in IL-8 were noted immediately after surgery and on day 1 ($P < 0.01$). No alterations were detected in the sample taken 4 weeks before operation, nor at day 4 or 5 or 5 weeks after operation. Comparing the two groups, we found significantly higher plasma IL-8 concentrations in the autologous blood group on day 1 after operation ($P < 0.05$) compared with the allogeneic blood group.

C3a was elevated in both groups after blood transfusion. In the allogeneic blood group, the plasma levels were significantly higher on day 4 or 5 after operation ($P < 0.001$) than before. In the autologous blood group, there was a significant increase in C3a plasma levels on day 1 ($P < 0.05$), day 4 or 5 ($P < 0.001$), and at 5 weeks after operation ($P < 0.05$), compared with preoperative levels. There were no significant differences between the groups.

C5a decreased significantly on postoperative day 1 ($P < 0.01$) compared with before operation in the allogeneic blood group. There were no significant differences between the groups.

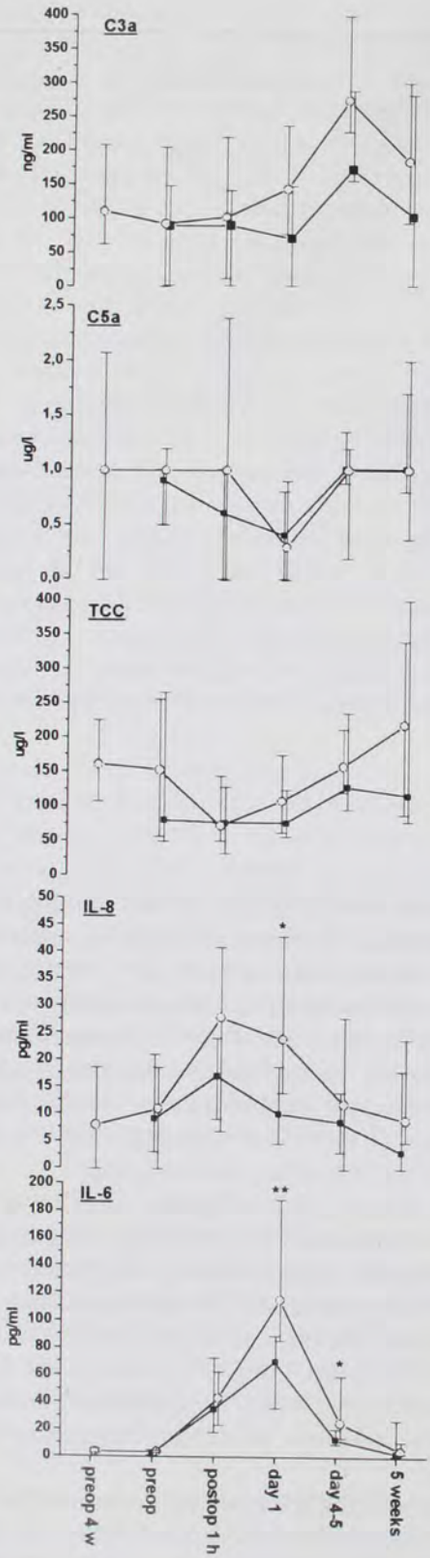

Fig. 1. Plasma concentrations of C3a, C5a, terminal C5b-9 complex, interleukin-8, and interleukin-6 in the allogeneic (filled squares) and autologous (open circles) blood groups. Median values and 25–75% percentiles are given. *$P < 0.05$ and **$P < 0.01$ denote significant differences between the groups.

Terminal C5b-9 complex levels in the autologous blood group were significantly decreased on day 1 ($P < 0.05$) and significantly increased 5 weeks after operation ($P < 0.05$) compared with preoperative levels. No other significant differences were found.

Discussion

Our study shows a significant increase in the concentration of IL-6 in recipients of allogeneic and autologous blood. The increase was significantly higher in the autologous group, with a peak on the first postoperative day (fig. 1). Interleukin-8 followed a similar pattern in the autologous group, but the increase was less and there was no significant increase of IL-8 in the allogeneic blood group (fig. 1). An increased concentration of IL-6 and IL-8 indicates an inflammatory or proinflammatory response. The cytokines IL-6 and IL-8 are released in sepsis and in association with trauma and hemorrhage.[19,20] In such situations, the concentrations of IL-6 and IL-8 increase to > 300 pg/ml and 1,000–5,000 pg/ml, respectively. Similar concentrations have been found in autologous shed blood.[10] The moderate increases in IL-6 and IL-8 concentrations that we found are less impressive but do indicate an inflammatory response. Surgical trauma leads to release of cytokines. After laparoscopic and abdominal hysterectomy, IL-6 levels of approximately 50 pg/ml are seen.[21] The IL-6 concentration after hip replacement surgery without blood transfusion has not been studied extensively, but Arnestad *et al.*[10] found a concentration of 56 pg/ml just after surgery. In an attempt to elucidate the degree to which hip surgery is responsible for the IL-6 release, we did a *post hoc* study of six patients who did not receive blood transfusion. The same surgical and anesthetic protocol was used. On day 1 the IL-6 concentration (median) was 97 pg/ml. No statistical conclusions can be drawn, but the levels are similar to those we found after surgery and transfusion. The lower increase in IL-6 and IL-8 in the allogeneic blood group could be a result of transfusion-depressed immune function.[3,18]

Kalechman *et al.*[22] showed that transfusion of one single unit of allogeneic whole blood generated a sharp decrease in cytokine production.[22] There was a moderate but significant increase in C3a concentration in both groups. This merely indicates complement activation in the recipient, possibly due to the surgical trauma and the blood transfusion. The increase in the autologous group could result from high concentrations of C3a in stored whole blood.[9] C5a and terminal C5b-9 complex did not show any major changes.

A limitation of this study is that we compared red blood cells (allogeneic blood group) and whole blood (autologous blood group). Arnestad *et al.*[10] showed that when retransfusing shed whole blood containing high concentrations of IL-6, a peak appeared in the patient's IL-6 plasma concentration as soon as 1 min after completed retransfusion. Sixty minutes after retransfusion, the IL-6 concentration was regressing. In the present study, the peak value for IL-6 occurred on day 1 after operation; for IL-8, the peak value occurred 1 h after operation. This would suggest that the cytokine content of whole blood is not the explanation of the significantly greater increase in cytokine concentration in the autologous group.

Techniques for autologous blood transfusion have been developed to minimize the need for allogeneic transfusions. Retransfusion with autologous shed blood is associated with infusion of large concentrations of cytokines, with significant increases in the IL-6 plasma concentration[10] and infusion of large amounts of anaphylatoxins.

Allogeneic erythrocytes, the most common form of blood transfusion, offers benefits such as good availability and less activation of the complement system during storage.[7] Its known disadvantages, compared with autologous blood, are virus transmission, immunosuppression, and possibly increased rates of cancer recurrence.

Transfusion of autologous blood collected before operation is becoming an important method. Storage of whole blood is most common, probably because it is cheaper than separating the products.[23] Known advantages are the absence of virus transmission and immunologic transfusion reactions. Possible advantages might be less immunosuppression and fewer postoperative infections. One possible disadvantage of using whole blood is an increase in anaphylatoxins and perhaps cytokines. If blood components are separated, these agents will be present in relatively high contents in the plasma fraction.

In conclusion, this study shows that the plasma concentrations of IL-6 and IL-8 are greater in patients receiving autologous whole blood than in patients receiving allogeneic red blood cells. This could be a result of transfusion-depressed immune function in the allogeneic group.

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