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Complement Activation and Reinfusion of Wound Drainage Blood

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Eighteen patients undergoing total hip replacement (n = 13) or knee arthroplasty (n = 5) due to osteoarthritis or osteoarthrosis were prospectively studied in an investigation of complement activation and anaphylatoxin release in association with reinfusion of aspirated wound blood. Twelve of the patients needed blood transfusions and received an average of 390 ± 75 ml (±SD) of autologous blood within 45 min. Plasma complement components, anaphylatoxins, and inhibitors were studied 1 min before and 15 min after the start of and 15 min after the completion of autologous transfusion. Samples also were taken from the collected blood, before and after passing it through a microporous filter. Blood gases and systemic complement samples were drawn simultaneously. There were no significant changes in systemic complement variables before, during, or after transfusion of autologous blood. However, in the aspirated blood, increased concentration of anaphylatoxins (C3a and C5a) and terminal complement complexes (TCC) were present ($P < 0.001$). There were no differences observed between samples drawn before and after filtration of the blood. The concentration of C5 was less in the collected blood than in the systemic blood ($P < 0.05$). No changes in blood gases were observed. This study demonstrated that postoperatively salvaged whole blood underwent anaphylatoxin formation and complement activation. However, after reinfusion of this blood, neither systemic complement activation nor clinical complications were observed. (Key words: Transfusion, autologous blood: Anaphylatoxins, complement.)

THE RISKS of transmitting infections and of adverse immunologic reactions from homologous blood transfusions have increased the need for alternatives to conventional blood transfusion.[1] Although autologous blood transfusion first was reported in 1886,[2] this technique was not used extensively until the 1970s.[3] Since then, peri- and postoperative autologous transfusions have been used in various kinds of emergency and elective surgery.[4–6] Different techniques for autologous transfusions have been developed. Reinfusion of whole blood and reinfusion of erythrocyte concentrate are currently practiced.[7,8] However, several studies have demonstrated changes in the blood transfused.[9–11] The coagulation and the fibrinolytic systems seem to be activated in association with the transfusion of autologous blood.[10,11] Transfusion of autologous blood also has been associated with complications such as hemolysis and embolization of air and microaggregates.[12] It is well known that blood in contact with foreign material activates the complement cascade. Dialysis membranes, heart–lung machines, and contrast agents activate the alternative pathway[13–15] (fig. 1). The classical complement pathway can be initiated by immunocomplexes and by bacteria[16,17] (fig. 1).

When complement is activated, the anaphylatoxins C3a and C5a are formed.[18] They induce smooth muscle contraction, enhance vascular permeability, and release histamine from mast cells and basophils.[19] C5a and C5a desArginine are chemotactically attractive for neutrophils.[20,21] C5a may induce secretion of lysosomal enzymes and induce production of interleukin and prostaglandin from macrophages.[22,23] Activation of complement has also been proposed as one etiologic factor behind the development of respiratory distress syndrome and multi-organ failure.[24,25] The terminal complement complex is generated as an end-product of complement activation.[26] This complex may be responsible for reactive lysis.

This study was performed to investigate whether the reinfusion of aspirated wound blood influences the complement cascade and the concentration of systemic anaphylatoxins.

Materials and Methods

Eighteen patients (11 females, 7 males, age range 35–80 yr, mean 66 yr) undergoing elective hip (n = 13) or knee arthroplasty (n = 5) were studied. Twelve of these patients required blood transfusions and received autologous blood postoperatively. The indications for reinfusion of aspirated blood were the same as for transfusion of bank blood. The reinfusion was started when the patient had a hemoglobin value below 100 g/l or when the patient showed signs of hypovolemic or circulatory insta-

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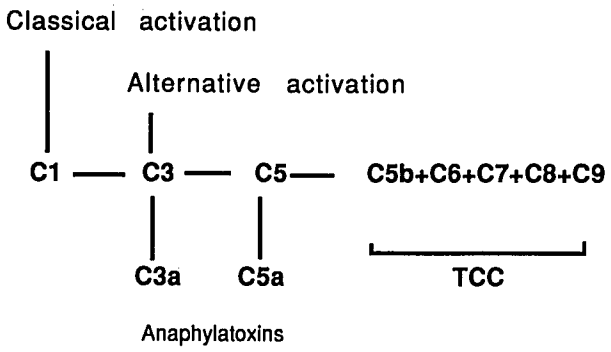

FIG. 1. The complement cascade.

bility due to bleeding. Shed blood was mixed with 40 ml anti-coagulating citrate–dextrose solution (ACD) in the suction equipment (40 ml ACD contain 0.32 g citric acid, 0.88 g sodium citrase, and 0.98 g glucose). A drainage suction system allowing reinfusion of aspirated wound blood was used (Solcotrans®, Solco Basle Ltd., UK). An aspiration tube of 100-cm length and 3-mm diameter was connected to the entry port of the blood bag. A maximum negative pressure of 100 mmHg was applied through a 15-cm-long tubing connected to the opposite part of the bag. The material of the tubes and bags was polyvinylchloride. When the bag was filled, the aspirated salvaged blood was reinfused to the patient through a microaggregate filter by gravity. The filter used was a Pall SQ 49-S micron microporous filter. The patients received one unit of aspirated wound blood, and the blood was infused *via* a peripheral venous catheter. The reinfusion was started within 4 h from the start of the blood collection, and the reinfusion was performed during a period of maximum length 45 min.

Blood samples from the patients were taken prior to transfusion of autologous blood, 15 min after the start, and 15 min after the end of the transfusion of autologous blood. Samples also were taken from the side port of the infusion bag and distal to the microporous filter just prior to the infusion. None of the patients had been given any bank blood at the time of the sampling. No bank blood was given before the infusion of shed blood or before the sampling procedure was completed. All samples for complement determinations were drawn into tubes with EDTA. The quantity was 0.054 ml 0.34 M EDTA per 4.5 ml blood. All determinations were performed in duplicate. The tubes were immediately centrifuged at room temperature (20–22° C) to remove the cells. The plasma was frozen within 30 min and kept at −80° C until analyzed for C3a, C5a, terminal complement complexes (TCC), C1 esterase inhibitor (C1INH), C3, C4, and C5.

The plasma concentrations of C3a and C5a antigens were determined by radioimmunoassay.[27] All samples were analyzed immediately after thawing, and none was refrozen. The samples were stored in different tubes for the different analyses. The plasma concentrations of TCC were determined by ELISA.[28] The ranges of C3a and C5a determinations in plasma from 25 healthy individuals were 156–230 ng/ml and <10 ng/ml, respectively.[29] The range of TCC determinations in plasma from 25 healthy individuals was 2.2–4.6 arbitrary units per ml.[29]

The complement components C1INH, C3, C4, and C5 were determined with a rocket immunoelectrophoresis technique.[30] The standard deviations of the duplicate determinations of C1INH, C3, C4, and C5 were less than 6% of the mean.

Pa_{O_2}, Pa_{CO_2}, *p*H, base excess (BE) and oxygen saturation were analyzed 1 min before the start of autotransfusion, 15 min after the start of autotransfusion, and 15 min after the completion of autotransfusion.

STATISTICS

Values are given as mean values and standard errors of the mean. The Wilcoxon test for paired comparisons was used for the comparison of plasma values 1 min before start of autotransfusion, 15 min after the start of autotransfusion, and 15 min after the completion of autotransfusion, and for comparison of complement variables in collected blood before and after filtration. The Wilcoxon test for paired comparisons was used also for comparison of the concentrations of complement variables in collected blood after filtration and in patient plasma 15 min after completed autotransfusion. The statistical analysis of C3a differences were performed with and without a logarithmic conversion on the values. Differences were considered as significant when P was less than 0.05.

Results

The mean blood volume collected through the drainage system was 260 ml (n = 18). Twelve of the 18 patients fulfilled clinical criteria for blood transfusion and received autologous blood. The mean autologous transfusion volume, including 40 ml ACD solution, given to these 12 patients was 390 ± 75 ml (range 280–500 ml).

The concentrations of C3a, C5a, TCC, C1INH, C3, C4, and C5 in plasma 1 min before and 15 min after the start of the transfusion of autologous blood, and 15 min after the transfusion, and the concentrations in the transfused blood before and after filtration are given in Table 1.

No significant alterations in plasma C3a, C5a, TCC, C1INH, C3, C4, and C5 could be observed in association with reinfusion of aspirated blood. The plasma concentrations of the complement components remained within the normal ranges 15 min after the start of reinfusion and 15 min after the completion of reinfusion of aspirated blood. In the aspirated blood the concentrations of C3a,

TABLE 1. Complement Variables

	C1INH (μg/ml)	C3 (mg/ml)	C4 (μg/ml)	C5 (μg/ml)	C3a (ng/ml)	C5a (ng/ml)	TCC (AU/ml)
Ex vivo blood analyses							
Collected blood before filtration	19 ± 3	3.0 ± 0.3	524 ± 90	152 ± 14	4,510 ± 1,710	28.4 ± 7.6	32.8 ± 9.6
Collected blood after filtration	17 ± 2	2.7 ± 0.2	497 ± 52	147* ± 12	4,210† ± 1,540	32.2† ± 8.8	28.8† ± 8.6
Patient plasma analyses							
1 min before start of autotransfusion	19 ± 2	3.9 ± 0.2	628 ± 86	207 ± 23	254 ± 75	<10	1.3 ± 0.4
15 min after start of autotransfusion	18 ± 5	4.1 ± 0.7	782 ± 208	223 ± 52	268 ± 90	<10	1.7 ± 0.6
15 min after completed autotransfusion	20 ± 4	3.4 ± 0.5	560 ± 117	182* ± 14	340† ± 98	<10†	1.2† ± 0.1

No significant changes were found between concentrations of C1INH, C3, C4, C5, C3a, C5a, or TCC 1 min before start of transfusion and 15 min after start of or 15 min after complete autotransfusion. Similarly, no significant differences were found in collected blood before or after filtration.

*,† Indicates statistically significant difference between collected blood after filtration and systemic plasma concentration 15 min after completed autotransfusion with a P value of <0.05 and <0.001, respectively.

Mean values and standard errors of the mean are given.

C5a, and TCC were increased compared to systemic plasma concentrations ($P < 0.001$). There were no significant differences before and after filtration.

The logarithmic conversion of the C3a values did not change the statistical significance ($P > 0.001$). The concentrations of C1INH, C3, and C4 in the aspirated blood were not significantly changed compared to those in plasma. The C5 concentration was lower in aspirated blood than in plasma ($P < 0.05$).

In association with the reinfusion of the aspirated blood no alterations in Pa_{O_2}, Pa_{CO_2}, pH, BE, or oxygen saturation were observed. All values remained unchanged compared to those found 1 min before the start of the reinfusion.

Discussion

The current study demonstrated that elevated concentrations of anaphylatoxins and TCC were infused into the patient when reinfusion of aspirated whole blood was performed. Also, the low concentrations of C5 in aspirated blood compared to systemic blood indicated that these complement products were consumed in an activation of the complement cascade. This activation of the cascade may occur in the collection system or in the wound, where the blood is in contact with extravasal tissues.

There were, however, no signs of systemic complement activation in association with the reinfusion. Arterial oxygen tension, pH, and oxygen saturation remained unchanged during and after the reinfusion of aspirated wound blood. In the current study a maximum of 500 ml aspirated blood was reinfused. No clinical signs of complications were seen. However, a larger volume of reinfused aspirated blood may influence circulatory and respiratory stability. Complications due to complement activation with anaphylatoxin and TCC formation depend both on the plasma peak concentration and on the duration of elevated concentrations of C3a, C5a, and TCC. In the current study, the blood amounts of reinfused anaphylatoxin and TCC were too small to significantly increase the concentrations in systemic blood. However, the concentrations of C3a, C5a, and TCC in the collected blood were of magnitude similar to those recorded in plasma in patients undergoing coronary bypass surgery, in septic patients, and in patients with acute pancreatitis.[29,31,32,**]

There are very few reports on elevated plasma C5a levels. Since C5a binds with great avidity to leukocytes,[20] it is difficult to detect concentrations higher than 10 ng/ml in plasma. The high concentrations of C5a in shed blood may activate leukocytes and platelets, which may lead to release of prostaglandins, leukotrienes, and free-oxygen radicals. Because C3a and TCC were within normal ranges in the circulation, dilution of released complement split products may be of importance in the current study. Future investigations will explain if it is possible to inhibit or prevent complement activation and anaphylatoxin release by some type of complement inhibitor. Anti-C5a antibodies tests *in vitro* have been documented to inhibit C5a-induced neutrophil chemotaxis.[33] Also, septic primates have been effectively treated with anti-C5a antibodies.[34,35] Earlier studies of collected blood have

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shown that free plasma hemoglobin and fibrin degradation products (FDP) are increased, whereas platelet count and fibrinogen (Factor 1) are decreased in collected blood compared to venous blood.[36,37]

The increased amount of free plasma hemoglobin in collected blood is due to hemolysis.††,‡‡ The hemolysis may be caused by mechanical damage to erythrocytes by negative pressure during suction, foaming, and contact with air and artificial surfaces. Complement activation and formation of TCC may also be an etiologic factor behind the red cell destruction. The changes in FDP and fibrinogen in collected blood also indicate that the coagulation system is activated. However, erythrocytes collected for retransfusion are unchanged regarding osmotic and energetic quality.§§ The 2,3-diphosphoglycerate (2,3-DPG) level and oxygen transport seem to be better than those in preserved blood.§§

In conclusion, the current study demonstrated that the complement cascade was activated as evidenced by biochemical changes in the aspirated blood, collected for autologous transfusion. However, during the transfusion of one unit of collected blood there were no signs of complement activation in the systemic blood, nor were there any adverse clinical signs or symptoms when this blood was reinfused.

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