

Anaphylactoid Reactions to Vascular Graft Material Presenting with Vasodilation and Subsequent Disseminated Intravascular Coagulation

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This report describes five patients who had immediate adverse reactions following placement of a vascular graft. All had unusually persistent decreases in systemic vascular resistance, and four of these patients had bleeding as an early manifestation of this reaction. In two of three patients in whom the graft was replaced, uneventful recovery followed. Both patients in whom the graft was not replaced died. Blood samples from two of the patients demonstrated activation of complement and of the kinin system, whereas control patients did not demonstrate increased levels of activation products from these cascade systems. Recognition of this syndrome is important to patient survival, which appears to depend on rapid replacement of the graft. (Key words: Anaphylactoid. Disseminated intravascular coagulation. Fibrin splint products. Kallikrein. Surgery, vascular. Systemic vascular resistance.)

TRANSIENT HYPOTENSION and decreased systemic vascular resistance (SVR) following restoration of blood flow after aortic reconstruction grafting are common. How-

ever, persistently decreased blood pressure is much less common and is usually related to surgical bleeding.¹ We describe an unreported complication following vascular graft replacement in five patients. Immediately after restoration of blood flow across the prosthetic graft, these patients developed persistent decreases in blood pressure associated with peripheral vasodilation and erythema followed later by clinically evident disseminated intravascular coagulation (DIC). In two of these cases, blood samples diverted from studies fortuitously in progress permitted biochemical verification of contact activation and DIC. To elucidate the mechanisms underlying this syndrome, we quantified a variety of mediators that induce hypotension, vasodilation, or DIC. We specifically looked for evidence of activation of kallikrein, complement, and arachidonic acid oxygenation pathways.²⁻⁴

Case Reports

Case 1. A 55-yr-old woman who had visceral angina 8 yr after receiving an aortofemoral bypass graft underwent aortoceliac and aortosuperior mesenteric revascularization on June 28, 1981 (table 1). The patient had no allergic history or evidence of sepsis or clotting disorder. Surgery proceeded uneventfully until blood flow through the bifurcation graft (preclotted knitted Dacron Milliknit® 10 × 5 mm, lot 12B; Golaski Medical Products, Philadelphia, Pennsylvania) was begun. As expected, SVR decreased from 1,800 before declamping to 1,200 postdeclamping (dynes · s · cm⁻⁵) but unexpectedly did not increase after 4–5 min.¹ It continued to decrease to 833 (dynes · s · cm⁻⁵) over 10 min without evidence of bleeding. Ten minutes later, blood began to leak rapidly from graft interstices (not particularly from the suture lines). Protamine was not given. White blood cell (WBC) and polymorphonuclear leukocyte counts (PMN) were within normal limits preoperatively and after the reaction occurred. Bleeding continued for 3 h, and laboratory evaluation confirmed DIC: fibrin split products (FSP) exceeded 200 µg/ml (normal, 0–10 µg/ml), platelet count was 70,000/ml⁹ of blood (normal, 150,000–400,000/ml⁹ of blood), and fibrinogen level measured 70 mg/dl (normal, 200–400 mg/dl). The graft was replaced with a graft of different material 3 h later, but not before the patient had been given 76 units of blood. Bacterial cultures of graft and patient's blood proved sterile. This amount of bleeding made us suspect "surgical" bleeding, but we observed bleeding from every raw surface and from graft interstices—a clear indication of bleeding from a nonsurgical cause. No laboratory evidence of a transfusion reaction was present (the units were retested; free hemoglobin was not present in serum or urine). Bleeding and hypotension markedly decreased after the graft was replaced. The patient died eight days later of complications from DIC.

Case 2. A 58-yr-old woman underwent aortic reconstruction on December 21, 1983 (table 1). The patient had no allergic history or ev-

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TABLE 1. Summary of Presentations and Outcomes of Patients with Immediate Adverse Reactions to Vascular Grafts

Patient	Age (yr)	Sex	Diagnosis	Graft Material	Manifestations of Reaction	Outcome
1	55	F	Visceral angina	Knitted dacron; Milliknit	Hypotension; decreased SVR; DIC-76 unit blood loss	Graft replaced; died 8 days later
2	58	F	Atherosclerotic aortoiliac disease	Knitted dacron; Milliknit	Hypotension; decreased SVR; cutaneous erythema; bleeding	Graft replaced; full recovery
3	70	F	Atherosclerotic aortic disease	Knitted dacron; Milliknit	Hypotension; decreased SVR; cutaneous erythema; DIC with bleeding	Graft replaced; full recovery
4	70	M	Atherosclerotic aortoiliac disease	Knitted dacron; Milliknit	Hypotension; decreased SVR; cutaneous erythema; bleeding	Died 2.5 weeks later
5	66	M	Aortic disease	Woven dacron; Meadox- Cooley	Hypotension; decreased SVR; DIC with bleeding	Died 2.5 hours later

idence of sepsis or clotting disorder. Surgery proceeded uneventfully until blood flow through the preclotted knitted Dacron graft (Milliknit® 12 × 6 mm, lot K8B) was begun. SVR was 1,800 before declamping and 860 (dynes · s · cm⁻⁵) 3 min after blood flow was restored. Seven minutes later, SVR continued to decline to 611 (dynes · s · cm⁻⁵), whereas usually, after 4 min of reactive hyperemia, SVR begins to increase.¹ No surgical bleeding was found and no protamine had been administered. Both units of blood given to this point were retested and found compatible with blood of the patient. Free hemoglobin was not present in the patient's blood or urine. WBC and PMN counts were within normal limits both prior to and 60 min into the reaction. Aerobic and anaerobic cultures of graft and blood proved sterile. The patient developed generalized erythema, and 10 min later bleeding occurred through the interstices of the graft. FSP exceeded 200 µg/ml. Fibrinogen level was 100 mg/dl and platelet count was 40,000/ml³ of blood. SVR was restored 3 h later after 12 units of blood had been transfused and the graft was replaced with another of different material. Her postoperative course was uneventful.

Case 3. On December 22, 1983, a 70-yr-old woman with no prior history of allergy, sepsis, or clotting disorder had aortic reconstruction for atherosclerosis (table 1). The procedure was uneventful until blood flow through the bifurcation graft (preclotted knitted Dacron-Milliknit® 12 × 6 mm, lot K8B) was begun. SVR decreased from 1,824 before removal of the aortic cross clamp to 1,200 (dynes · s · cm⁻⁵) 3 min after flow was restored and to 580 (dynes · s · cm⁻⁵) over 20 min. In addition, generalized erythema occurred, and bleeding through the graft interstices began. Protamine had not been given, a 20-unit blood loss ensued over the next 30 min, and DIC secondary to an abnormal reaction to the graft material was suspected. Evidence documenting DIC included presence of FSP > 200 µg/ml, a fibrinogen level of 40 mg/dl, and a platelet count of 50,000/ml³ of blood. No incompatible units of blood were found on retesting, and free hemoglobin was not present in blood or urine. Clot lysis studies confirmed that the patient did not have primary fibrinolysis. WBC and PMN counts were normal prior to and after this event. Aerobic and anaerobic cultures of graft and patient's blood proved sterile. The graft was replaced with one of another material and the remainder of the patient's hospital course was unremarkable.

Case 4. At another hospital, on January 20, 1984, a 70-yr-old man without a history of bleeding or allergy underwent aortic bifurcation grafting for atherosclerosis with a Meadox-Cooley Double-Velour Knitted Dacron 18 × 10 mm graft (lot #081010; Meadox Medicals, Inc., Oakland, New Jersey) (table 1). Soon after restoration of blood flow, SVR decreased, generalized erythema occurred, and excessive blood loss ensued through the interstices of the graft. Protamine had

not been given, and laboratory examination confirmed DIC: FSP exceeded 200 µg/ml, fibrin monomer (measured by a protamine sulfate method) was positive, and fibrinogen level was less than 40 mg/dl. It was assumed that the patient had a transfusion reaction, and he was given diuretics, fresh-frozen plasma, epinephrine, platelets, and 24 units of blood in the next 24 h. Over the next 2.5 weeks, 500–1,000 mg of iv epinephrine was required every 24 h (20–50 mg every hour) to keep the patient's SVR at normal levels. He died of acute respiratory distress syndrome. Graft material was not examined microscopically at autopsy.

Case 5. After the events described in Case 3, we obtained a manufacturer's list of purchasers of lot K8B 12 × 6 mm Milliknit® grafts. Our subsequent inquiries did not uncover similar cases that could be unequivocally related to Milliknit® grafts. However, a doctor (S.G.) at another medical center was puzzling over a recent unexpected death that occurred under circumstances similar to those we have just described.

On October 9, 1984, a 66-yr-old man underwent repeat abdominal aortic aneurysmectomy at a level superior to a previous vascular graft inserted in 1972 (table 1). Surgery was accomplished using a 25-mm woven Dacron graft. After the proximal clamp was released, values for hemodynamic variables became normal. Ten minutes later, and before protamine was given, severe hypotension occurred (mean arterial blood pressure decreased from 107 to 38 mmHg) despite the fact that normal cardiac output and filling pressures were maintained (cardiac index = 3.55 l · min⁻¹ · m⁻²; pulmonary artery occlusion pressure = 15 mmHg). SVR decreased from 1,437 to 239 (dynes · s · cm⁻⁵). Ten minutes later the patient had severe consumption coagulopathy. Values for FSP and fibrinogen were unavailable, but platelet count had fallen from 225,000/ml³ of blood prior to surgery to 119,000/ml³ of blood. After 1 g of ε-aminocaproic acid (EACA) was infused and blood replacement given, the coagulopathy was less severe as demonstrated clinically and by thromboelastography. A definite (but temporary) reduction of bleeding through the interstices was observed. Additional data on clotting status (obtained by Thromboelastograph D, Haemoscope Corp.) revealed lysis of the clot, consistent with fibrinolysis and DIC^{5,6} because during the coagulopathy total lysis of the clot was observed during the second minute of clot formation. After EACA infusion total lysis of the clot was observed during the fourth minute of thromboelastography. This patient, who did not have his graft replaced by another, subsequently died from DIC and uncontrolled bleeding.

Methods

Patients 2 and 3 (and two control patients, similar in age, with scheduled operative procedures, and vascular

TABLE 2. Levels of Substances in Blood in Relation to Time of Vascular Graft Placement

Patient No.	Sample Time in Relation to		C3a (ng/ml)	C4a (ng/ml)	C5a (ng/ml)	C3 (mg/dl)	C4 (mg/dl)	C100 (units/ml)	AP100 (units/ml)	Tx B ₂ (pg/ml)	Histamine (ng/ml) Fluorometric Measurements
	Placement of Proximal Suture Line* (min)	Restoration of Flow† (min)									
877 (Patient 2)	7/before	23/before	3,947	2,457	726	40	7.8	16	<2 §	190	NM
	7/after	9/before	47,943	2,620	225	46	9.4	20	10	NM	NM
	18/after	2/after	>50,000	2,284	910	62	11.6	28	7.0	1,200	NM
	26/after	10/after	35,454	2,358	787	46	6.1	20	<2 §	NM	NM
	(30/after graft removal)		3,659	2,302	806	58	8.7	11.5	<2 §	NM	NM
876 (Patient 3)	22/after	7/after	387	314	0.4	7.5	15.6	NM	5.2	175	1.32‡
	45/after	30/after	1,004	514	0.8	7.5	10.6	NM	NM	7,300	1.52‡
	(30/after replacement with another graft)		679	255	3.9	3.5	10.6	13.0	6.2	220	0.76‡
897 (Control)	60/before	43/before	450	543	1.2	62	20.1	160	3.5	195	<.20§
	10/after	7/before	482	457	2.3	107	5.5	120	2.0	220	<.20§
	24/after	7/after	580	683	0.5	92	15.6	8.5	6.2	340	<.20§
	47/after	30/after	701	887	0.0§	71	17.8	6.8	4.8	310	<.20§
904 (Control)	60/before	46/before	475	673	1.9	88	5.1	8.9	3.0	140	<.20§
	7/after	7/before	414	NM	0.0§	88	15.6	5.4	NM	170	<.20§
	21/after	7/before	435	456	1.1	75	10.6	8.5	2.5	230	<.20§
	44/after	30/after	544	713	1.2	71	11.6	13.0	3.5	210	<.20§

Tx B₂ = thromboxane B₂.

NM = not measured.

* Time in relation to insertion of graft and placement of proximal suture line without blood flow through the graft.

† Time in relation to the restoration of blood through the graft to

at least one limb.

‡ These three histamine levels (ng/ml) by radioenzymatic assay are 1.140 (1.32 in fluorometric), 1.638 (1.52), and 0.412 (0.76).

§ Below detectable levels of assay.

problems who were receiving their second graft, a graft made by Milliknit) gave informed consent to a protocol approved by the UCSF Committee on Human Experimentation. These patients had been participating in a study in which blood samples were obtained before and shortly after graft replacement (before major bleeding occurred; table 2 contains times). With patient permission, we used some of those blood samples to evaluate the possibility of a reaction to graft materials. A control graft material (Meadox vascular prosthesis, Veri-Soft, Dacron polyester) was used in certain assays described herein.

To determine contact activation of plasma and other biochemical markers, blood was collected in siliconized tubes containing citrate, and these tubes were immediately placed on ice; citrated plasma was separated in a refrigerated centrifuge and frozen in polypropylene tubes at -70° C until assayed. Control and patient plasmas were obtained similarly, and these plasmas were processed and stored in a similar manner. Replicate assays were performed within one week. Plasma kallikrein activity (expressed as picomoles of substrate hydrolyzed per milliliter of plasma per minute) was measured using the radioisotopic amidolytic assay described by Rodgers *et al.*⁷ Kallikrein activity was characterized by cleavage of the radio-labeled tripeptide substrate N-benzoyl-L-prolyl-L-phenylalanyl-L-arginyl-[¹⁴C]-anilide (New England Nuclear, Boston, Massachusetts) and by inhibition of amidolytic activity with soybean trypsin inhibitor (obtained from Sigma Chemical Co., St. Louis, Missouri) but not lima

bean trypsin inhibitor.^{7,8} Total plasma kallikrein activity was measured; plasma samples were not processed to remove inhibitors of kallikrein. To compare kallikrein activity generated in patient plasma *in vivo* with that generated *in vitro* by an artificial surface, a plastic petri dish (Falcon Plastics #3001, Oxnard, California) with a negatively charged surface was used. Concentrations of the plasma inhibitors of kallikrein (α_2 -macroglobulin and C₁-inhibitor) were determined immunologically by Ouchterlony gel diffusion using antibodies to these proteins (Calbiochem, San Diego, California). Additionally, quantitative measurement (nephelometric immunologic assay) of plasma C₁-inhibitor was performed in one patient (Smith Kline Bio-Science, Van Nuys, California).

Lipid mediators were recovered from aliquots of heparinized plasma by acidification to a pH of 3.5 with 0.1 M of acetic acid and extraction of the lipids on columns of octadecylsilane (Sep-Pak-columns, Waters Associates, Milford, Massachusetts) prior to quantification by specific radioimmunoassays for leukotrienes B₄, C₄, D₄, and E₄,⁹⁻¹¹ and for thromboxane B₂ and 6-keto-PGF₁ (Sara-gen, Inc., Boston, Massachusetts). Histamine in heparinized plasma was quantified by a sensitive fluorometric technique using α -phthalaldehyde as the fluorophore.¹² Values obtained in patient 3 and in one of the two control patients were later validated by histamine N-methyl transferase (HNMT)-based radioenzymatic assay with thin layer chromatography separation.^{13,14}

The degree of complement activation was assessed by

TABLE 3. Studies on Plasma Obtained during and after Coagulopathy of Patient 3

Kallikrein activity ($\text{pmol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$)	
During coagulopathy	4,228
1 h after graft replacement	108
Inhibition of plasma amidolytic activity (% reduction*)	
Soybean trypsin inhibitor	60% (patient 3); 100% (control)
Lima bean trypsin inhibitor	9% (patient 3); 6% (control)

* Data are expressed as the average of two experiments in terms of percent reduction from the original kallikrein activity (patient 3, original activity = $4,228 \text{ pmol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$; control patient, original value = $1,694 \text{ pmol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$). Contact activation of control plasma occurred in a glass test tube.

measuring the anaphylatoxins: C3a and C3a des-arginine (desArg), C4a and C4a desArg, and C5a and C5a desArg. Radioimmunoassay of these products was carried out at Upjohn Diagnostics (Kalamazoo, Michigan), using techniques developed by Hugli and Chenoweth,¹⁵ for radioimmunoassay measurements of C3a desArg and C5a desArg, and by Gorski.¹⁶ The method consists of first precipitating intact C3, C4, or C5 and then incubating the supernatant containing C3a desArg, C4a desArg, or C5a desArg with specific antibodies and ¹²⁵I-labeled purified peptides in a standard radioimmunoassay. Cross-reactivity of C3a desArg, C4a desArg, and C5a desArg was estimated to be less than 1%. Standard curves for each radioimmunoassay were determined using ranges from 50 to 1,000 mg/ml for C3a desArg, 80 to 2,600 for C4a desArg, and 10 to 400 for C5a desArg. Samples producing values that did not fall within these ranges were diluted appropriately. Alternate pathway (AP100) and classical pathway (C100) hemolytic activity was also measured by the Upjohn Diagnostic Laboratory.

The ability of graft materials to contact activate plasma was measured by incubating plasma with a 1 × 2 cm section of graft material in a siliconized glass tube. Plasma was taken from patient 3 and from two control patients. The blood from patient 3 was drawn four months after the reaction, at a time when the patient's condition was clinically stable. Kallikrein activity was then assayed at intervals. In some of the experiments, platelet-rich plasma or plasma reconstituted with neutrophils or monocytes was also tested. Control plasma was obtained from pre-matched patients with similar vascular problems.

Results

BIOCHEMICAL MARKER ASSAYS

After graft placement patients 2 and 3 had sixfold and 35-fold increases, respectively, in thromboxane B₂; such

increases did not occur in the control patients. Histamine levels were markedly elevated in patient 3 (table 2), but no alteration in leukotrienes or 6-keto-PGF₁ was noted. Insufficient plasma was available to evaluate these last three substances in patient 2. Patients 2 and 3 had substantial increases in C3a after graft insertion (table 2).

Table 3 summarizes results of contact activation studies of the plasma of patient 3. This plasma was assayed for total kallikrein activity during the time of coagulopathy and 1 h after removal of the initial graft and replacement with a different prosthesis. At the time of bleeding and hypotension, plasma kallikrein activity was markedly elevated at $4,228 \text{ pmol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$; 1 h after removal of the questionable graft material, plasma kallikrein activity had decreased to $108 \text{ pmol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$, a value similar to that observed in plasma of control patients (*i.e.*, $154 \text{ pmol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$). These results are similar to results obtained in normal controls.⁷ For comparison, exposure of normal plasma to a negatively charged plastic surface resulted in kallikrein activity of $920 \text{ pmol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ after 10 min of exposure. Contact activation of control plasma by a glass surface generated kallikrein activity of $1,694 \text{ pmol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ after 10 min of exposure.⁷ This comparison of the patient's plasma kallikrein activity with that generated *in vitro* with normal plasma and two artificial thrombogenic surfaces (plastic or glass) indicates that substantial contact activation occurred *in vivo* at the time the patient's blood was exposed to the suspect graft material. Also shown in table 3 are results of experiments characterizing the amidolytic activity in this patient's plasma.

When the plasma sample from patient 3 (obtained during DIC, kallikrein activity $4,228 \text{ pmol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$) was incubated with soybean trypsin inhibitor (2 mg/ml, 20 min in a siliconized tube), kallikrein activity was inhibited 60%. Activity was not significantly inhibited when plasma was incubated with lima bean trypsin inhibitor (50 $\mu\text{g}/\text{ml}$, 20 min in a siliconized tube). In contrast, when normal plasma was exposed to glass and then treated with soybean trypsin inhibitor, kallikrein activity was substantially inhibited (table 3), as has been previously reported.² Inhibition by soybean trypsin inhibitor, but not lima bean trypsin inhibitor, suggests that the majority of proteolytic activity in the sample from patient 3 was kallikrein.^{2,7} The observation that inhibition by soybean trypsin inhibitor was incomplete suggests that other proteases capable of cleaving the radiolabeled substrate may also be present.⁷ Another interpretation of these data is that increased amounts of α_2 -macroglobulin complex were formed in this patient. This complex may have resulted from decreased levels of functional C₁-inhibitor activity.

The percent conversion of plasma prekallikrein to kallikrein can be calculated using the radioisotopic assay technique and a standard curve generated with purified kallikrein.⁷ Assuming a plasma prekallikrein level of 15

$\mu\text{g/ml}$,¹⁷ the plasma of patient 3 exhibited approximately 26% conversion of prekallikrein to kallikrein.

STUDIES OF *IN VITRO* CONTACT ACTIVATION

A segment of control graft and a segment of the suspect graft from patient 3 were tested for their ability to contact activate either normal plasma or platelet-rich plasma. The material suspected of triggering the reaction in patient 3 had been removed from the original graft. This segment had not been preclotted or placed in the patient. Control plasma was obtained from age-matched patients who had similar vascular problems. Minimal contact activation of normal (control) plasma occurred in the presence of either graft material (*i.e.*, plasma kallikrein activity rose less than 25% above baseline values). When plasma obtained from patient 3 four months after the reaction was assayed in the presence of the suspect graft material, significant generation of kallikrein was observed (fig. 1). The amount of kallikrein activity generated by plasma from patient 3 was similar to that generated by normal plasma exposed to a negatively charged plastic surface for 10 min ($920 \text{ pmol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$).⁷ Addition of physiologic concentrations of platelets, neutrophils, or monocytes to the plasma of patient 3 resulted in kallikrein activity that was approximately 10% higher than that in plasma alone. Plasma from control patients did not generate kallikrein, nor did plasma of patient 3 when exposed to the control graft material.

To determine if patient 3 might be deficient in inhibitors of kallikrein, such as α_2 -macroglobulin and C_1 -inhibitor, Ouchterlony immunodiffusions were performed. Both α_2 -macroglobulin and C_1 -inhibitor were present in the plasma. A nephelometric immunologic assay to quantitate plasma levels of C_1 -inhibitor revealed patient 3 had 100% of normal C_1 -inhibitor level (Smith Kline Bio-Science).

The amount of thromboxane B_2 generated by platelets from patient 3 increased from a baseline of 10,000 and 12,500 pg per 8×10^8 platelets to 32,500, 54,500, and 62,500 pg per 8×10^8 after 2, 10, and 30 min of exposure to the suspect graft material. This contrasts with the amount of thromboxane B_2 generated by platelets from the plasma of a control patient, which did not change appreciably when exposed for equal periods of time to the suspect graft material.

Discussion

An adverse reaction to graft material due to release of chemical mediators of hypersensitivity and DIC is rarely suspected, and no report of such a reaction to vascular grafting material exists in the literature. The tendency not to suspect an adverse reaction to graft materials is reinforced by the historical inertness of the substances

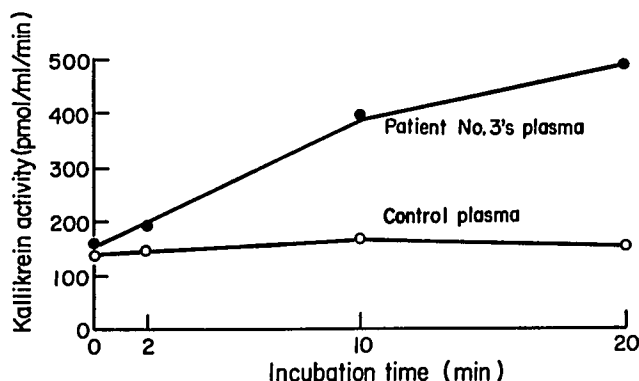


FIG. 1. Significant kallikrein activity occurred when a piece of vascular graft material suspected of causing contact activation of blood coagulation in a patient (described in case 3) was incubated with plasma taken from that patient four months later. No kallikrein activity resulted from incubating the suspect material with plasma from control patients.

used—either woven or knitted Dacron.*** Because graft materials are exposed only to saline, or substances in the patient's own blood, it is logical to assume that they would remain inert. Although the matrix of both types of grafts used was Dacron, the combinations and identity of plasticizers, *etc.*, used to bind and combine the Dacron together are the proprietary information of each company and presumably different.*** If this reaction were triggered by hypersensitivity, it presumably was to those plasticizers because one graft material triggered the reaction, whereas contact with the other company's graft material did not trigger the reaction.

Plasma kallikrein levels were elevated during the reactions in the two patients described above; in addition, in one patient, plasma obtained four months after the reaction generated kallikrein activity when incubated *in vitro* with the questionable graft material but not control graft material. Such biochemical markers and *in vitro* kallikrein activation were not present in plasma of control patients.

The relationships between contact activation, the coagulation and fibrinolytic mechanisms, and the complement and kinin pathways are complex (fig. 2). Factor XIIa and kallikrein are important regulators of these systems; when Factor XII is activated by an abnormal surface or by complement, kallikrein is generated and coagulation, fibrinolysis, kinin formation, and complement activation are amplified.²⁻⁴

The possible *in vivo* roles of contact activation of coagulation pathways in blood are controversial. *In vitro* Factor XII, prekallikrein, and high-molecular weight kininogen assemble upon surfaces to initiate intrinsic co-

*** Tyson RR, Derrick BM: Vascular prostheses today. Resident and Staff Physician 29:104-110, 1983

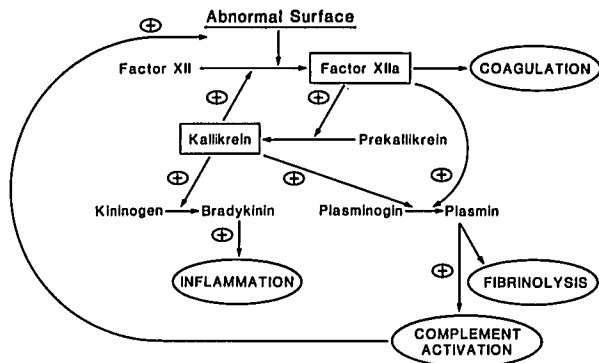
INTERACTIONS BETWEEN THE COAGULATION KININ-GENERATING,
FIBRINOLYTIC AND COMPLEMENT SYSTEMS

FIG. 2. Relationships between contact activation, coagulation, fibrinolysis, kinin, and complement pathways. In this model, Factor XIIa and kallikrein are key protease regulators and depicted within boxes. Factor XII is activated either by an abnormal surface directly or by complement activation. This activation results in generation of kallikrein and initiation of coagulation. Kallikrein further increases activation (shown as a \oplus arrow) of Factor XII, generates bradykinin from high-molecular weight kininogen, and activates plasminogen, leading to fibrinolysis. Factor XIIa also generates plasmin. In addition to lysing fibrin, plasmin can also cleave complement factor C_3 , further increasing or initiating complement activation.

agulation, fibrinolysis, kinin formation, and complement activation.^{2,17} However, patients deficient in these proteins have no clinically apparent disorder. Massive release of kinins has been reported in septic shock¹⁸ and in the carcinoid syndrome.¹⁹ Localized activation of coagulation has been described in aortic aneurysm²⁰ and giant hemangiomas.²¹ The placement of a vascular prosthesis that has not been endothelialized might be considered to place patients at risk for DIC initiated by contact activation of plasma by the graft material. However, the constellation of clinical events, including kinin release and DIC, has not been noted in patients undergoing prosthetic vascular graft surgery in the absence of sepsis, transfusion reaction, or other predisposing conditions for DIC. Endotoxin and complement activation could also explain this constellation of events. However, there was no evidence of gram-negative sepsis and no other explanation for activation of complement. Therefore, we are left with the possibility that the graft material was responsible, and the rapid improvement in clinical course following graft replacement makes this likely. We have observed five instances of this syndrome that were associated with two types of graft material. Three of the five reactions occurred with two different lots of Milliknit® grafts, and two with other forms of Dacron.

Kallikrein activity was markedly increased in plasma obtained during the reaction. This activity was identified by cleavage of the tripeptide substrate and typical inhi-

bition profile with soybean and lima bean trypsin inhibitors. Other investigators have noted a larger percentage of soybean trypsin inhibitor-resistant kallikrein activity than that noted in this study.²² In any event, increased kallikrein activity indicates that contact activation of plasma was occurring. After placement of the graft, circulating levels of kallikrein were found to be higher in plasma from patients 2 and 3 but not in that from the two control subjects undergoing identical operations for identical indications. Blood was not collected using optimal techniques for measuring specific vasoactive mediators. Nevertheless, because both control and patient plasmas were handled similarly, the increased kallikrein activity and complement and thromboxane levels seen in the patient plasmas in association with development of DIC are not likely due to a collection or storage artifact and suggest a pathogenic role for these mediators. *In vitro* studies confirmed that prekallikrein in the plasma of one patient was activated by the graft material that had elicited the hypersensitivity reaction, but not by material of a different type.

Activation of prekallikrein leads to production of kinin,^{2,17} activation of the complement system, and possibly DIC. The involvement of this chain of events in the observed clinical syndrome is further substantiated by the fact that levels of complement fragment C3a increased after insertion of the graft. Although it is not possible to perform statistical evaluation between two data points, the difference between 580 and greater than 50,000 is striking. The human complement system consists of proteins activated by either classical or alternative pathways. Measurement of activated products of C3 and C5 reflects activation of either pathway, whereas activation of C4 marks participation of the classical pathway.^{15,16} Thus, in patients 2 and 3 alternate pathway and possible classical pathway activation are demonstrated (table 2); this activation was not seen in control patients undergoing similar operations. The levels of C3a seen after graft insertion in patient 3 are high. Complement activation by Dacron has been reported *in vitro*.²³ Once activated, these complement proteins are potent anaphylatoxins with the ability to cause contraction of smooth muscle, increase vascular permeability, and promote formation of lipid mediators.^{14,24,25} The massive increases in levels of thromboxane B₂ seen during the reaction in patients 2 and 3 (table 2) further support this hypothesis. Thromboxane B₂ is a relatively biologically inactive metabolite of thromboxane A₂. Thromboxane A₂ derived from platelets induces irreversible platelet aggregation, is a potent stimulus for contraction of vascular smooth muscle, and promotes release of other vasoactive mediators.²⁴ It has been difficult to demonstrate the release of other arachidonic acid metabolites (*e.g.*, leukotrienes) into the blood during immunobiologic reactions. Thus, our failure

to demonstrate elevated leukotriene levels during the reactions may reflect limitations of the assays rather than lack of activation of the lipoxygenase pathway. Resolution of the syndrome when the graft was replaced, and the occurrence of death when it was not, are also consonant with the hypothesis of contact activation of plasma by graft material, followed by kinin generation, activation of the complement system, formation of lipid mediators, and intravascular coagulation.

It is unclear why these patients are susceptible to contact activation of plasma by a prosthetic graft. None of these five patients received protamine sulfate prior to initiation of the reaction; consequently, an anaphylactic or anaphylactoid reaction to protamine sulfate or a protamine sulfate-graft complex is not probable.²⁶ Although critically reduced concentrations of inhibitors of kallikrein would be one explanation, the inhibitors C₁-inhibitor and α_2 -macroglobulin were present at normal levels, judged by an immunoassay. However, this observation does not exclude the possibility that these inhibitory proteins might be dysfunctional. This latter possibility was not investigated. Alternatively, our patients could have a contact activating cofactor that becomes functional when adsorbed to grafts. Another possibility is that substances used to weave, polymerize, or sterilize certain grafts are capable of activating contact pathways in specific patients. This hypothesis would be supported by the success obtained after replacing one graft with that of other manufacturers. It is also possible that bacterial or endotoxin contamination of a graft could lead to development of DIC. The observation that the offending graft activated the kallikrein pathway *in vitro* in patient 3 but not in two control patients argues against such a mechanism. At least patient 3 appears to have a unique hypersensitivity to that specific graft.

Bleeding after vascular graft replacement is most often attributed to surgical technique. The fact that the anesthesiologist decided that surgical technique was not the cause for bleeding in the first three patients led us to search for another mechanism for this bleeding. In all five of these cases DIC occurred, initiated we believe by contact activation of plasma by graft material, kinin generation, activation of the complement system, formation of lipid mediators, and intravascular coagulation. These reactions were manifested initially by persistently decreased SVR and erythema, which may be mediated by release of kinins and anaphylatoxins of the complement cascade. Markedly higher plasma kallikrein activity and levels of complement fragment C3a during the episode with a decrease toward normal levels after replacement of the graft support this hypothesis. Bleeding through the graft may also occur because of accelerated fibrinolysis because kallikrein activates plasminogen.^{4,27} This mechanism may have contributed to rapid blood loss in patient

5. The primary reasons for suspecting contact activation initiation of DIC were the association of hypotension and erythema with flow into the new graft, laboratory evidence of DIC in the absence of any other explanation for DIC (sepsis, transfusion reaction, massive tissue damage, *etc.*), and resolution of DIC by switching to a graft made with other materials. Quick recognition of such reactions is important to survival, which appears to depend on rapid replacement of the graft.

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