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PUBLICATIONS

C5 Chemotactic Fragment Induces Leukocyte Production of Tissue Factor Activity

A LINK BETWEEN COMPLEMENT AND COAGULATION

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ABSTRACT Complement-activated human plasma causes generation of tissue factor in human leukocytes. This phenomenon appears to be related to the fifth component of complement (C5) as demonstrated by the use of C5 deficient-plasma and suppression of activity with antibody to C5. Isolation of the chemotactic factor from activated serum or trypsinization of purified C5 reproduces the phenomenon. These data provide evidence for a direct link between complement products and activation of the coagulation system. Because chemotactic peptides from C5 can be generated by a variety of enzymes, our findings suggest a relationship between complement, coagulation, and inflammation.

INTRODUCTION

Blood coagulation has long been recognized to be a part of the inflammatory response. Examples include the defibrination syndrome and the demonstration of fibrin deposits, leukocytes, and complement components in tissues of patients with a variety of inflammatory conditions. A similar process is found in experimental immune complex injury of kidneys and blood vessels, conditions which are associated with complement activation. Several of these in vivo reactions can be diminished or abolished if coagulation is impeded or leukopenia induced (1–3).

Fibrin deposition may result by activation of either the intrinsic- or extrinsic- (tissue factor) dependent pathway. Leukocytes can be induced to generate tissue factor activity by incubation with bacterial endotoxin, phytohemagglutinin, antigen-antibody complexes, platelets, and renal dialysis membranes (4–7).

In the following report we demonstrate that activation of the complement system in human plasma, and in particular of the fifth component of complement (C5), causes a marked increase in tissue factor content of human leukocytes.

METHODS

Leukocytes from human blood were prepared after sedimentation with dextran, by the method of Boyum as described (6). Platelet-rich plasma was prepared as previously described (6). Sterile heparinized plasma was obtained from blood anticoagulated with 5 U heparin/ml blood. The blood was centrifuged at 2,000 *g* for 20 min, the plasma pipetted off, and this step repeated. Plasma deficient in the fifth component of complement was obtained from the previously described patient (8).

Sterile, pyrogen-free, renal dialysis membranes (8 cm^2) (Gambro Inc., Newport News, Va.) were introduced into sterile plastic tubes (Falcon Plastics, Division of BioQuest, Oxnard, Calif.). The following reagents were obtained:

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zymosan, inulin, limulus lysate, trypsin, soybean trypsin inhibitor, Dextran 250 (Sigma Chemical Co., St. Louis, Mo.), antibody to C5 and the third component of complement (C3) (Behring Diagnostics, American Hoechst Somerville, Corp., N. J.). The chemotactic factor of C5 was prepared from serum treated with 1 M epsilon aminocaproic acid (Sigma Chemical Co.) and zymosan 10 mg/ml. After acidification, the sera was chromatographed on a Sephadex G-100 column (Pharmacia Fine Chemicals, Piscataway, N. J.) (9), the C3 fragment was prepared as described (9), and cobra factor (10) and citrated saline (6) were prepared as described. Purified C5 was prepared by the method of Nilsson et al. (11).

Gamma globulins from anti-C5 sera (2980F, University of Connecticut) and control sera were the breakthrough fraction from DEAE-cellulose columns. The sera heated to 56°C for 30 min, dialyzed against pyrogen-free 0.02 M phosphate buffer pH 6.2, were applied to a DEAE-23 (Whatman Inc., Clifton, N. J.) column equilibrated with same buffer, and the breakthrough fraction collected. Antisera to C5 (2341B) and C3 (5104C) were used after heating at 56°C for 30 min, dialyzing for 48 h, and adsorption with tricalcium phosphate (6). Meticulous care was taken to preserve sterility and apyrogenicity (6). Tests for endotoxin were as described (12).

Chemotaxis was measured in the modified Boyden chamber and results expressed as ED_{50}[1] (9), which is the amount of chemotactic factor that gives 50% of a maximal chemotactic response for that factor. The coagulant activity was assayed by the two-stage assay (6); the standard preparation of thromboplastin (Difco Laboratories, Detroit, Mich.) was arbitrarily assigned a value of 1,000 U/ml (6).

RESULTS

The first experiments have involved the incubation of human peripheral blood leukocytes with human plasma and other factors. As shown in Table I, incubation of human leukocytes with normal human plasma caused moderate generation of tissue factor activity. However, complement activation of the plasma, either by addition of cobra venom factor, zymosan, inulin, or contact with renal dialysis membranes, in each case, led to the production of much higher amounts of tissue factor. To assess whether platelets were responsible for the generation of tissue factor, leukocytes were incubated with membrane-contacted heparinized plasma that was platelet poor or platelet rich. As the data in Table I indicate, generation of tissue factor occurs with plasma whether or not platelets are present. Furthermore, washed leukocytes, in medium 199, contacted with membrane generated no activity. The presence of a chelator (EDTA) of divalent metal cations, thus blocking complement activation, or the use of preheated plasma (56°C, 0.5 h) before contact with dialysis membranes, prevented appearance of tissue factor activity. Addition of antibody to C5 after activation of plasma with dialysis membranes also prevented, significantly, generation in leukocytes of tissue factor activity.

The ability of the C5 chemotactic factor, which was isolated from activated human serum, to cause generation of tissue factor from leukocytes is shown in

TABLE I

Tissue Factor Production by Leukocytes Contacted with Complement Activation Products

Material incubated with leukocytes	Generation of tissue factor activity by leukocytes			
	Expts.	A	B	C
			U	
Plasma		23	35	11
+ Cobra factor*		180	240	140
+ Zymosan		260	160	160
+ Inulin		240	350	140
Platelet-poor plasma		27	18	25
+ Renal dialysis membrane‡		82	56	75
Platelet-rich plasma		32	65	22
+ Renal dialysis membrane		157	100	80
Heated plasma + renal dialysis membrane		3	3	—
EDTA (10 mM) plasma + renal dialysis membrane		1	1	—
EDTA plasma + renal dialysis membrane, then dialyzed		1	—	—
Medium 199 + renal dialysis membrane		1	1	—
Dialysis membrane activated plasma				
+ saline		60	50	—
+ Anti C5		10	10	—
C5 chemotactic factor				
+ Control		104	107	200
+ Anti C5		64	18	74
+ Control		128	36	106
+ Anti C3		184	43	136
C5 (40 μg) + trypsin§		400	340	360
C5 (40 μg)		180	180	250

Approximately 2×10^7 leukocytes/ml were incubated with plasma or fractions thereof and medium 199. After 16 h at 37°C, samples were centrifuged at 2,000 *g* for 10 min, the plasma was decanted and the leukocytes washed four times in citrated saline, brought to initial volume, sonically disrupted, and assayed for tissue factor activity.

* Plasma treated with cobra factor (10 U/ml, 0.5 h), zymosan (5 mg/ml, 1 h), or inulin (10 mg/ml, 1 h) (40% of final incubation mixture).

‡ Plasma (1.2 ml for 8 cm^2 renal dialysis membrane) was incubated at 37°C for 3 h (80% of final incubation mixture).

§ Purified C5, 100 μg, was incubated with 1 μg trypsin for 2 min, at 37°C followed by soybean trypsin inhibitor, 5 μg, for 0.5 h at 25°C.

[1] *Abbreviation used in this paper*: ED_{50}, the amount of chemotactic factor that gives 50% of a maximal chemotactic response for that factor.

Fig. 1. A linear dose response is demonstrated. In contrast, C3 fragments with similar chemotactic activity had very weak stimulatory effect. To rule out that the stimulatory effect was caused by the presence of contaminating bacterial endotoxin, two experiments were done. Titration of the C5 chemotactic factor preparation indicated 0.1 μg endotoxin present when 40 ED_{50} U of chemotactic factor were used. This amount of endotoxin caused about 55 U of tissue factor activity to be elaborated from leukocytes. With higher amounts of endotoxin (up to 1,000-fold), a plateau of tissue factor activity was reached at about 60 U (Fig. 2). Furthermore, the addition of three different antibody preparations led to 49, 63, and 83% suppression in generation of tissue factor activity, whereas the addition of antibody to the third component of complement had no inhibitory effect (Table I). These data reinforce the conclusion that the generation of tissue factor is not caused by the presence of contaminating bacterial endotoxin. Furthermore, the antibody to C5 had no effect on the stimulatory activity of endotoxin.

The data in Table I show that the trypsinization of C5 also produces an activity that will induce the generation of tissue factor. The control for the preparation, intact C5, also resulted in the generation of significant amounts of tissue factor, albeit less than when the trypsinized C5 preparation was used. These findings are probably explicable by the release of a C5-cleaving enzyme from lysosomal granules of leukocytes (13).

The possibility that complement activation is required for plasma to induce formation in leukocytes of tissue factor was explored with the use of human plasma genetically deficient in C5. The incubation of

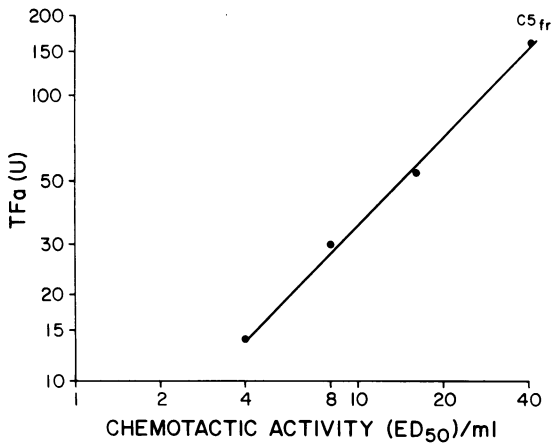

FIGURE 1 Stimulatory effect of C5 chemotactic factor. Approximately 2×10^7 leukocytes/ml were incubated with various concentrations of chemotactically active C5 in medium 199. After incubation for 16 h at 37°C, the leukocytes were sonically disrupted and assayed for tissue factor activity (TFa).

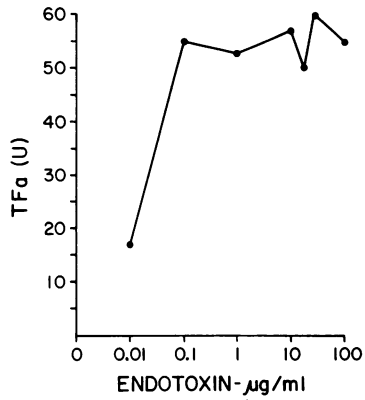

FIGURE 2 Stimulatory effect of endotoxin. Approximately 2×10^7 leukocytes/ml were incubated with endotoxin in medium 199. Same conditions as for Fig. 1. TFa, tissue factor activity.

this plasma with renal dialysis membranes did not cause generation of tissue factor activity by leukocytes. However, the reconstitution with control plasma of the C5-deficient plasma resulted in the production of tissue factor from leukocytes (Fig. 3).

DISCUSSION

Fragments of the C5 molecule play a major role in inflammation-inducing chemotactic, and anaphylatoxic reactions; membrane and shape changes in leukocyte and release of leukocytic enzymes (13–15).

A direct linkage between the complement and coagulation system has been difficult to demonstrate. Although rabbits deficient in the sixth component of complement (C6) have some coagulation abnormalities, which are correctible with the addition of purified C6 (16), similar abnormalities have not been found in humans (17).

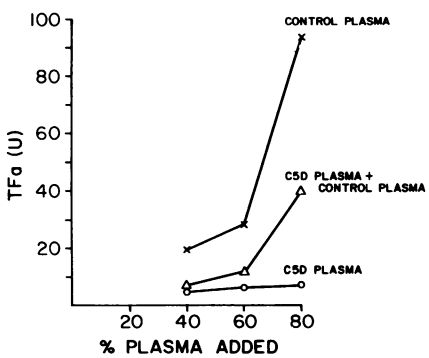

FIGURE 3 Lack of stimulatory effect of C5-deficient plasma (C5D). C5D was incubated with dialysis membranes alone, or 1:1 with control plasma. Plasmas were then incubated with leukocytes as described for Table I. TFa, tissue factor activity.

A brief report suggested that C3b may induce leukocyte coagulant activity. However, no evidence was provided that contamination with endotoxin was not the inducing agent (18).

The results presented here, taken together, strongly suggest that complement's C5-derived fragments can induce generation of tissue factor in leukocytes and point to a direct link between complement activation and triggering of the clotting system via the extrinsic pathway.

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