The Moran Foundation
DEPARTMENT OF PATHOLOGY
BAYLOR COLLEGE OF MEDICINE
TEXAS MEDICAL CENTER
HOUSTON, TEXAS 77025

March 3, 1986

I. TITLE OF RESEARCH PROPOSAL: The Role of Complement Activation in Myocardial Infarction (2-85-0816)

II. PRINCIPAL INVESTIGATOR: William R. Bennett, M.D.

ASSOCIATE INVESTIGATORS: James B. Young, M.D.
Roberto Bolli, M.D.
David H. Yawn, M.D.
Phillip J. Migliore, M.D.
Robert Roberts, M.D.

III. BACKGROUND INFORMATION AND STUDY DESIGN:

During the course of our investigation, it became apparent that when TPA (tissue plasmagen activator) was administered to patients with acute myocardial infarction, there was a marked rise in C_{3a}, C_{4a}, and C_{5a}. The increase levels of complement anaphylatoxins persisted for at least an hour after the administration of TPA. At this time we have only studied a small number of patients and would like to pursue the study of the activation of complement due to the administration of tissue plasmagen activator factor. Therefore we request an extension of our original grant to study this phenomenon. We intend to study additional patients who have received tissue plasmagen activator. We also plan to conduct <u>in vitro</u> experiments with the addition of tissue plasmagen activator factor to plasma to delineate further the extent of complement activation by TPA.

IV. See attached COMPLEMENT WORK SHEET.

V. BUDGET REQUIREMENTS:

Kits for radioimmunoassay for C_{3a}, C_{4a}, and C_{5a}. Total: $1500.

Estimated technician time is $200.

Total budget request is $1700.

VI: BIOLOGRAPHY:

See attached abstract.

TIMI

D. COMPLEMENT WORK SHEET

Pt's Name: _____ Hosp Number: _____ Date: _____

PLEASE FILL IN THE FOLLOWING TIMES:

(1) Onset of pain: _____

(2) Time TPA started: _____

(3) Time TPA finished: _____

SAMPLES TO BE OBTAINED AT THE FOLLOWING INTERVALS:

(1) Immediately upon arrival in ER of TIMI personnel
(2) Immediately before TPA started
(3) 5 min after TPA started
(4) 20 min after TPA started
(5) 1 h after TPA started
(6) 2 h after TPA started
(7) 3 h after TPA started
(8) 4 h after TPA started
(9) 5 h after TPA started
(10) 6 h after TPA started

SAMPLE NUMBER	TIME OBTAINED
#1	_____
#2	_____
#3	_____
#4	_____
#5	_____
#6	_____
#7	_____
#8	_____
#9	_____
#10	_____

PLEASE COLLECT 4 - 5 CC OF BLOOD FOR EACH SAMPLE. KEEP ON ICE AND PLACE IN PURPLE TOP TUBES WITH WHITE POWDER INSIDE.

TURN IN AT 3RD FLOOR STAT LAB (FONDREN-BROWN BUILDING) WITH GENERAL LAB SLIP MARKED: "COMPLEMENT STUDY C_3a, C_4a & C_5a", PATIENT'S NAME AND DATE.

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A

Name Roberto Bolli, M.D.

Address Baylor College of Medicine; Section of Cardiology
6535 Fannin; Mail Station F-905
Houston, Texas 77030

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RECOMBINANT TISSUE PLASMINOGEN ACTIVATOR INDUCES COMPLEMENT ACTIVATION. WR Bennett*, R Bolli, A Raizner**, C Pratt, P Migliore*, J Young, D Yawn*, R Roberts**, Baylor College of Medicine, Houston, TX.

Tissue plasminogen activator (rt-PA) is undergoing intense investigation in patients with acute myocardial infarction (AMI) subsequent to initial trials showing it to be an effective thrombolytic agent. Since rt-PA converts plasminogen to plasmin, an activator of complement (C_1), we tested the hypothesis that rt-PA induces complement activation. Seven patients with AMI underwent coronary angiography within 9 h of onset of symptoms; 3 received rt-PA (80-100 mg i.v.) and 4 did not. Blood samples collected in EDTA, on the average 15 min before and 45 min after initiating rt-PA were immediately put on ice, as were samples during comparable intervals in controls (Group I). Serum complement levels were assessed by radioimmunoassay for components C_3a, C_4a and C_5a. In patients receiving rt-PA all samples were obtained before reperfusion was detected by angiography.

($\bar{X}$ ± SEM) (¶P < .05 vs Group I)	C_3a	C_4a	C_5a
Normal Range (ng/ml)	144-166	136-680	14-14.8
Group I (n=4) (no rt-PA)	263±61	427±106	11.2±0.6
Group II (n=3) Before rt-PA	295±85	409±46	11.8±2.7
After rt-PA	597±125¶	2088±392¶	42.3±21.6

In the absence of rt-PA, C_3a was slightly increased but C_4a and C_5a were normal. Thus, rt-PA causes a rapid, striking increase in 3 components of the complement system. Serum complement levels may be a useful means of detecting activity of rt-PA and the level of activity may reflect the extent of thrombolysis.

BOTH THIS FORM AND THE FORM LETTER OF TRANSMITTAL MUST BE SIGNED BY A MEMBER (RULE 2)

Craig M. Pratt, M.D.
(please type name)

MEMBER'S SIGNATURE .....

Revised October 1985

The Moran Foundation
DEPARTMENT OF PATHOLOGY
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William Bennett, M.D.
Fellow in Cardiology
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Department of Internal Medicine
Baylor College of Medicine
One Baylor Plaza
Houston, Texas 77030

April 23, 1986

Dear Dr. Bennett:

At a recent committee meeting, the Scientific Advisory Committee of The Moran Foundation voted, on behalf of the Foundation's Board of Directors, to:

1. Approve a recently submitted addendum to project 2-85-0016, and to support it with the amount requested ($1,700.00). To minimize confusion, the addendum request will be titled and coded with the original project titled "Activiation of the Immune System in the Acute Myocardial Infarction". The code of the latter will be modified to 2-85-0016A.

2. Approve your new protocol entitled, "The Role of Complement Activation in Rejection of Heart Transplants and Myocarditis", and to support it per your request with $5,820.00. This project will be coded 2-86-0020.

Details of budgetary management should be worked out with Mr. Wes Moreland in the Baylor Department of Pathology Office (799-4661). Any specific item(s) not listed in your budget request which may be needed later for continuation of your project should be submitted to the Research Director for approval prior to purchase, especially if it is a capital acquisition.

As you know, progress reports summarizing the status of your investigative efforts are required annually and should be submitted by December 1. Continuing support of your project is, at least in part, dependent on this report. Any resulting publications should, of course, include a statement reflecting support received from the Foundation.

Sincerely yours,

Philip J. Migliore

Philip J. Migliore, M.D.
Research Director
The Moran Foundation

c: Jack L. Titus, M.D., Ph.D.
Mr. John Moran
Mr. Wes Moreland
Dr. David Yawn

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December 12, 1986

Simon Dack, M.D., F.A.C.C.
Editor-in-Chief
Journal of the American College of Cardiology
52 Vanderbilt Avenue, New York, NY 10017

Dear Dr. Dack:

Enclosed please find the original and two copies of the manuscript entitled "Activation of the Complement System by Recombinant Tissue Plasminogen Activator" by Bennett et al. We would like this paper to be considered for publication in the Journal of the American College of Cardiology. Although this study was presented in preliminary form at the National Meeting of the American Federation For Clinical Research, Washingtion, D.C., in May 1986, the manuscript has not been submitted and is not being considered for publication in another journal.

Thank you very much in advance for your consideration.

Sincerely,

Roberto Bolli

Roberto Bolli, M.D.
Assistant Professor of Medicine
Director, Experimental Animal Laboratory,
Methodist Hospital
Director, Coronary Care Unit,
VA Medical Center

ACTIVATION OF THE COMPLEMENT SYSTEM BY RECOMBINANT TISSUE PLASMINOGEN ACTIVATOR

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Roberto Bolli, M.D.

From the Section of Cardiology, Department of Medicine and the Department of Pathology, Baylor College of Medicine, Houston, Texas, 77030.

Running Title: Plasminogen Activator and Complement

2-85-0016

This study was supported in part by the Moran Foundation and NHLBI Contract HV38034.

Presented in preliminary form at the AFCR National Meeting, Washington, D.C., May, 1986.

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ABSTRACT

Recent trials have shown that recombinant tissue plasminogen activator (rt-PA) is an effective thrombolytic agent in patients with acute myocardial infarction. Since rt-PA converts plasminogen to plasmin, which is known to activate complement *in vitro*, we tested the hypothesis that rt-PA can induce *in vivo* activation of complement. Studies were performed in 12 patients with acute myocardial infarction. Six patients (controls) had patent coronary arteries and did not receive rt-PA; these patients had normal values of C4a (409 ± 111 ng/ml) and C5a (8.8 ± 1.8 ng/ml) with a slight elevation of C3a (204 ± 6.6 ng/ml) in samples collected prior to coronary arteriography (253 ± 25 min after onset of pain). After coronary arteriography, there was a slight decrease in the values of C4a (224 ± 37 ng/ml), C5a (7.3 ± 1.3 ng/ml) and C3a (164 ± 35 ng/ml). The remaining six patients had complete coronary occlusion and received rt-PA (80 to 150 mg i.v.). In this treated group, before coronary arteriography the values of C4a (406 ± 51.6 ng/ml) and C5a (8.1 ± 1.9 ng/ml) were normal, and those of C3a were slightly elevated (250 ± 76 ng/ml). All complement values obtained before rt-PA were similar to those in the untreated group. However, after administration of rt-PA (but before any angiographically detectable reperfusion), there was a striking increase in C4a (2265 ± 480 ng/ml; $p < .01$), C3a (600 ± 89 ng/ml; $p < .05$) and C5a (30.0 ± 4.5 ng/ml; $p < .05$).

Thus, rt-PA causes an immediate, marked activation of complement in patients with acute myocardial infarction. Measurement of plasma anaphylatoxin levels may be a useful means of assessing the activity of rt-PA, and the degree of complement activation might reflect the extent of thrombolysis. The significance of rt-PA-induced complement activation with

respect to the extent of ischemic injury remains to be determined, but it is conceivable that the anaphylatoxin release might limit the otherwise beneficial effects of rt-PA.

Key Words: Recombinant tissue plasminogen activator
Complement
Thrombolysis
Acute Myocardial Infarction

INTRODUCTION

Administration of thrombolytic therapy is becoming an increasingly common approach to the treatment of patients with acute myocardial infaction. Recombinant tissue plasminogen activator (rt-PA) is more effective than streptokinase in producing recanalization of acutely occluded coronary arteries (6), and may become the thrombolytic agent of choice. Recombinant tissue plasminogen activator induces thrombolysis by activating plasminogen to plasmin (1). However, the proteolytic activity of plasmin is not limited to fibrin and fibrinogen, but also involves a number of other proteins including components of the complement system. Specifically, in vitro studies have shown that plasmin can activate C1 with subsequent activation of the entire complement cascade (2) and that plasmin also has the ability to directly cleave and activate C3 (3) and C5 (4). Although the thrombolytic action of rt-PA has been well characterized, the potential effects of this plasmin-generating agent on the complement cascade are unknown. Accordingly, this study was performed to determine whether administration of rt-PA to patients with acute myocardial infarction results in activation of the complement system in vivo.

The study was performed in 12 patients with documented acute myocardial infarction. The plasma level of various complement components were analyzed serially before and after arteriography and after administration of rt-PA. Due to the large amount of complement proteins normally present in the blood, there can be acute activation of complement without measurable changes in the levels of C3 and C4. Therefore, in addition to determining the concentrations of C3 and C4, we also measured C4a, C3a and C5a (the anaphylatoxins). Since these peptides are normally present in small quantities and are produced when

the complement cascade is activated, their concentration is a more sensitive marker of complement activation than the levels of C3 and C4 (5).

METHODS

Patient Population

The patient population consisted of 12 patients who were enrolled in the National Heart, Lung, and Blood Institute Thrombolysis in Myocardial Infarction (TIMI) trial (6). Criteria for enrollment included age less than 76 years, chest pain consistent with myocardial ischemia lasting for at least 30 minutes, and 0.1 mV ST-segment elevation in at least two electrocardiographic leads. Patients were enrolled within 7 hours from the onset of pain. Myocardial infarction was confirmed by elevated plasma MBCK activity in all patients.

Protocol

Cardiac catheterization was performed in all patients using the Judkins technique. Heparin (5000 U) was given intravenously prior to any contrast medium injection and blood sample collection. The protocol consisted of a left ventriculogram followed by arteriography of the non-infarct-related coronary artery and then of the infarct-related artery. Only patients with complete occlusion of the infarct-related artery received rt-PA. Six of our 12 patients were found to have a completely occluded infarct-related artery and were given rt-PA. In these patients repeat arteriograms of the infarct-related artery were obtained 30, 60, and 90 minutes after the initiation of rt-PA therapy. Samples of arterial blood for C3, C4, C3a, C4a and C5a assay were obtained before and after arteriography of the infarct-related artery, immediately after rt-PA, and then serially 5 to 15

minutes after each repeat arteriography up to 90 minutes after rt-PA administration. Measurements were included in this study only as long as the arteriograms obtained after collection of the samples demonstrated persisting complete obstruction of the infarct-related artery. This ensured that the complement values were not affected by reperfusion.

Due to changes in the TIMI protocol, the six patients treated with rt-PA received different intravenous doses of the drug. One patient received 40 mg of rt-PA over 1 hour and then 20 mg/hour for 2 hours (total dose: 80 mg). Two patients received a 6 mg bolus of rt-PA followed by 54 mg over 1 hour and then 20 mg/hour for 2 hours (total dose: 100 mg). Three patients received a 9 mg bolus followed by 81 mg over the first hour, 20 mg over the second hour and 10 mg/hour for the ensuing four hours (total dose: 150 mg).

The remaining six patients were found to have a patent infarct-related artery on the initial arteriogram and were not given rt-PA. These patients served as a control group. In this cohort, samples for C3, C4, C3a, C4a, and C5a assay were obtained before and after arteriography of the infarct-related artery and every 5 to 15 minutes, up to 30 minutes, after arteriography of the infarct-related artery.

In most patients, multiple (up to 4) measurements of complement components were obtained before arteriography of the infarct-related artery, after arteriography of the infarct-related artery, and after rt-PA administration. For the sake of clarity, the results of these multiple determinations were pooled before arteriography, after arteriography, and after rt-PA, and the mean of each subset of values was used to express the concentrations of complement components during these intervals.

All blood samples (6 to 8 ml) were collected in vials containing disodium-ethylene diamine tetracetic acid. Immediately after collection, the

specimens were placed on ice and the plasma was separated in a refrigerated centrifuge. The specimens were then frozen at -70°C until analysis was performed. The concentration of C4 and C3 was measured using a rate-limited nephelometric immunoassay (Beckman Immunochemistry System). Values of the anaphylatoxins C4a, C3a, and C5a were determined by radioimmunoassay using a commercially available kit (Upjohn Co.) (7, 8). Normal values in our laboratory are 75-150 mg/dl for C3, 13-35 mg/dl for C4, 136-680 ng/ml for C4a, 44-116 ng/ml for C3a, and < 10 ng/ml for C5a.

All values are expressed as mean ± standard error of the mean. Means were compared by the paired or unpaired Student's t-test, as appropriate.

RESULTS

The initial values for C4 and C3 were normal in all patients. Table 1 shows the values in the control group and in the rt-PA-treated group. There was no significant change in C3 or C4 in either group during the course of the study.

Figure 1 shows the values of C4a, C3a and C5a in the control group. The baseline values of C4a (409 ± 111 ng/ml), and C5a (8.8 ± 1.8 ng/ml) were within normal limits, while there was a slight increase in the values of C3a (204 ± 6.6 ng/ml). These values were obtained a mean of 26 ± 9 minutes before arteriography of the infarct-related artery and 253 ± 25 minutes after the onset of chest pain. After arteriography of the infarct-related artery there was a slight decrease in all three anaphylatoxins (C4a = 224 ± 37 ng/ml; C3a = 164 ± 35 ng/ml; and C5a = 7.3 ± 1.3 ng/ml). These values were obtained 14 ± 2 minutes after arteriography of the infarct artery. In four patients, additional late measurements were obtained a mean of 25 ± 3 minutes after

arteriography of the infarct artery. These values (C4a = 203 ± 23 ng/ml; C3a = 112 ± 12 ng/ml; and C5a = 5.6 ± 1.6 ng/ml) were lower than the previous measurements. Thus, no increase in plasma concentration of anaphylatoxins was observed during the course of the cardiac catheterization in these patients who were not treated with rt-PA.

Figure 2 depicts the values of C4a, C3a and C5a in the six patients who received rt-PA. As in the control group, the baseline values of C4a (406 ± 51 ng/ml) and C5a (8.1 ± 1.9 ng/ml) were normal, while there was a slight increase in C3a (250 ± 76 ng/ml). These values were obtained an average of 19 ± 4 minutes before arteriography of the infarct-related artery and 276 ± 27 minutes after the onset of pain. Repeat complement values (C4a = 317 ± 126 ng/ml; C3a = 137 ± 43 ng/ml; and C5a = 6.7 ± 1.5 ng/ml) were obtained an average of 13 ± 3 minutes after arteriography of the infarct-related artery in four patients. Similar to the pattern in the control group, these values tended to be lower than those measured before arteriography. There was no discernible difference in any of the complement measurements between the rt-PA group and the control group either before or after arteriography of the infarct-related artery.

Administration of rt-PA was followed by a striking increase in C3a, C4a and C5a. The greatest change occurred in C4a, which increased from 317 ± 126 ng/ml to 2265 ± 480 ng/ml ($p < .01$). C3a increased from 137 ± 43 ng/ml to 600 ± 89 ng/ml ($p < .05$) and C5a from 6.7 ± 1.5 to 30.0 ± 4.5 ng/ml ($p < .05$). These values were obtained an average of 30 ± 4 minutes after initiation of rt-PA infusion and 48 ± 4 minutes after arteriography of the infarct-related artery.

The values for C3a, C4a and C5a in individual patients are demonstrated in Figure 3. Before administration of rt-PA, there was no consistent change

in individual levels of anaphylatoxins. After administration of rt-PA, however, an immediate increase in C4a, C3a and C5a was consistently observed in all patients. To exclude the possibility that this phenomenon reflected differences in the timing of samples between control and treated groups, we compared the results obtained in the two cohorts at corresponding intervals from arteriography of the infarct-related artery. In the four patients in which post-rt-PA measurements were obtained 22 ± 2 minutes from the arteriography of the infarct-related artery, the values for C4a (1665 ± 465 ng/ml), C3a (351 ± 67 ng/ml) and C5a (16.5 ± 3.6 ng/ml) were considerably greater than those measured 25 ± 3 minutes after arteriography of the infarct-related artery in the control group (C4a: 203 ± 22 ng/ml; C3a: 112 ± 13 ng/ml; and C5a: 5.6 ± 1.6 ng/ml).

Three different dosages of rt-PA were used in the treated group. The values of C4a, C3a and C5a with each dose regimen are illustrated in Figure 4. There was no discernible trend suggesting a relation between the dose of rt-PA and the magnitude of complement activation as assessed by the rise in anaphylatoxin concentration. However, the small sample size precludes any definitive conclusions.

DISCUSSION

This study demonstrates that administration of rt-PA is associated with an immediate and marked activation of the complement system as reflected by elevations of C4a, C3a and C5a. These elevations persist for up to 90 minutes after rt-PA administration. Since all measurements were obtained before any angiographic evidence of coronary artery recanalization, the rt-PA-induced activation of the complement cascade appears to be independent of reperfusion.

The lack of increase in anaphylatoxins in control patients subjected to heparin administration and repeated contrast medium injections indicates that the rise in C3a, C4a and C5a observed in the rt-PA-treated group was not the result of cardiac catheterization per se.

Figure 5 illustrates the sequence of complement activation. Plasmin can directly activate C1, which initiates the classic complement cascade. This nonantigen-antibody activation of C1 is an example of fluid phase complement activation. After C1 there is activation of C4, which liberates the anaphylatoxin C4a. C2 is then activated, followed by C3, which produces C3a and C3b. C3 is an important step in the complement cascade because one of its products, C3b, can further activate C3, thereby causing an amplification loop; in fluid phase activation, however, this amplification loop is characteristically inhibited due to the increased activity of the C3b inhibitory protein (9, 10). Therefore, the increase in C3a and C5a would be expected to be relatively less in fluid phase activation as compared to antigen- antibody-induced activation. Once activated, C3 can activate C5 to produce C5a. After C5 activation, the complement cascade continues to completion with the production of the C5b,6,7,8,9 complex (membrane attack complex).

In this study, the major increase among the anaphylatoxins after rt-PA (relative to pre-angiography values) was observed for C4a, the initial anaphylatoxin produced in the complement cascade. There was relatively less production of C3a and C5a, the second and third anaphylatoxins generated in the complement cascade, respectively. As discussed earlier, this pattern of decreasing activation of the complement cascade from C4 to C5 is consistent with a fluid phase activation of the complement system.

Due to the small number of patients with each drug dose, we were unable to establish whether a relationship exists between the amount of complement activation and the dose of rt-PA. Since activation of complement and clot lysis are both mediated by the same enzyme (plasmin), it is possible that the level of complement activation may reflect the amount of thrombolytic activity. Accordingly, measurement of plasma anaphylatoxin levels may be useful in that it may provide a noninvasive index of the therapeutic activity of rt-PA. This potentially important implication of our findings will require confirmation in future studies.

Although acute myocardial ischemia is associated with activation of the complement system in experimental animals (11), it is unknown whether a similar phenomenon occurs in humans during the initial hours following the onset of acute myocardial infarction. Previous studies in patients have evaluated the complement system 1 to 7 days after the onset of infarction and have shown increased complement activity at these times (12, 13). Based on clinical and experimental data, however, it would appear that myocardial necrosis is completed within four to five hours after coronary artery occlusion (14). In exploring the potential pathogenetic role of the complement system in myocellular injury, it is important to determine whether complement activation occurs during this critical interval. In the present study, the first complement sample was obtained an average of 276 ± 27 minutes after the onset of chest pain in the rt-PA group and 253 ± 25 minutes in the control group. At these time points there was no significant elevation of C4a or C5a and there was only a slight increase in C3a in both the control and rt-PA groups. It is possible that this pattern resulted from an earlier activation of the complement cascade that had almost resolved due to the short half-life of the anaphylatoxins. It is also possible that a localized

complement reaction in the myocardium may not generate sufficient C4a, C3a and C5a to be detected in the systemic circulation. Thus, we cannot, on the basis of the present data, exclude significant activation of complement in the early phase of myocardial infarction.

Considerable experimental evidence suggests that complement activation may be detrimental in the setting of acute myocardial ischemia. Anaphylatoxins have been demonstrated to cause white blood cell clumping, activation, and migration in vitro as well as in vivo (5, 15). Complement-mediated influx of inflammatory cells into reperfused myocardium may extend the amount of necrosis initially caused by ischemia and play an important role in the so called "reperfusion injury" (14,16). Indeed, recent data suggest that activated leukocytes exacerbate myocardial injury associated with ischemia and reflow (16). Leukocytes can obstruct ischemic capillaries, causing the "no reflow" phenomenon after reperfusion (17), and can release cytotoxic oxygen free radicals, lytic enzymes and leukotrienes, which may mediate reperfusion injury (16). In addition, depletion of complement has been demonstrated to decrease infarct size in dogs (18) and baboons (19). In light of these considerations, it is conceivable that the activation of complement by rt-PA may limit the otherwise beneficial effects of this thrombolytic agent. It is unknown, however, whether the level of anaphylatoxin production induced by rt-PA is sufficient to cause activation of leukocytes and to produce any deleterious influence on the extent of injury during myocardial infarction in man. Characterization of the potential nonspecific effects of plasmin warrants further investigation, particularly in view of the widespread use of thrombolytic therapy.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1: Plasma concentration of anaphylatoxins in the six untreated patients (mean ± SEM). Before angiography of the "infarct" artery, C4a and C5a were normal and C3a was only slightly elevated. No increase was observed during the course of cardiac catheterization. Measurements were obtained 26 ± 9 minutes before arteriography of the "infarct" artery and 14 ± 2 minutes after arteriography of the "infarct" artery. In addition, in four of the six patients "late" values were obtained 25 ± 3 minutes after arteriography of the "infarct" artery. The dashed line represents the upper limit of normal for C4a, C3a and C5a. The number of patients studied at each time point is specified above the bar.

Figure 2: Plasma concentrations of anaphylatoxins in the six patients given rt-PA (mean ± SEM). Before angiography of the "infarct" artery, the values of C4a and C5a were normal and C3a was only slightly increased. No significant change was observed after arteriography of the "infarct" artery. A striking increase in all three anaphylatoxins, however, was observed after rt-PA. Measurements were obtained at the following time points: 19 ± 4 minutes before arteriography of the "infarct" artery, 13 ± 3 minutes after arteriography of the "infarct" artery, and 30 ± 4 minutes after rt-PA. The dashed line represents the upper limit of normal for each anaphylatoxin. The number of patients studied at each time point is specified above the bar. The p values refer to the differences between levels obtained before angiography of the "infarct" artery and after rt-PA.

Figure 3: Time course of plasma anaphylatoxin concentration in individual patients given rt-PA. The upper panel represents C5a values, the middle panel C3a values and the lower panel C4a values. Note that the vertical scale is interrupted for C3a and C5a. Striking increases in all three anaphylatoxins occurred within minutes of administration of rt-PA.

Figure 4: Relationship between plasma concentration of anaphylatoxins and dose of rt-PA. One patient received a "low dose" (80 mg), two patients received a "medium dose" (100 mg), and three patients received a "high dose" (150 mg). No correlation was observed between anaphylatoxin concentration and dose of rt-PA. Letters next to symbols identify individual patients.

Figure 5: Schematic representation of complement activation (see text for explanation).

Table 1. Plasma Concentrations of C3 and C4 in Control and rt-PA-Treated Patients

	C4 (mg/dl)	C3 (mg/dl)
Control Group		
Before arteriography of infarct-related artery	23 ± 4	122 ± 8
After arteriography of infarct-related artery	19 ± 3	113 ± 6
rt-PA-Treated Group		
Before arteriography of infarct-related artery	18 ± 4	101 ± 3
After rt-PA	17 ± 2	104 ± 4

Values are mean ± SEM.

rt-PA = Recombinant tissue plasminogen activator

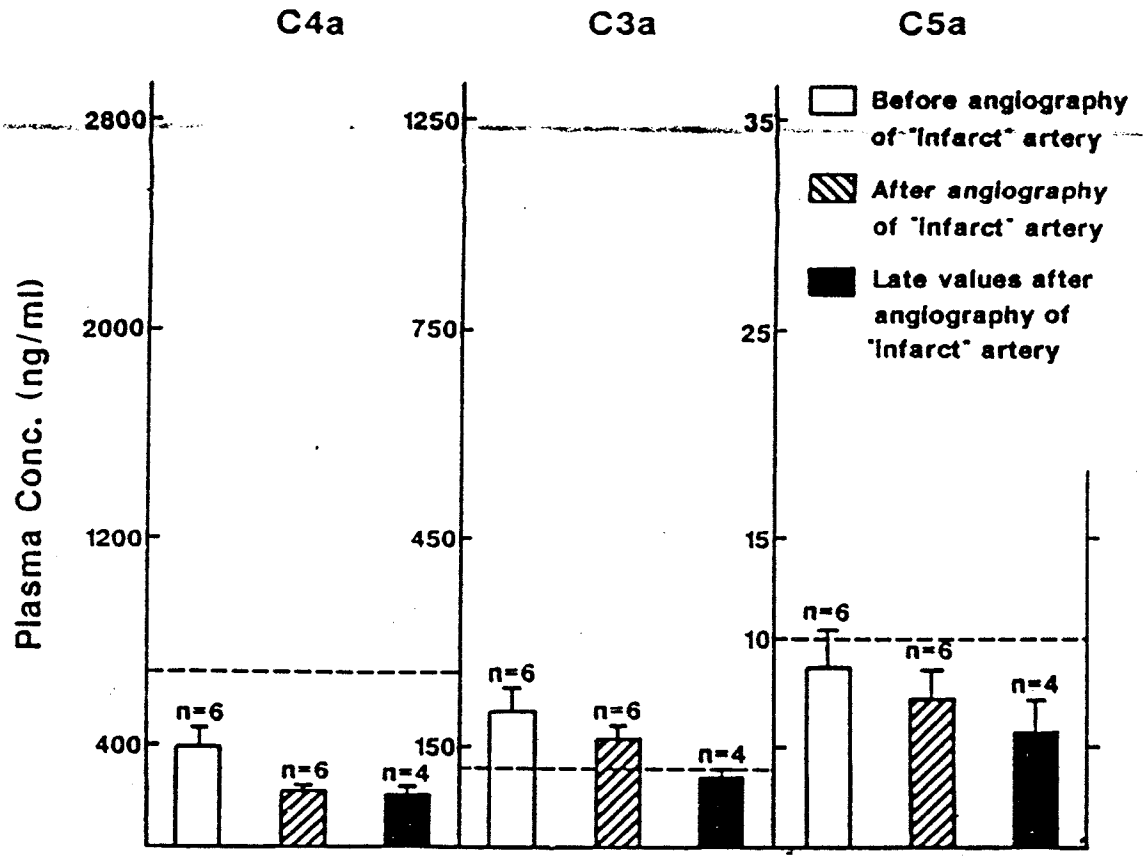

FIGURE 1

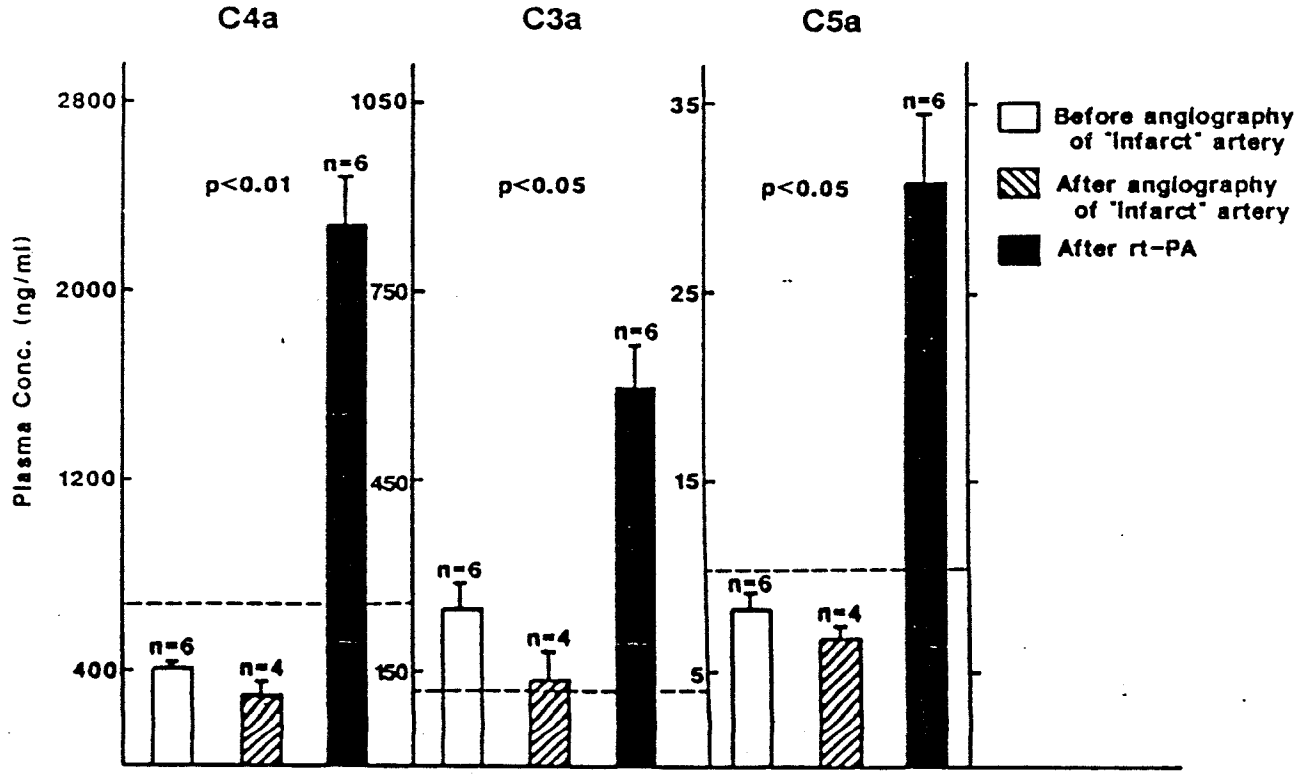

FIGURE 2

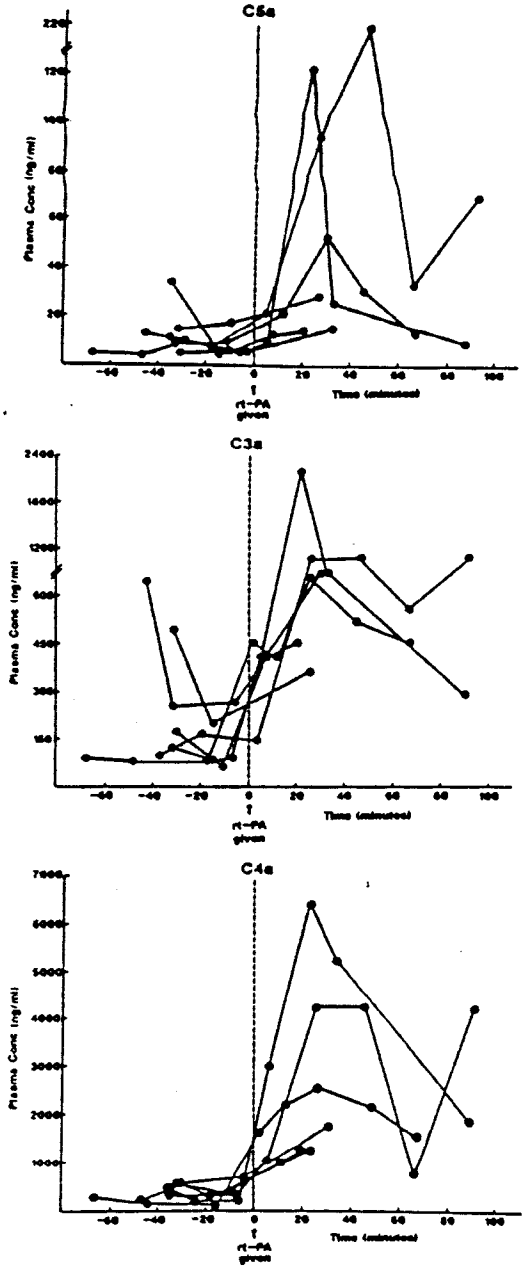
C5a
Plasma Conc (ng/ml)
Time (minutes)
rt-PA given
C3a
Plasma Conc (ng/ml)
Time (minutes)
rt-PA given
C4a
Plasma Conc (ng/ml)
Time (minutes)
rt-PA given

FIGURE 3

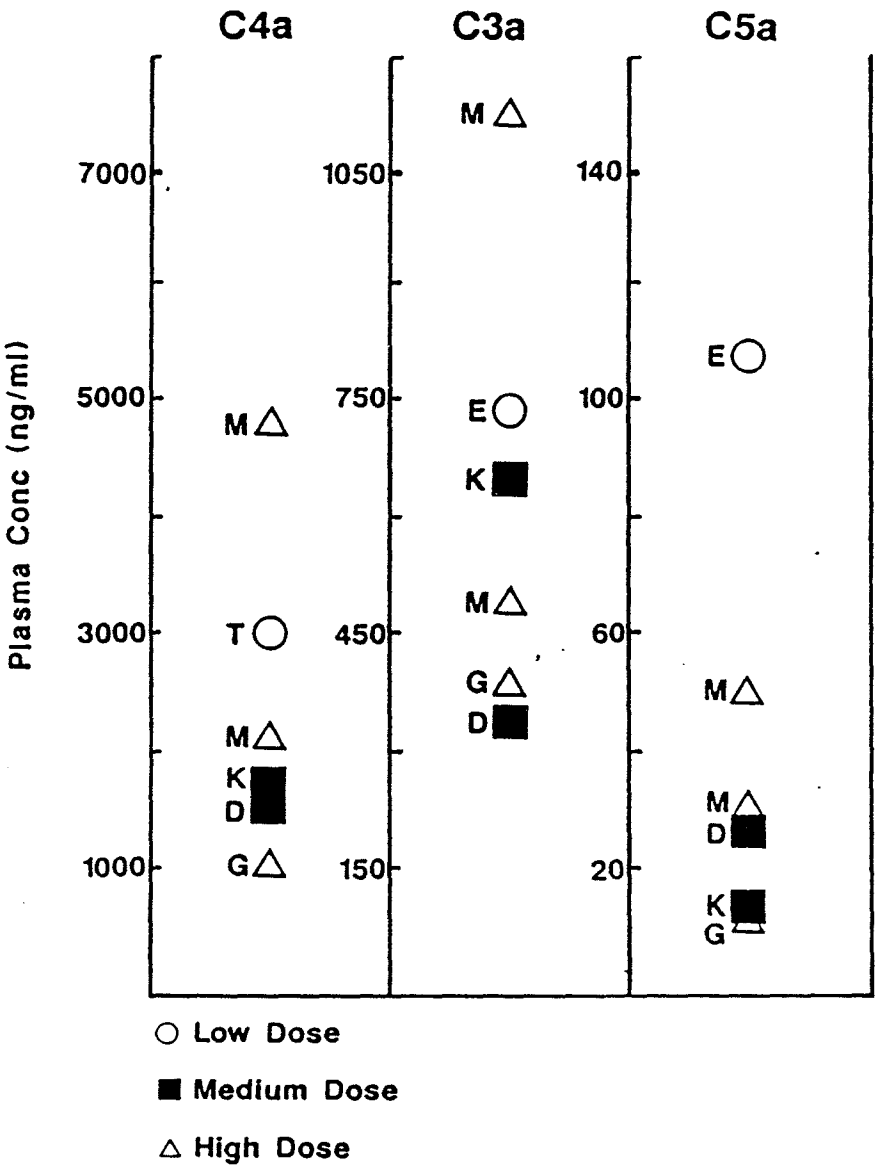
C4a
C3a
C5a
Plasma Conc (ng/ml)
7000
5000
3000
1000
1050
750
450
150
140
100
60
20
M
E
K
T
D
G
Low Dose
Medium Dose
High Dose

FIGURE 4

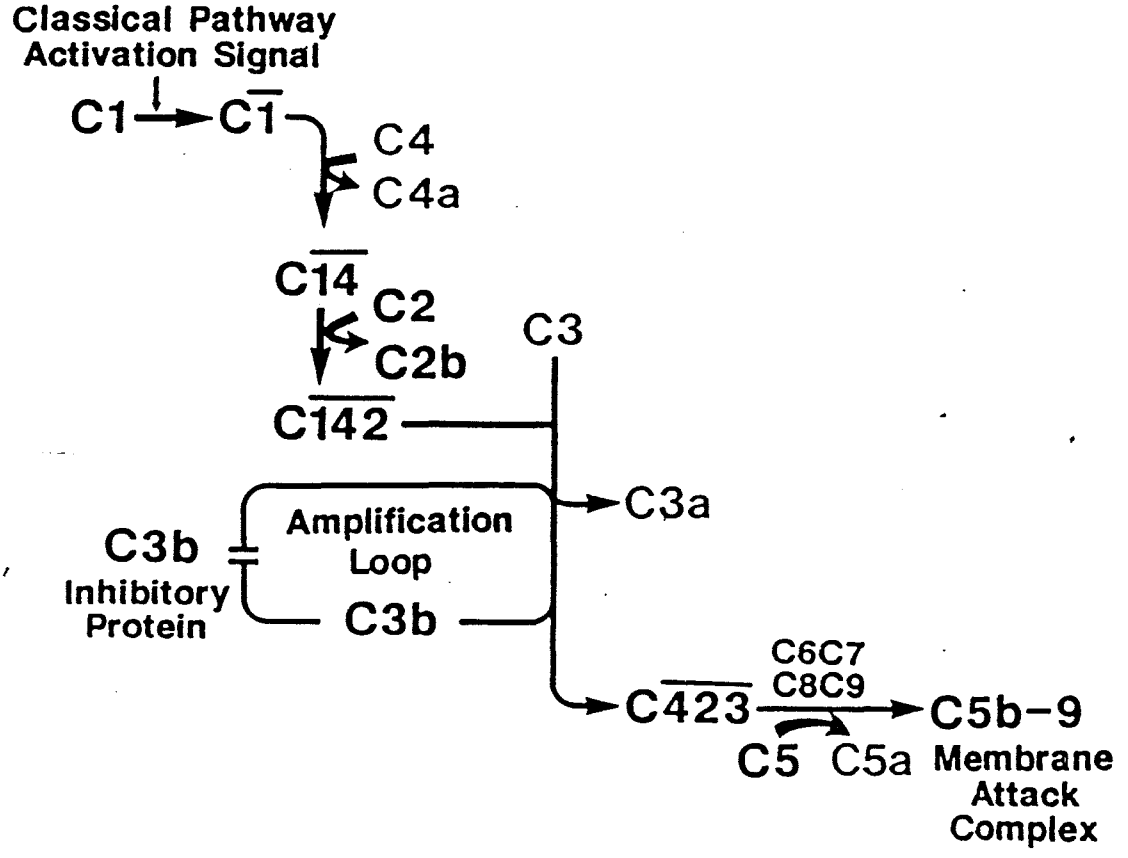
Classical Pathway
Activation Signal
C1
C1
C4
C4a
C14
C2
C2b
C142
C3
C3a
Amplification
Loop
C3b
Inhibitory
Protein
C3b
C6C7
C8C9
C423
C5b-9
C5
C5a
Membrane
Attack
Complex

FIGURE 5