Inhibition of Heparin/Protamine Complex-Induced Complement Activation by Compstatin in Baboons

Athena M. Soulika,*,[1] Mohammad M. Khan,†,[1] Takashi Hattori,† Frank W. Bowen,† Bradford A. Richardson,†
C. Erik Hack,‡ Arvind Sahu,*,[2] L. Henry Edmunds Jr.,† and John D. Lambris*,[3]

*Department of Pathology and Laboratory Medicine School of Medicine and †Harrison Department of Surgery School of Medicine,
University of Pennsylvania, Philadelphia, Pennsylvania 19104; and ‡Department of Pathophysiology of Plasma Proteins,
Central Laboratory for Blood Transfusion, Amsterdam, The Netherlands

Complement activation products are major components of the inflammatory response induced by cardiac surgery and cardiopulmonary bypass which contribute to postoperative organ dysfunction, fluid accumulation, and morbidity. Activation of the complement system occurs during extracorporeal circulation, during reperfusion of ischemic tissue, and after the formation of heparin–protamine complexes. In this study we examine the efficacy of Compstatin, a recently discovered peptide inhibitor of complement, in preventing heparin/protamine-induced complement activation in baboons. The study was performed in baboons because Compstatin binds to baboon C3 and is resistant to proteolytic cleavage in baboon blood (similar to humans); Compstatin inhibits only the activation of primates' complement system. After testing various doses and administration regimens, Compstatin produced complete inhibition at a total dose of 21 mg/kg when given as a combination of bolus injection and infusion. Compstatin completely inhibited *in vivo* heparin/protamine-induced complement activation without adverse effects on heart rate or systemic arterial, central venous, and pulmonary arterial pressures. This study indicates that Compstatin is a safe and effective complement inhibitor that has the potential to prevent complement activation during and after clinical cardiac surgery. Furthermore, Compstatin can serve as the prototype for designing an orally administered drug.   © 2000 Academic Press

Key Words: Compstatin; heparin/protamine complexes; cyclic peptides; complement inhibitors; anticoagulant; cardiopulmonary bypass; primates.

---

[1] Equal contribution.
[2] Present address: National Centre for Cell Science, Pune University Campus, Ganeshkhind, Pune 411007, India.
[3] To whom reprint requests should be addressed at 401 Stellar-Chance Labs, 422 Curie Blvd., School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6100. Fax: (215) 573-8738. E-mail: lambris@mail.med.upenn.edu.

INTRODUCTION

Clinical cardiac surgery using cardiopulmonary bypass produces a massive inflammatory response, which involves activation of platelets (1, 2) endothelial cells (3), neutrophils (4, 5), monocytes (6), and lymphocytes (7, 8) and the contact (9), intrinsic (10, 11), and extrinsic (12) coagulation, fibrinolytic (13), and complement plasma protein systems (14, 15). Activation of the complement system during cardiopulmonary bypass occurs at three different times: first, during blood contact with biomaterial and gas (16, 17) via the alternative and classical pathways; second, after protamine administration (18) and formation of heparin–protamine complexes that activate mainly the classical pathway; and, third, after reperfusion of the arrested heart (19). Myocardial reperfusion activates both the alternative and the classical pathways. Complement activation during clinical cardiac surgery is a major component of the "whole body inflammatory response" that is associated with temporary organ dysfunction (20, 21), fluid accumulation, and morbidity associated with these operations (22).

Activated complement intermediates that are vasoactive or cytotoxic are anaphylatoxins (C3a, C4a, and C5a) and the C5b-9 complex. The anaphylatoxic peptides induce damage through their effect on neutrophils, mast cells, eosinophils, and basophils. Previous studies suggest that C5a is the only peptide that has a chemotactic effect on the aforementioned cells. However, recent work indicates that C3a also has a chemotactic effect on eosinophils (23) and mast cells (24, 25). C5b-9 is a major cytoxin.

C5a is at least 10 times more potent than C3a in inducing most biological responses. Therefore, many investigators suggest that complement inhibition at the C5 level will eliminate the damaging effects of activation. However, since the concentration of C3 (and C3a) is 15 times higher in plasma than that of C5 (and C5a), we suggest that inhibition at the level of C3 will be more effective. In fact, Rinder and co-workers (26),

using a model of extracorporeal circulation, demonstrated that inhibition at the C3 level inhibits both monocyte and neutrophil CD11b upregulation and platelet activation. In a similar model Rinder *et al.* (27), observed that inhibition of C5a and C5b-9 generation do not inhibit monocyte CD11b upregulation.

Several compliment inhibitors have been developed: antibodies against C5 (28); proteases (29); recombinant forms of natural inhibitors, such as complement receptor 1 (CR1) (30); decay accelerating factor (31); and membrane cofactor protein (32). The most promising inhibitor at the C3 level appears to be a soluble form of CR1 (33), which is currently being evaluated in clinical trials. However, the high molecular weight and relatively complex structure of CR1 may impede the development of an oral drug. Furthermore, its size and complexity may defeat large-scale synthesis.

Other approaches to suppress complement activation use heparin-coated (34) or heparin removal devices (35). The disadvantage of these efforts is that these devices only inhibit activation on biomaterials and have no effect on complement activation caused by gas surfaces or reperfusion of previously ischemic tissues.

Compstatin, a novel and potent inhibitor of the complement system, is a promising alternative (36). Compstatin is a 13-residue cyclic peptide that was developed in our laboratory using a combinatorial phage-displayed random peptide library. It has the ability to bind specifically to primates' native C3 and inhibit further activation.

Compstatin has already been tested in a model of hyperacute rejection in discordant kidney xenotransplantation (37) and in a model of extracorporeal circulation (38). In the first model, porcine kidneys were perfused with human blood; kidneys treated with a control peptide survived 90 min whereas kidneys treated with Compstatin survived up to 380 min (the duration of the experiment). In the extracorporeal circulation model, Compstatin inhibited the generation of C3a and C5a, the formation of the membrane attack complex, and CD11b expression on neutrophils (PMNs). The peptide also inhibited binding of C3/C3 fragments, PMNs, and monocytes to the polymer surface.

These data encouraged us to test the safety and efficacy of Compstatin in an *in vivo* primate model. The results of this investigation clearly indicate that Compstatin is both safe and effective in inhibiting the activation of the complement system *in vivo*.

MATERIALS AND METHODS

Preparation of Baboon C3b

Baboon (*Papio anubis*) C3 was purified as previously described for human C3 (39, 40). In brief, baboon plasma was precipitated with 5% PEG, and the supernatant was precipitated a second time with 16% PEG. The pellet was resuspended in 0.01 M Na_2HPO_4, (pH 7.9) and passed through a Mono Q column (Pharmacia). Fractions containing C3 were pooled, concentrated, and passed through a gel filtration column (1.6 × 50 cm, Pharmacia). C3 was converted to C3b by limited proteolysis with trypsin (41).

Biotransformation of Compstatin

Freshly drawn baboon blood or plasma containing heparin (3.75 units/ml) was incubated at 37°C with Compstatin at a final concentration of 1 mg/ml. Samples of 500 μl were taken at various time points and centrifuged in order to separate cells; 100 μl of the supernatant was mixed with an equal volume of H_2O/TFA 0.1% and centrifuged for 1 h at 14000 rpm through a 5000-MW-cutoff filter. The flow-through was analyzed using an analytical HPLC connected to a reverse phase C-18 column.

In Vivo Studies

Juvenile baboons (*P. anubis*) weighing 10.5–28.8 kg were used. A total of six baboons were used for 13 studies; one was studied once, three were studied twice, and two were studied three times. Animals were rested for 6 to 8 weeks between serial studies. For each study, the baboon was placed in a squeeze cage, sedated with 10 mg/kg ketamine hydrochloride intramuscularly and induced with 5 mg/kg thiopental sodium intravenously (IV). The animal was then intubated; general anesthesia was maintained with inhalation isoflurane (1–2%). The electrocardiogram was continuously monitored. The right or left groin was prepared and draped appropriately for a sterile vascular cutdown and/or cannulation. Hemodynamic monitoring was accomplished using an arterial line with a 22-G catheter placed in the femoral artery and a 5-Fr Swan-Ganz catheter placed via a femoral vein. This study was approved by the University of Pennsylvania Committee on Animal Care and Utilization.

All animals received a bolus dose of porcine intestinal heparin (3 mg/kg; Elkins-Sinn, Inc., Cherry Hill, NJ). Sixty minutes later each animal received protamine (3 mg/kg, Elkins-Sinn) over a 5-min period. Six animals served as controls; they received 7.2 ml/kg of PBS 60 min after heparin administration and then 29 ml/h IV for 45 min beginning 15 min after the protamine infusion. All injections were done through the peripheral vein.

Eight animals received Compstatin in various doses by bolus injection, infusion, or both (Table 1). Three baboons received a 50 mg/kg bolus of Compstatin 60

TABLE 1

Doses and Administration Regimens of Compstatin Used

Animal	Experiment number	Corresponding panels in Fig. 3	Bolus (mg/kg)	Infusion (mg/kg/min)	Total dose (mg/kg)
I	1	A	50	—	50
II	2	B	50	—	50
III	3	C	50	0.3 15 to 60 min	63.5
IV	4	D	25	0.3 8 to 53 min	38.5
V	5	E	25	0.3 8 to 53 min	38.5
VI	6	F	12	0.2 0 to 45 min	21
III	7	G	5	0.2 0 to 60 min	17
VI	8	F	—	0.4 −10 to 75 min	34

Note. Time represents minutes after the end of protamine administration.

min after heparin and 10 min before protamine administration. One of these three animals also received an infusion of Compstatin (0.3 mg/kg) for 45 min beginning 15 min after the bolus of Compstatin.

Two baboons received a bolus of 25 mg/kg of Compstatin 60 min after heparin and 2 min before protamine and both also received an infusion of Compstatin (0.3 mg/kg/min) for 45 min beginning 8 min after the bolus.

Two baboons received an infusion of Compstatin (0.2 mg/kg/min), one for 45 min and one for 60 min, beginning immediately after a bolus dose of the drug. The first animal received a bolus dose of 12 mg/kg; and the second received a bolus of 5 mg/kg.

A single animal received an infusion of Compstatin (0.4 mg/kg/min) for 85 min beginning 60 min after heparin and 5 min before protamine and did not receive a bolus dose.

Baboons were awakened 240 min after protamine administration and extubated. Catheters were removed, and cutdown wounds were closed with subcuticular polyglycolate sutures.

Heart rate by electrocardiogram, systemic (systolic, diastolic, mean) arterial blood pressure, central venous pressure, and pulmonary arterial pressure were continuously monitored.

Collection of Blood Samples

Blood samples for the evaluation of hematological parameters were collected in glass tubes containing heparin. Blood samples for the assay of complement activation products were collected in 5-ml plastic tubes containing 3.8% Na citrate (9:1, vol:vol) and centrifuged at 2000g for 20 min at 4°C for plasma separation. All samples were taken by venous puncture and as-

sayed immediately. Aliquots were also stored at −70°C for further examination. Blood collection time points were as follows: after induction of anesthesia (baseline); 5 min after heparin injection (heparin); 55 min after heparin (before bolus); 1, 5, 10, 15, 30, 45, 60, 90, 120, 180, and 240 min after protamine administration (Fig. 1).

Biochemical Parameters

Concentrations of activated fragments of baboon C3 were measured in plasma by a sandwich ELISA as described previously (42), with slight modifications: microtiter plates were coated with 50 μl of 1 μg/ml of the capture anti-C3-28 antibody solution in PBS. This antibody specifically recognizes neoepitopes exposed only in cleaved products of primate native C3 (C3b, iC3b, C3c). The wells were then blocked with 1% BSA in PBS for 1 h, and serial dilutions of the plasma samples along with duplicates of the standard (baboon C3b) were added and incubated for 1 h. The plates were washed twice with 0.05% PBS–Tween 20 (washing buffer), and a polyclonal rabbit anti-human C3b was added. After 50 min of incubation wells were washed twice, and bound C3 fragments were detected by addition of 1:1000 anti-rabbit horseradish peroxidase (HRP). After 40 min the wells were washed three times and the HRP substrate was added (ABTS 0.05% in 0.1 M Na citrate, pH 4.2, with H_2O_2 diluted 1:1000). The plates were read at 405 nm. All steps were performed at room temperature.

Hematological Parameters

Hematological parameters were measured using a STKS, Coulter (VCS). Specifically, we measured the
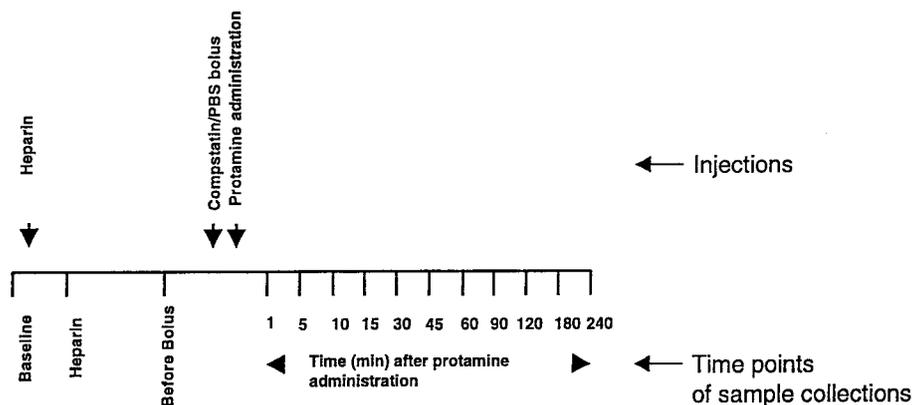
FIG. 1. Representative scheme for the procedure. The animal was sedated and 3 mg/kg of heparin was injected. One hour later Compstatin/PBS was injected followed by administration of protamine as an infusion (3 mg/kg) for 5 min. Infusions of Compstatin/PBS of various duration and amounts were also given. Samples were collected before the beginning of the procedure (Baseline), 5 min after the heparin injection (Heparin), 2 min before the bolus injection of Compstatin/PBS (Before Bolus), and 1, 5, 10, 15, 30, 45, 60, 90, 120, 180, and 240 min after the end of protamine administration. Arrows represent injections.

values of hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), red cell distribution width (RCDW), red blood cell (RBC), platelets, and white blood cell counts (WBC). In addition, we measured the hematocrit (Hct) and the activated clotting time (ACT).

RESULTS

Biotransformation of Compstatin

Analysis by HPLC of samples of Compstatin incubated at 37°C showed that Compstatin is stable in baboon plasma for more than 24 h. The same profile was observed when Compstatin was incubated with baboon blood, suggesting that the Compstatin was not adsorbed to blood cells (Fig. 2).

Complement Activation

Our ELISA was designed to detect the activation products of C3 (C3b, C3c, and iC3b), hereafter referred to as C3b/c. Although C3a, C5a, and/or C5b-9 are the usual indicators of activation of the human complement system, the analogous parameters could not be measured in our study, since reagents for the respective baboon fragments are not easily available. The results we obtained, however, reflect an acceptable level of complement activation after exposure to heparin–protamine complexes.

The levels of C3b/c generated in response to heparin–protamine complexes in the presence of Compstatin or PBS (control) are shown in Fig. 3. In our system heparin–protamine complexes induce an immediate spe-

cific activation of the complement system that slowly decreases to baseline levels (Fig. 3).

We tested a wide range (total doses 17–63.5 mg/kg) of Compstatin dosages administered under various experimental regimens (Table 1). Initially a bolus injection of 50 mg/kg of Compstatin was used. This dose inhibited complement activation when administered to one animal (Fig. 3A), but when given to a second baboon (Fig. 3B), there was noticeable activation beginning at 15 min after protamine administration. The administration regimen was then changed to 50 mg/kg of Compstatin followed by an infusion at a rate of 0.3 mg/kg/min for 45 min (15–60 min) after protamine administration (Fig. 3C). When administered according to this regimen, Compstatin completely inhibited complement activation induced by heparin–protamine complexes.

The total dose of the inhibitor then was progressively lowered to the following levels:

(1) 38.5 mg/kg (bolus, 25 mg/ml and infusion, 0.3 mg/kg/min for 45 min [8 to 53 min]; Figs. 3D and 3E;

(2) 34 mg/kg (infusion of 0.4 mg/kg/min for 85 min [−10 to 75 min]; Fig. 3F, —▲—);

(3) 21 mg/kg (bolus, 12 mg/kg and infusion, 0.2 mg/kg/min for 45 min [0–45 min] Fig. 3F);

(4) 17 mg/kg (bolus, 5 mg/kg and infusion, 0.2 mg/kg/min for 60 min [0–60 min] Fig. 3G).

Although constant infusion of 0.4 mg/kg/min for 85 min (34 mg/kg total dose) had no significant effect on complement activation, we observed complete inhibition with a bolus of 12 mg/kg combined with an infusion of 0.2 mg/kg/min for 45 min (Fig. 3D). When the total dose was lowered to 17 mg/kg, with a 5 mg/kg
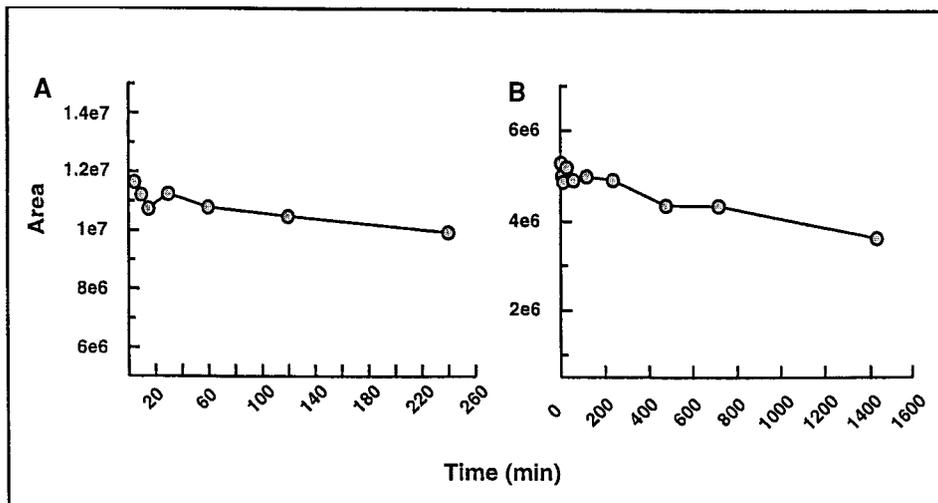
FIG. 2. Biotransformation of Compstatin in baboon blood (A) and plasma (B). Incubations were carried out at 37°C, and the final concentration of Compstatin was 1 mg/ml.

bolus and an infusion of 0.2 mg/kg/min, attenuated inhibition was observed (Fig. 3G).

Hematological Parameters

No significant differences between the baboons receiving Compstatin and those receiving PBS in the electrocardiogram, systemic arterial, central venous and pulmonary arterial pressures, or various hematological parameters (Hb, MCH, MCHC, MCV, RCDW, RBC, and Hct) were observed at any time during the studies (Fig. 4). ACT increased as expected after heparin injection and decreased to control values after protamine administration (data not shown). Platelet counts (Fig. 5A) decreased slightly after heparin administration and remained at that level until the end of the experiment. White blood cell counts (Fig. 5B) decreased in both groups at 1 min after protamine administration and increased 30 min later.

DISCUSSION

In this report we demonstrate that a newly discovered complement inhibitor, Compstatin, effectively inhibits activation of the complement system when tested in an *in vivo* model. Compstatin is a low-molecular-weight peptide (1593 Da), with a simple structure effective *in vivo* in concentrations comparable to those of other pharmacologic peptides (i.e., cyclosporin).

In this study we extended the previous observation of Sahu *et al.* (43), who found that Compstatin is not degraded in human blood, to baboon plasma and blood. The pharmacokinetics of Compstatin require further study.

In our *in vivo* model, juvenile baboons that received vehicle (PBS) after exposure to heparin and protamine showed significant activation of the complement system. Activation levels corresponded to 1–3% of the total C3 plasma concentration. Although this range is lower than that observed during cardiopulmonary bypass (7–10%) or after formation of immunocomplexes (~10%), ranges of activation were adequate to test the efficacy and safety of Compstatin *in vivo.* Our inhibitor successfully inhibited this activation of the complement system at a total dose as low as 21 mg/kg.

In our first animal in which Compstatin was administered as a single bolus injection, we observed complete inhibition of complement activation. However, when this experiment was repeated in another animal, we saw activation, beginning 15 min after protamine administration. This discrepancy can be attributed to a variation in the intensity of complement activation between animals or the clearance rate of Compstatin. HPLC analysis of the plasma samples showed only trace amounts of the intact form of Compstatin at 20 min after injection (data not shown). Subsequent experiments using a combination of bolus injection and infusion achieved complete inhibition of the comple-

FIG. 3. Activation of complement in response to heparin–protamine complexes *in vivo* in the presence or absence of Compstatin. Each panel represents a different animal treated with a various regimens of Compstatin or PBS. The same animal was used for the experiments shown in C and G.

Y-axis label: μg/ml of C3b/c generated

X-axis label: TIMEPOINTS
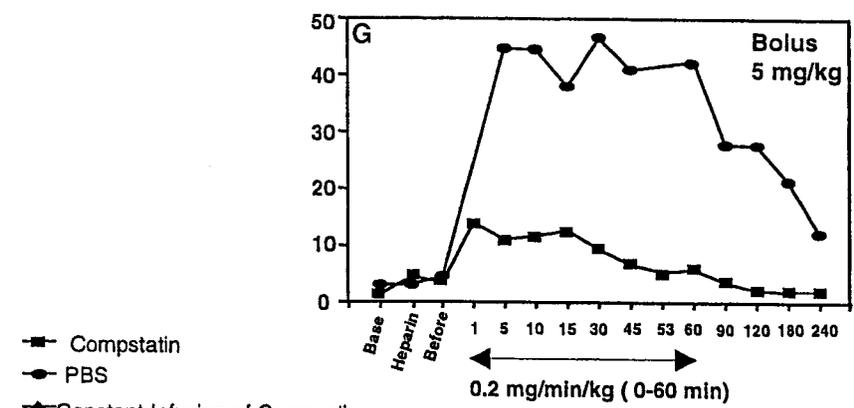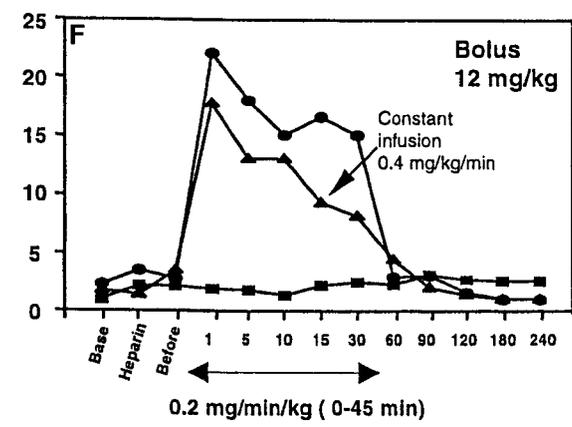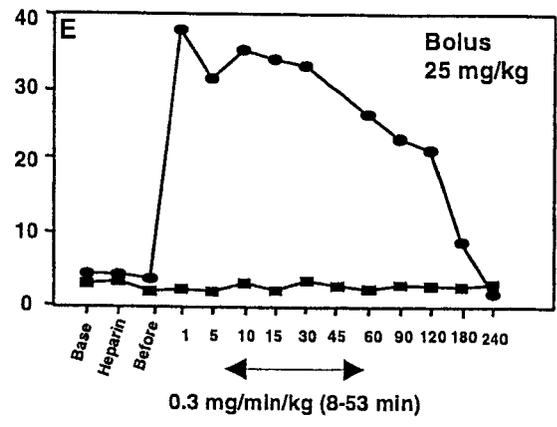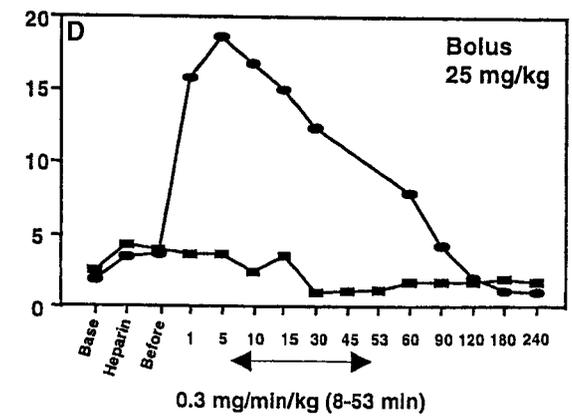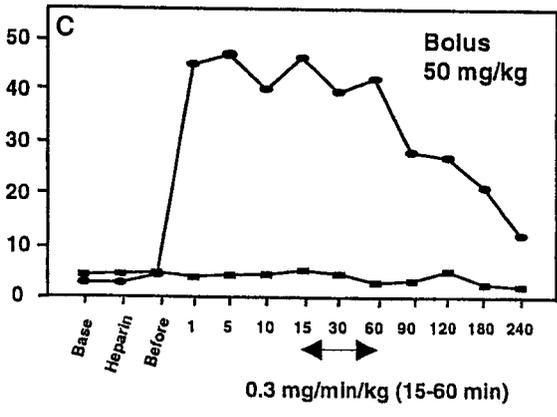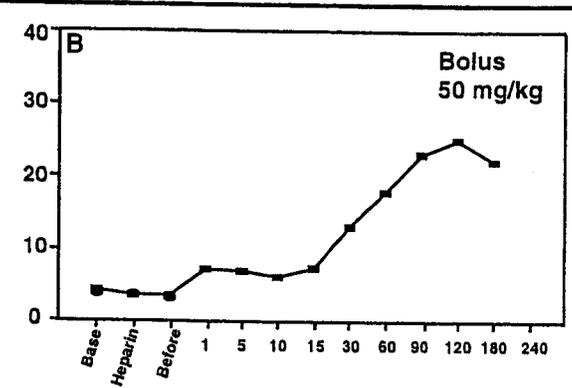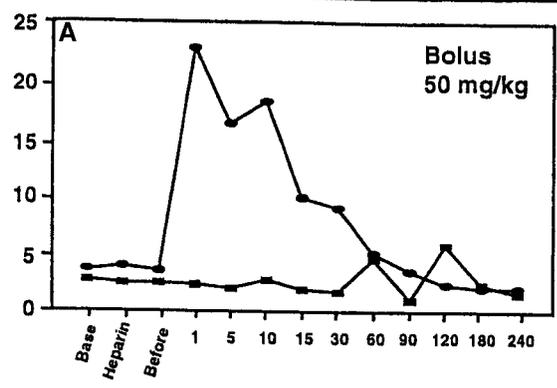
Legend:
- ■ Compstatin
- ● PBS
- ▲ Constant Infusion of Compsatin without bolus (0.4 mg/kg/min) See table 1 experiment 8

Panel A: Bolus 50 mg/kg

Panel B: Bolus 50 mg/kg

Panel C: Bolus 50 mg/kg — 0.3 mg/min/kg (15-60 min)

Panel D: Bolus 25 mg/kg — 0.3 mg/min/kg (8-53 min)

Panel E: Bolus 25 mg/kg — 0.3 mg/min/kg (8-53 min)

Panel F: Bolus 12 mg/kg — Constant infusion 0.4 mg/kg/min — 0.2 mg/min/kg (0-45 min)

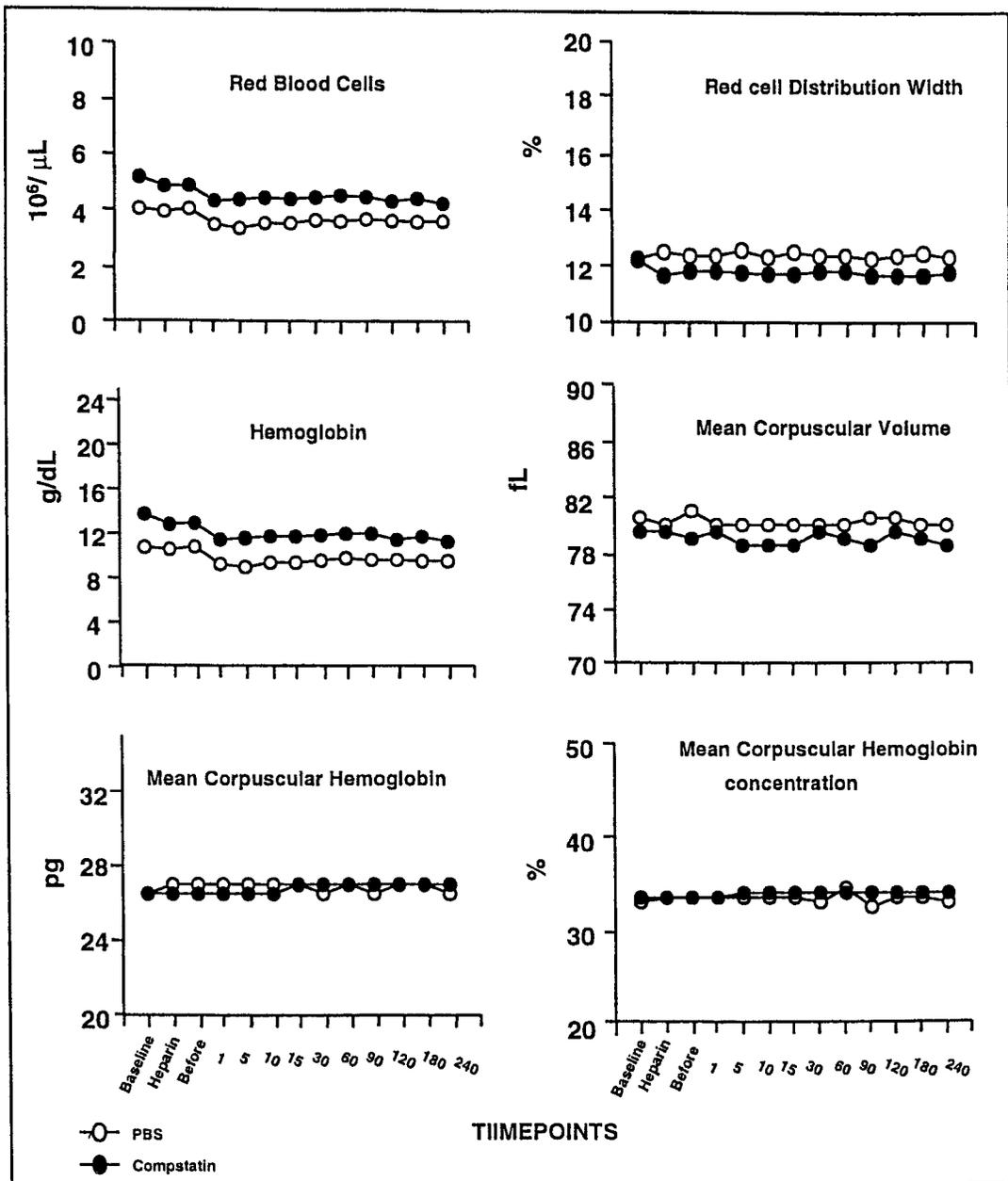Panel G: Bolus 5 mg/kg — 0.2 mg/min/kg (0-60 min)

FIG. 4. Effect of Compstatin on various hematological parameters. Changes in levels of red blood cell count, red cell distribution width, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular volume during the experiment in the presence or absence of Compstatin.

ment system throughout the experiment. At the lowest dose tested, a total of 17 mg/kg (5 mg/kg bolus and 0.2 mg/kg/min, 0–60 min infusion), minimal activation was observed, and this activation occurred only during the first 10 min of protamine administration. Our observation that Compstatin has a minimal effect when administered by constant infusion suggests that an initial bolus is critical for inhibiting peak activation immediately after protamine administration.

White blood counts showed a decrease after protamine administration, followed later by an increase in both groups; therefore, these changes are independent of complement activation. The same pattern has previously been observed by Gillinov and co-workers (44) in a piglet cardiopulmonary bypass model with sCR1 as inhibitor and by Tonz et al. (45) in patients. This initial decrease may reflect the adhesion of circulating white blood cells to the vessel wall or aggregation re-
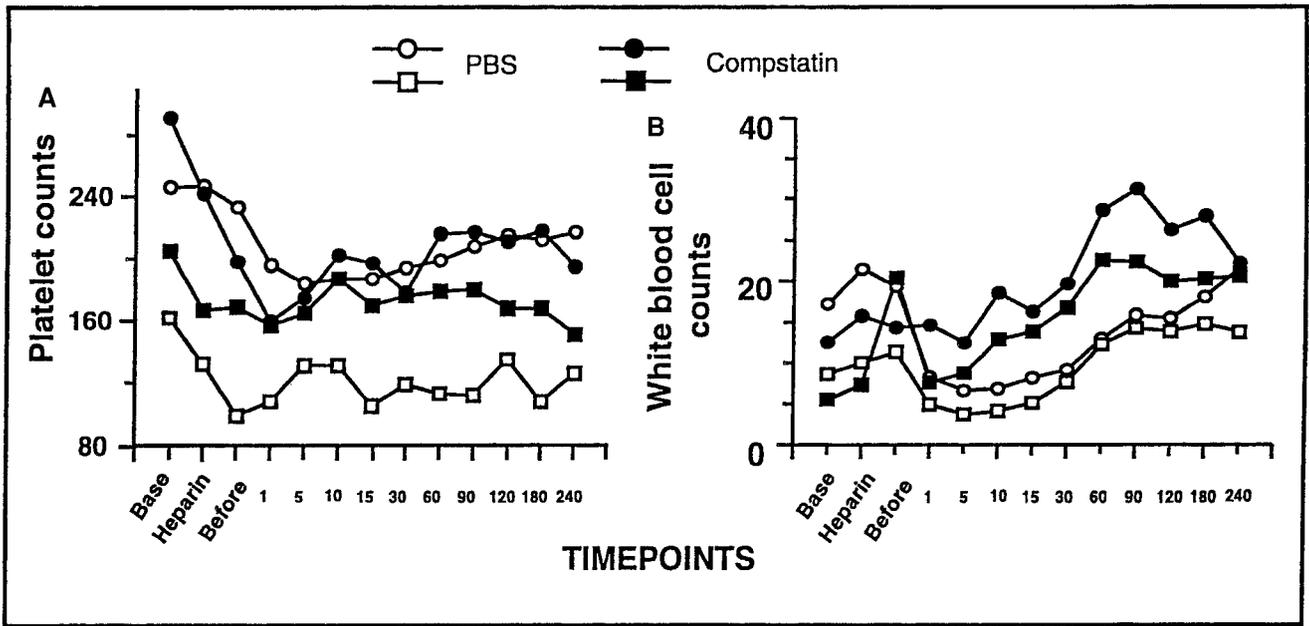
FIG. 5. Effect of Compstatin on platelets and white blood cell counts. Changes in the platelet (A) and white blood cells (B) counts during the experiment. The data are representative from two different animals treated with Compstatin (filled symbols) or PBS (open symbols).

lated to the expression of adhesion molecules on neutrophils. Platelet counts also showed the same pattern in both groups. There was an initial drop of about 20% after heparin administration and then a return to the original levels at 10 min after protamine administration. These observations are in agreement with those of Muriithi *et al.* (46), who suggest that heparinization causes a spontaneous *in vivo* microaggregation of platelets.

We observed no significant differences in hematologic measurements (Hb, MCH, MCHC, MCV, RCDW, RBC, Hct, ACT, WBC, and platelets) between control and Compstatin-treated animals, a finding which suggests that Compstatin has no toxic effect at the concentrations used in this study.

Taken together, our results indicate that Compstatin has a great potential for clinical application as a safe inhibitor of complement activation.

REFERENCES

1. Edmunds, L. H., Jr., Ellison, N., Colman, R. W., Niewiarowski, S., Rao, A. K., Addonizio, V. P., Jr., Stephenson, L. W., and Edie, R. N., Platelet function during cardiac operation: Comparison of membrane and bubble oxygenators. *J. Thorac. Cardiovasc. Surg.* **83,** 805–812, 1982.

2. Zilla, P., Fasol, R., Groscurth, P., Klepetko, W., Reichenspurner, H., and Wolner, E., Blood platelets in cardiopulmonary bypass operations. Recovery occurs after initial stimulation, rather than continual activation [see comments]. *J. Thorac. Cardiovasc. Surg.* **97,** 379–388, 1989.

3. Asimakopoulos, G., and Taylor, K. M., Effects of cardiopulmonary bypass on leukocyte and endothelial adhesion molecules. *Ann. Thorac. Surg.* **66,** 2135–2144, 1998.

4. Kappelmayer, J., Bernabei, A., Gikakis, N., Edmunds, L. H., Jr., and Colman, R. W., Upregulation of Mac-1 surface expression on neutrophils during simulated extracorporeal circulation. *J. Lab. Clin. Med.* **121,** 118–126, 1993.

5. Colman, R. W., Surface-mediated defense reactions. The plasma contact activation system. *J. Clin. Invest.* **73,** 1249–1253, 1984.

6. Kappelmayer, J., Bernabei, A., Edmunds, L. H., Jr., Edgington, T. S., and Colman, R. W., Tissue factor is expressed on monocytes during simulated extracorporeal circulation. *Circ. Res.* **72,** 1075–1081, 1993.

7. Roth, J. A., Golub, S. H., Cukingnan, R. A., Brazier, J., and Morton, D. L., Cell-mediated immunity is depressed following cardiopulmonary bypass. *Ann. Thorac. Surg.* **31,** 350–356, 1981.

8. Nguyen, D. M., Mulder, D. S., and Shennib, H., Effect of cardiopulmonary bypass on circulating lymphocyte function. *Ann. Thorac. Surg.* **53,** 611–616, 1992.

9. Wachtfogel, Y. T., Harpel, P. C., Edmunds, L. H., Jr., and Colman, R. W., Formation of C1s–C1-inhibitor, kallikrein–C1-inhibitor, and plasmin–alpha 2-plasmin-inhibitor complexes during cardiopulmonary bypass. *Blood* **73,** 468–471, 1989.

10. Gikakis, N., Khan, M. M., Hiramatsu, Y., Gorman, J. H., III, Hack, C. E., Sun, L., Rao, A. K., Niewiarowski, S., Colman, R. W., and Edmunds, L. H., Jr., Effect of factor Xa inhibitors on thrombin formation and complement and neutrophil activation during

in vitro extracorporeal circulation. *Circulation* **94,** 11341–11346, 1996.

11. Chung, J. H., Gikakis, N., Rao, A. K., Drake, T. A., Colman, R. W., and Edmunds, L. H., Jr., Pericardial blood activates the extrinsic coagulation pathway during clinical cardiopulmonary bypass. *Circulation* **93,** 2014–2018, 1996.

12. Boisclair, M. D., Lane, D. A., Philippou, H., Esnouf, M. P., Sheikh, S., Hunt, B., and Smith, K. J., Mechanisms of thrombin generation during surgery and cardiopulmonary bypass [see comments]. *Blood,* **82,** 3350–3357, 1993.

13. Gram, J., Janetzko, T., Jespersen, J., and Bruhn, H. D., Enhanced effective fibrinolysis following the neutralization of heparin in open heart surgery increases the risk of post-surgical bleeding. *Thromb. Haemost.* **63,** 241–245, 1990.

14. Johnson, R. J., Complement activation by biomaterials. *Prog. Clin. Biol. Res.* **337,** 507–512, 1991.

15. Pekna, M., Nilsson, L., Nilssonekdahl, K., Nilsson, U. R., and Nilsson, B., Evidence for iC3 generation during cardiopulmonary bypass as the result of blood–gas interaction. *Clin. Exp. Immunol.* **91,** 404–409, 1993.

16. Cavarocchi, N. C., Pluth, J. R., Schaff, H. V., Orszulak, T. A., Homburger, H. A., Solis, E., Kaye, M. P., Clancy, M. S., Kolff, J., and Deeb, G. M., Complement activation during cardiopulmonary bypass. Comparison of bubble and membrane oxygenators. *J. Thorac. Cardiovasc. Surg.* **91,** 252–258, 1986.

17. van Oeveren, W., Kazatchkine, M. D., Descamps-Latscha, B., Maillet, F., Fischer, E., Carpentier, A., and Wildevuur, C. R., Deleterious effects of cardiopulmonary bypass. A prospective study of bubble versus membrane oxygenation. *J. Thorac. Cardiovasc. Surg.* **89,** 888–899, 1985.

18. Cavarocchi, N. C., Schaff, H. V., Orszulak, T. A., Homburger, H. A., Schnell, W. A., Jr., and Pluth, J. R., Evidence for complement activation by protamine–heparin interaction after cardiopulmonary bypass. *Surgery* **98,** 525–531, 1985.

19. Chenoweth, D. E., Cooper, S. W., Hugli, T. E., Stewart, R. W., Blackstone, E. H., and Kirklin, J. W., Complement activation during cardiopulmonary bypass: Evidence for generation of C3a and C5a anaphylatoxins. *N. Engl. J. Med.* **304,** 497–503, 1981.

20. del Balzo, U., Engler, R. L., and Ito, B. R., Complement C5a-mediated myocardial ischemia and neutrophil sequestration: Two independent phenomena. *Am. J. Physiol.* **264,** H336–H344, 1993.

21. Bruins, P., Te, V. H., Yazdanbakhsh, A. P., Jansen, P. G., van Hardevelt, F. W., de Beaumont, E. M., Wildevuur, C. R., Eijsman, L., Trouwborst, A., and Hack, C. E., Activation of the complement system during and after cardiopulmonary bypass surgery: Postsurgery activation involves C-reactive protein and is associated with postoperative arrhythmia. *Circulation* **96,** 3542–3548, 1997.

22. Kirklin, J. K., Westaby, S., Blackstone, E. H., Kirklin, J. W., Chenoweth, D. E., and Pacifico, A. D., Complement and the damaging effects of cardiopulmonary bypass. *J. Thorac. Cardiovasc. Surg.* **86,** 845–857, 1983.

23. Daffern, P. J., Pfeifer, P. H., Ember, J. A., and Hugli, T. E., C3a is a chemotaxin for human eosinophils but not for neutrophils. I. C3a stimulation of neutrophils is secondary to eosinophil activation. *J. Exp. Med.* **181,** 2119–2127, 1995.

24. Legler, D. F., Loetscher, M., Jones, S. A., Dahinden, C. A., Arock, M., and Moser, B., Expression of high- and low-affinity receptors for C3a on the human mast cell line HMC-1. *Eur. J. Immunol.* **26,** 753–758, 1996.

25. Nilsson, G., Johnell, M., Hammer, C. H., Tiffany, H. L., Nilsson, K., Metcalfe, D. D., Siegbahn, A., and Murphy, P. M., C3a and C5a are chemotaxins for human mast cells and act through distinct receptors via a pertussis toxin-sensitive signal transduction pathway. *J. Immunol.* **157,** 1693–1698, 1996.

26. Rinder, C. S., Rinder, H. M., Johnson, K., Smith, M., Lee, D. L., Tracey, J., Polack, G., Higgins, P., Yeh, C. G., and Smith, B. R., Role of C3 cleavage in monocyte activation during extracorporeal circulation. *Circulation* **100,** 553–558, 1999.

27. Rinder, C. S., Rinder, H. M., Smith, B. R., Fitch, J. C., Smith, M. J., Tracey, J. B., Matis, L. A., Squinto, S. P., and Rollins, S. A., Blockade of C5a and C5b-9 generation inhibits leukocyte and platelet activation during extracorporeal circulation. *J. Clin. Invest.* **96,** 1564–1572, 1995.

28. Fitch, J. C., Rollins, S., Matis, L., Alford, B., Aranki, S., Collard, C. D., Dewar, M., Elefteriades, J., Hines, R., Kopf, G., Kraker, P., Li, L., O'Hara, R., Rinder, C., Rinder, H., Shaw, R., Smith, B., Stahl, G., and Shernan, S. K., Pharmacology and biological efficacy of a recombinant, humanized, single-chain antibody C5 complement inhibitor in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass. *Circulation* **100,** 2499–2506, 1999.

29. Fujii, S., and Hitomi, Y., New synthetic inhibitors of C1r, C1 esterase, thrombin, plasmin, kallikrein and trypsin. *Biochim. Biophys. Acta* **661,** 342–345, 1981.

30. Larsson, R., Elgue, G., Larsson, A., Ekdahl, K. N., Nilsson, U. R., and Nilsson, B., Inhibition of complement activation by soluble recombinant CR1 under conditions resembling those in a cardiopulmonary circuit: Reduced up-regulation of CD11b and complete abrogation of binding of PMNs to the biomaterial surface. *Immunopharmacology* **38,** 119–127, 1997.

31. Moran, P., Beasley, H., Gorrell, A., Martin, E., Gribling, P., Fuchs, H., Gillett, N., Burton, L. E., and Caras, I. W., Human recombinant soluble decay accelerating factor inhibits complement activation in vitro and in vivo. *J. Immunol.* **149,** 1736–1743, 1992.

32. Christiansen, D., Milland, J., Thorley, B. R., Mckenzie, I. F. C., and Loveland, B. E., A functional analysis of recombinant soluble CD46 in vivo and a comparison with recombinant soluble forms of CD55 and CD35 in vitro. *Eur. J. Immunol.* **26,** 578–585, 1996.

33. Lazar, H. L., Bao, Y., Gaudiani, J., Rivers, S., and Marsh, H., Total complement inhibition: An effective strategy to limit ischemic injury during coronary revascularization on cardiopulmonary bypass. *Circulation* **100,** 1438–1442, 1999.

34. Fukutomi, M., Kobayashi, S., Niwaya, K., Hamada, Y., and Kitamura, S., Changes in platelet, granulocyte, and complement activation during cardiopulmonary bypass using heparin-coated equipment. *Artif. Organs* **20,** 767–776, 1996.

35. Hendrikx, M., Leunens, V., Vandezande, E., Wallyn, A., and Flameng, W., The use of a heparin removal device: A valid alternative to protamine. *Int. J. Artif. Organs* **20,** 166–174, 1997.

36. Sahu, A., Kay, B. K., and Lambris, J. D., Inhibition of human complement by a C3-binding peptide isolated from a phage displayed random peptide library. *J. Immunol.* **157,** 884–891, 1996.

37. Fiane, A. E., Mollnes, T. E., Videm, V., Hovig, T., Hogasen, K., Mellbye, O. J., Spruce, L., Moore, W. T., Sahu, A., and Lambris, J. D., Compstatin, a peptide inhibitor of C3, prolongs survival of ex vivo perfused pig xenografts. *Xenotransplantation* **6,** 52–65, 1999.

38. Nilsson, B., Larsson, R., Hong, J., Elgue, G., Ekdahl, K. N., Sahu, A., and Lambris, J. D., Compstatin inhibits complement and cellular activation in whole blood in two models of extracorporeal circulation. *Blood* **92,** 1661–1667, 1998.

39. Hammer, C. H., Wirtz, G. H., Renfer, L., Gresham, H. D., and Tack, B. F., Large scale isolation of functionally active compo-

nents of the human complement system. *J. Biol. Chem.* **256,** 3995–4006, 1981.

40. Alsenz, J., Avila, D., Huemer, H. P., Esparza, I., Becherer, J. D., Kinoshita, T. W. Y., Oppermann, S., and Lambris, J. D., Phylogeny of the third component of complement, C3: Analysis of the conservation of human CR1, CR2, H, and B binding sites, concanavalin A binding sites, and thiolester bond in the C3 from different species. *Dev. Comp. Immunol.* **16,** 63–76, 1992.

41. Becherer, J. D., and Lambris, J. D., Identification of the C3b receptor-binding domain in third component of complement. *J. Biol. Chem.* **263,** 14586–14591, 1988.

42. Hiramatsu, Y., Gikakis, N., Gorman, J. H., III, Khan, M. M., Hack, C. E., Velthuis, H. T., Sun, L., Marcinkiewicz, C., Rao, A. K., Niewiarowski, S., Colman, R. W., Edmunds, L. H., Jr., and Anderson, H. L., III, A baboon model for hematologic studies of cardiopulmonary bypass. *J. Lab. Clin. Med.* **130,** 412–420, 1997.

43. Sahu, A., Morikis, D., Soulika, A. M., Spruce, L., Moore, W. T., and Lambris, J. D., Species specificity, structural functional analysis and biotransformation studies on Compstatin, a potent complement inhibitor. *Mol. Immunol.* **35,** 371–371, 1998.

44. Gillinov, A. M., DeValeria, P. A., Winkelstein, J. A., Wilson, I., Curtis, W. E., Shaw, D., Yeh, C. G., Rudolph, A. R., Baumgartner, W. A., and Herskowitz, A., Complement inhibition with soluble complement receptor type 1 in cardiopulmonary bypass. *Ann. Thorac. Surg.* **55,** 619–624, 1993.

45. Tonz, M., Mihaljevic, T., Von Segesser, L. K., Fehr, J., Schmid, E. R., and Turina, M. I., Acute lung injury during cardiopulmonary bypass. Are the neutrophils responsible? *Chest* **108,** 1551–1556, 1995.

46. Muriithi, E. W., Milne, E. M., Wanikiat, P., Armstrong, R. A., Wheatley, D. J., and Belcher, P. R., Heparinisation, platelet activation and cardiopulmonary bypass. http://www.ctsnet.org/doc/3001.