EFFECTS OF INHIBITION OF COMPLEMENT ACTIVATION USING RECOMBINANT SOLUBLE COMPLEMENT RECEPTOR 1 ON NEUTROPHIL CD11B/CD18 AND L-SELECTIN EXPRESSION AND RELEASE OF INTERLEUKIN-8 AND ELASTASE IN SIMULATED CARDIOPULMONARY BYPASS

The inflammatory response to cardiopulmonary bypass includes activation of complement and induction of several neutrophil activation pathways. A recombinant soluble form of complement receptor 1 was used as a specific inhibitor of complement activation in simulated cardiopulmonary bypass circuits. Substantial complement activation was observed in these circuits with progressive accumulation of both plasma C3a and terminal complement complex. Soluble complement receptor 1 resulted in a significant reduction in C3a levels ($p < 0.01$) but did not inhibit terminal complement complex generation. A marked rise in neutrophil CD11b/CD18 expression, simultaneous loss of L-selectin expression, and a progressive accumulation of plasma elastase–α_1-antitrypsin occurred and were not affected by soluble complement receptor. However, generation of interleukin-8 in the circuits was inhibited ($p < 0.05$) by pretreatment with soluble complement receptor. These data suggest that changes in neutrophil activation seen during cardiopulmonary bypass may not be induced directly by anaphylatoxin generation. (J THORAC CARDIOVASC SURG 1996;111:451-9)

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Activation of complement during cardiopulmonary bypass has been proposed as an important mediator of the adverse effects of cardiopulmonary bypass, particularly because of the known stimulatory effects of products of complement activation on neutrophils. Although the importance of complement activation is controversial,[1, 2] inhibition of complement activation during experimental cardiopulmonary bypass can attenuate cardiopulmonary bypass–induced lung injury and endothelial dysfunction.[3] Activation of both classic and alternative pathways occurs in cardiopulmonary bypass, and both culminate in cleavage of C3 and C5 with the generation of the anaphylatoxins C3a and C5a, which can activate neutrophils, enhancing chemotactic activity, respiratory burst, and degranulation, as well as inducing changes in adhesion molecule expression.[4] Although C5a is the more potent neutrophil stimulatory factor, it is rapidly bound to specific receptors expressed by neutrophils[5] and is thus removed from the circulation, which thereby restricts its value as an index of complement activation. The cleavage of C5 also results in the activation of the membrane attack pathway and the deposition of C5b-9 in the target cell membrane, which may result in cell lysis or cell activation. A soluble form of the complex, SC5b-9, is also formed as a by-product of the pathway and released into the plasma. It is relatively stable and therefore a good marker of activation of the terminal complement pathway.[6]

There is evidence that neutrophils mediate microvascular injury after cardiopulmonary bypass.[7-9] Their adhesion to endothelium is a prerequisite for this.[10] An initial tumbling or rolling adhesion medi-

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ated by selectin adhesion molecules expressed by both unactivated neutrophils (L-selectin)[11] and endothelial cells is followed by an activation step during which the neutrophil changes from a spherical, smooth shape to an elongated, bipolar structure leading to more avid adhesion mediated by leukocyte integrins (CD11a/CD18 and CD11b/CD18).[12] Numerous chemotactic agents, including C5a and interleukin-8 (IL-8), induce a rapid cleavage of the extracellular domain of L-selectin and a simultaneous rapid increase in the surface expression of CD11b/CD18 by mobilization of preformed granular stores.[13] The exact functional significance of these changes remains uncertain but they are sensitive markers of neutrophil activation.

Elastase, an endopeptidase (protease) released by neutrophils, is a component of the nonoxidative microbicidal response. The enzyme can degrade several elements of the endothelial extracellular matrix and thus may contribute to neutrophil-induced endothelial injury.[14, 15] Free elastase is rapidly bound to the plasma endopeptidase inhibitor α_1-antitrypsin to form a stable complex, and elevated levels of this can be detected in the blood of patients with acute inflammatory states[16] and have been demonstrated in cardiopulmonary bypass both in vivo[17, 18] and in simulated circuits.[19, 20]

IL-8 is member of a large family of related cytokines and has potent and specific effects on neutrophils.[21] In addition to chemotactic activity, IL-8 can induce enzyme release and the respiratory burst and cause changes in adhesion to endothelium. IL-8 has been shown bound to endothelium in immunohistochemical studies[22] and may regulate neutrophil transendothelial migration.[23, 24] Subcutaneous injection of the cytokine induces a rapid and specific neutrophil infiltration and associated endothelial injury.[25]

In the context of cardiopulmonary bypass, it has been widely stated that the adverse effects of complement activation are mediated via neutrophil activation.[26-28] In this study we examined the hypothesis that cardiopulmonary bypass–induced complement activation contributes to the induction of changes in neutrophil adhesive properties, to the release of neutrophil elastase, and to the generation of IL-8.

Methods

The study was approved by the ethics committee of the Royal Brompton National Heart and Lung Hospital. All volunteers gave informed consent.

Cardiopulmonary bypass circuits. Ten complete experiments were done and no samples were lost during collection or analysis. Circuits consisted of an oxygenator (model Wm Harvey H1700, CR Bard Inc., Tewksbury, Mass.) and reservoir connected by standard ⅜-inch polyvinyl chloride (PVC) tubing to an occlusive roller pump. The oxygenators were connected via a water circuit to a thermostatic heat exchanger and had filtered air passed through the oxygen port at 2 L/min throughout the experiment. Four hundred milliliters of fresh human blood, obtained by venipuncture from healthy adult male volunteers, was mixed immediately with 600 ml of Hartmann's solution and used to prime the circuit. Anticoagulation was achieved with heparin to a final concentration of 4 units/ml prime. A flow rate of 3 L/min was maintained for 2 hours at 37° C. A further 30 ml of blood was mixed with 45 ml of Hartmann's solution, identically anticoagulated, and used to prime a closed loop of identical circuit tubing, which was left static in a water bath at 37° C. In half the experiments a freshly reconstituted solution of recombinant soluble complement receptor 1 (sCR1) (final concentration 50 μg/ml) was added to the collection bag and syringe before venipuncture. In the remainder reconstitution solvent only was added. Previous pilot studies had shown that this concentration produced near maximal inhibition of C3a generation in similar circuits (data not shown).

Samples were taken immediately after hemodilution and 2, 30, 60, 90, and 120 minutes after commencement of circulation from both circuits and static loops. Blood anticoagulated with ethylenediaminetetraacetic acid was analyzed for hematocrit concentration, leukocyte count (Coulter counter), and manual leukocyte differential and centrifuged at 4° C; the plasma was separated and stored at −70° C for analysis of IL-8, elastase–α_1-antitrypsin, C3a, and terminal complement complex (TCC). A further four aliquots from each sample were anticoagulated with a 20 u/ml concentration of preservative-free heparin and cells stained immediately with the use of direct immunofluorescence.

Direct immunofluorescence staining. Four 25 μl aliquots of blood from each sample were incubated for 10 minutes at room temperature with saturating concentrations of one each of the following four fluorochrome-conjugated mouse immunoglobulin G1 anti-human monoclonal antibodies: anti-CD11a (25-3-1) (The Binding Site, Birmingham, United Kingdom), anti-CD11b (44) (Cymbus, Southampton, United Kingdom), anti–L-selectin (TQ1) (Coulter, Luton, United Kingdom), and a negative control antibody directed against a nonhuman epitope (X927) (Dako, High Wycombe, United Kingdom) as previously described.[29] Erythrocytes were then lysed with fluorescence-activated cell sorter lysing solution (Becton Dickinson, Oxford, United Kingdom), and samples were washed with Hanks' balanced salt solution without calcium, magnesium, or phenol red (Gibco, Paisley, United Kingdom), resuspended in fixative (1% formaldehyde, 2% glucose in phosphate buffered saline [PBS]), and held at 4° C in the dark until flow cytometry.

Flow cytometry. Flow cytometry was done with a Becton-Dickinson FACScan instrument. Green and red amplifier gains were calibrated before each experiment with

beads (Flow Cytometry Standards Corp., Research Triangle Park, N.C.) to ensure that relative fluorescence values were comparable between experiments and to monitor cytometer performance. Light scatter data amplifiers were set in linear mode whereas fluorescence detectors were set in (4 decade) logarithmic mode. Forward and orthogonal light scatter and green and red fluorescence data from 2000 events (leukocytes) from each sample were then acquired. Data were analyzed with the use of Datamate flow cytometry software (Applied Cytometry Systems, Sheffield, United Kingdom). For each experiment, a new analysis gate was created and verified to encompass the granulocyte cluster on the forward/orthogonal scatter dot plots for all samples. A median channel number was computed for each distinct population on the fluorescence histogram of each gated population (Fig. 1) and converted mathematically into a relative fluorescence value by the following formula: relative fluorescence = antilog_{10}(channel number/number of channels per decade). These values, with the relevant negative control antibody value subtracted in each case, were then used to represent the level of expression of the epitopes under study on the surface of the neutrophils.

Immunosorbent assays for TCC, elastase–α_1-antitrypsin, and IL-8. TCC was assayed with use of a monoclonal capture antibody (B7) that recognizes a neo-epitope on the complex not expressed by the component proteins before assembly.[30] PVC 96-well plates (Flow Laboratories, Herts, United Kingdom) were coated with antibody B7 (10 μg/ml) in carbonate/bicarbonate buffer (pH 9.6) for 16 hours at 4° C or for 2 hours at 37° C, washed, and then blocked for 1 hour. Standards prepared from dilutions of fully inulin-activated normal human serum and samples, diluted 1 in 100, were added and incubated for 2 hours. Wells were then washed and incubated with rabbit anti-S protein IgG (in-house), diluted 1 in 2000, for 1.5 hours. Wells were then washed and incubated with donkey anti-rabbit IgG conjugated to horseradish peroxidase (Jackson Immunoresearch Labs, West Grove, Pa.) diluted 1 in 5000, for 1 hour. After further washes, a substrate solution containing 0.7 mg/ml o-phenylenediamine dihydrochloride in a solution of 0.05% H_2O_2, 0.1 mol/L citric acid, and 0.2 mol/L Na_2HPO_4 was added; the reaction was stopped after 2 to 5 minutes with 10% H_2SO_4, and plates were read with use of a spectrophotometer (Titertek Multiscan MCC/340, Flow ICN, Rickmansworth, United Kingdom) set to 492 nm. PBS with 0.1% polysorbate 20 (Tween 20, BDH, Poole, United Kingdom) was used for all washes, with 1% bovine serum albumin (Sigma, Poole, United Kingdom) added for dilution of samples, standards, second and third antisera, and blocking steps. Incubation and blocking steps were done at 37° C. All samples were assayed in triplicate and percentage activation calculated from a standard curve prepared concurrently with each assay. The percentage activation in each sample was converted to micrograms per milliliter TCC using a conversion factor of 1% activation = 2.2 μg/ml, derived from Hugo, Kramer, and Bhakdi.[31]

Elastase-α_1-antitrypsin concentrations were measured by an assay modified after Brower and Harpel.[32] PVC 96-well plates (Greiner, Frickenhausen, Germany) were coated with sheep anti-human elastase (The Binding Site) (15 mg/ml) in carbonate/bicarbonate buffer (pH 9.6) for 24 hours, washed, and blocked for 30 minutes. Standards and samples, diluted 1 in 100 to 1 in 500, were added and incubated for 2 hours. Wells were then washed and incubated with peroxidase-conjugated sheep anti-human α_1-antitrypsin (The Binding Site), diluted 1 in 1000, for 1 hour. After further washes, a substrate solution containing a 0.5 mg/ml concentration of o-phenylenediamine dihydrochloride in a solution of 0.05% H_2O_2, 0.1 mol/L citric acid, and 0.2 mol/L Na_2HPO_4 was added; the reaction was stopped after 20 minutes with 4 mol/L H_2SO_4, and plates were read with use of a spectrophotometer (Titertek Multiscan; Flow ICN) set to 492 nm. PBS with 0.05% polysorbate 20 (Tween 20, BDH) was used for all washes and dilution of samples, standards, and second antisera, with 1% bovine serum albumin (BDH) added for blocking. Coating, incubation, and blocking steps were done at room temperature. All samples were assayed in duplicate and concentrations calculated from a standard curve prepared concurrently with each assay.

Assays for IL-8 were done with the use of kits (Quantikine, R & D Systems, Minneapolis, Minn.) according to the manufacturer's instructions. Samples were diluted 1 in 2 in the appropriate supplied diluent and incubated for 2 hours in wells coated with monoclonal anti-cytokine antibody. Wells were washed and then incubated for 2 hours with polyclonal anti-cytokine antibody conjugated to horseradish peroxidase. After further washes, a substrate solution containing tetramethylbenzidine and H_2O_2 was added; the reaction was stopped after 20 minutes with 2 mol/L H_2SO_4 and plates were read with use of a spectrophotometer (Dynatech MR4000, Billingshurst, United Kingdom) set to 450 nm with wavelength correction set to 570 nm. The supplied wash buffer was used for all washes. Incubation steps were done at room temperature. All samples were assayed in duplicate and concentrations calculated from a standard curve prepared concurrently with each assay.

The lower limit of these assays was defined, in all cases, as that concentration that corresponded to an optical density of 1.5 times the mean optical density of the blank wells of the plate.

Radioimmunoassays for C3a. Assays were done with the use of [^{125}I]radioimmunoassay kits obtained from Amersham International (Alysbury, Bucks, United Kingdom). Briefly, the assay is based on competition between the unknown C3a and C3a desArg in the sample and ^{125}I-labeled C3a desArg for a limited number of binding sites on a specific rabbit anti-C3a antibody. The antibody-bound fraction is reacted with a second antibody (goat anti-rabbit) and separated by centrifugation at 2000 *g*. The radioactivity in the pellet is measured with a gamma scintillation counter and the concentration of unlabeled C3a and C3a desArg in the sample determined by interpolation from a standard curve. The working range for the assay is 40 to 100 ng/ml: samples that contained more than this concentration were diluted appropriately and reassayed. The assay shows less than 1% cross-reactivity with C4a desArg and C5a desArg. The intraassay variation lies between 3% and 9% depending on the concentration of C3a in the sample.

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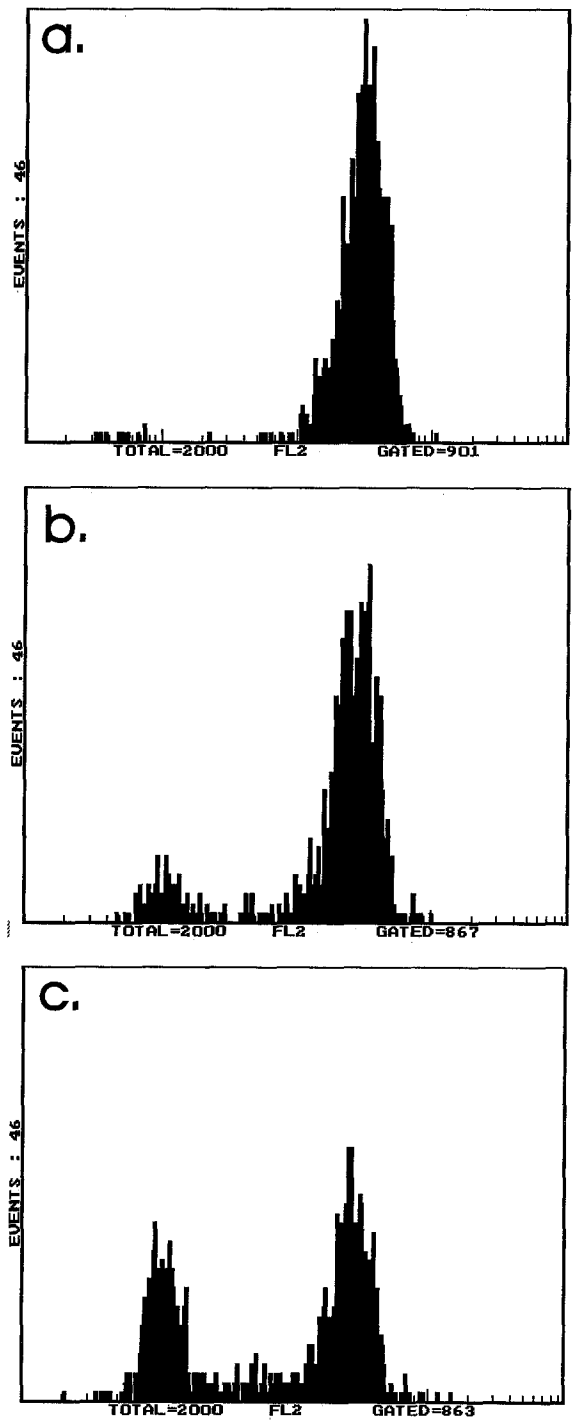

Fig. 1. Fluorescence histograms of gated granulocytes stained with phycoerythrin-conjugated anti–L-selectin in blood samples taken after 2 (**a**), 60 (**b**), and 120 (**c**) minutes of circulation in oxygenator circuit. Slight decrease in fluorescence of main population of cells *(right)* is observed with time whereas new population with low fluorescence emerges *(left)* at expense of main population. Note that fluorescence is shown on logarithmic scale.

Statistics. Summary measures statistics were used to incorporate the values from the multiple measurements made at different times without making multiple comparisons.

Differences between circulating and static blood. To assess the significance of differences in the various parameters under study between paired samples taken simultaneously from circuits and static loops, the difference was calculated for each time point in an experiment and plotted against time. For each set of points corresponding to a single experiment a regression line passing through the origin (because the difference at time zero was always nil) was fitted. Where the slopes of these lines are, on average, zero, this suggests, for the data observed in this study, that no difference exists between values for circulating and static blood. Slopes were calculated with the formula $b = \Sigma x_i y_i / \Sigma x^2$, where b is the slope, x is the time value, y is the parameter value (e.g., the difference in CD11b expression), and i equals 1, . . ., n representing serial measurements. A one-sample *t* test was then used to test the hypothesis that the slopes were zero on average. Where changes in the parameter under study were detected only at one point (e.g., IL-8) a paired *t* test was done comparing the differences between the circulating and static values for each experiment at that time. sCR1-treated and sCR1-untreated groups were assessed separately.

Effects of sCR1. To assess the effects of sCR1 on the parameters under investigation first-order regression lines (least squares) were fitted to plots of measured values against time. In this instance circulating values and static values were assessed separately because of the likelihood that sCR1 could be affecting changes in both, so that such effects could be masked in an analysis of the differences. If sCR1 was having no effect, the slopes of these lines would, on average, be identical in experiments with and without sCR1. An unpaired *t* test was then used to test this hypothesis. Where changes in the parameter under study were detected only at one point (i.e., IL-8) an unpaired *t* test was done instead on the two groups of values at that time. In all other cases comparisons between individual points were not made.

Results

A large progressive rise in plasma C3a level was observed in the circuits with time (Fig. 2). A smaller rise ($t = 5.31, p < 0.001$) was also observed in static control loops. These rises were both markedly reduced by treatment with sCR1 ($t = 3.69$ and 6.88, respectively; both $p < 0.01$). A rise in plasma TCC level was also observed in the circuits, although not in the static control loops (Fig. 3). Treatment with sCR1 had no effect on this rise in plasma TCC level.

Hematocrit concentrations and neutrophil counts fell to about 40% of values in undiluted venous blood when the blood was added to the priming solution and values remained at this level throughout the experiments in both circuits and static control loops. This was not altered by sCR1 treat-

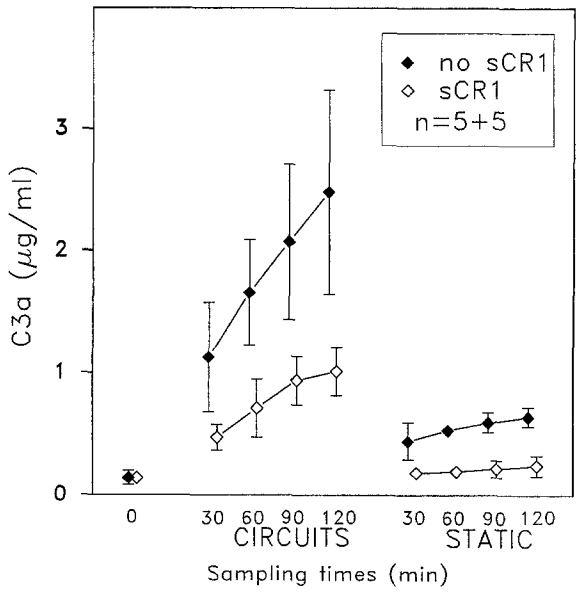

Fig. 2. Plasma concentrations of C3a in cardiopulmonary bypass circuits. Samples were taken at 30-minute intervals. Results from control loops of circuit tubing held static are shown on right-hand side. Data are shown as mean plus or minus 2 standard errors. Treatment with sCR1 (50 μg/ml) greatly reduced C3a generation ($p < 0.01$).

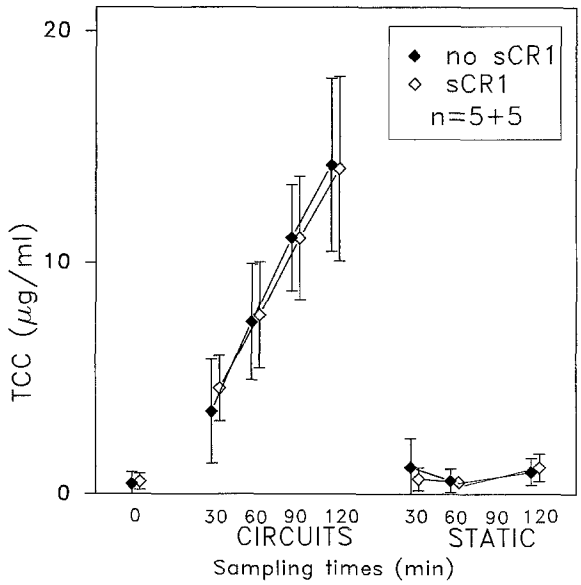

Fig. 3. Plasma concentrations of TCC in cardiopulmonary bypass circuits. Samples were taken at 30-minute intervals. Results from control loops of circuit tubing held static are shown on right-hand side. Data are shown as mean plus or minus 2 standard errors. Treatment with sCR1 (50 μg/ml) did not modify TCC generation.

ment (data not shown). No significant change in neutrophil CD11a/CD18 expression was observed under any conditions (data not shown). A marked homogeneous and progressive rise in neutrophil CD11b/CD18 expression occurred in the circuits and contrasted with significantly smaller rises in the static control loops. This increase in neutrophil CD11b expression was not significantly affected by treatment with sCR1 ($t = -0.62, p > 0.5$) (Fig. 4).

Two distinct patterns of L-selectin shedding from the surfaces of the circulating neutrophils were observed. A progressive fall in L-selectin expression was observed in the circuits but not in the static loops, mirroring the change in CD11b/CD18 expression, although the change was much less marked (Figs. 1 and 5). Additionally a population of neutrophils emerged that had lost nearly all expression of L-selectin, growing progressively in number with time at the expense of L-selectin-positive cells so that by 2 hours they numbered about 40% of the total (Fig. 1). Although the rate of L-selectin loss in the former process was significantly slower in sCR1-treated circuits ($t = -4.51, p < 0.01$) (Fig. 5), this difference was only apparent between 60 and 120 minutes and the levels of expression were the same after 2 hours in the treated and untreated circuits because the neutrophils in the treated circuits had lower mean initial expression. The loss of L-selectin-positive cells was not affected by sCR1 ($t = 0.43$, $p > 0.5$) (data not shown).

Plasma elastase–α_1-antitrypsin concentrations rose substantially in the circuits with time and again showed only small changes in static control loops (no sCR1: time = 0, 0.17 ± 0.02 μg/ml [mean ± 2 SE*]; time = 120 circuits, 0.52 ± 0.22 μg/ml; time = 120 static, 0.20 ± 0.02 μg/ml). Treatment with sCR1 had no observed effect on release of the enzyme (sCR1: time = 120 circuits, 0.44 ± 0.08 μg/ml; time = 120 static, 0.2 ± 0.02 μg/ml).

IL-8 was also detected in circuits after 2 hours of circulation and not in static control loops ($t = 4.39$, $p < 0.01$). The concentrations in the plasma from circuits treated with sCR1 were significantly lower than those in untreated circuits ($t = 2.49, p < 0.05$) (Fig. 6).

Discussion

CR1 is a regulator of complement activation protein that may prove useful as an effective specific inhibitor of complement activation in vivo.[33] It

*Standard error.

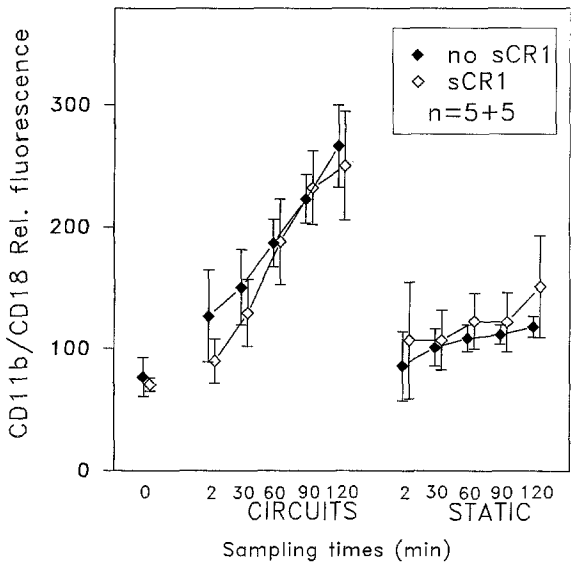

Fig. 4. Granulocyte expression of CD11b/CD18 in cardiopulmonary bypass circuits. Samples were taken at 30-minute intervals. Results from control loops of circuit tubing held static are shown on right-hand side. Data are shown as mean plus or minus 2 standard errors. Treatment with sCR1 (50 μg/ml) did not significantly modify changes. *Rel.,* Relative.

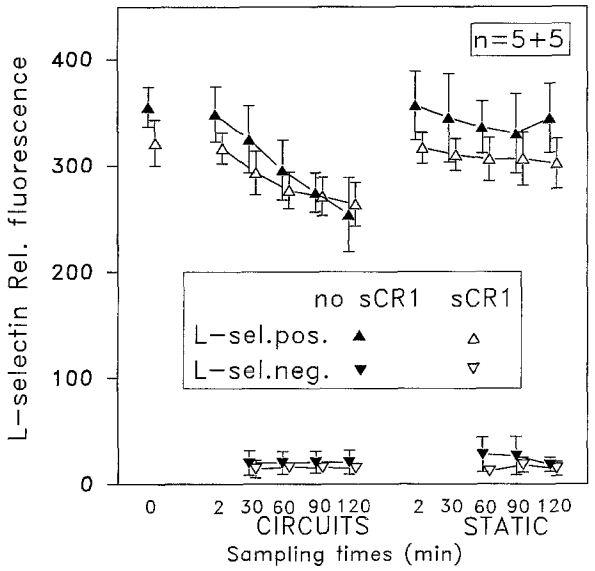

Fig. 5. Granulocyte expression of L-selectin *(L-sel.)* in cardiopulmonary bypass circuits. Samples were taken at 30-minute intervals. Results from control loops of circuit tubing held static are shown on right-hand side. Data from each time point are divided into two values corresponding to two populations seen in Fig. 1 (L-selectin negative [*neg.*] and positive [*pos.*]). Data are omitted where mean number of neutrophils is less than 21. Data are shown as mean plus or minus 2 standard errors. Treatment with sCR1 (50 μg/ml) resulted in little modification in changes in L-selectin expression. *Rel.,* Relative.

specifically binds to both C3b and C4b, thus exhibiting a capacity to displace the catalytic subunits of the C3 and C5 convertases of both activating enzymatic pathways. It also acts as a cofactor for the degradation of C3b and C4b by factor I.[34] Additionally, the proteolysis of C3b and C4b releases CR1 and allows it to recycle in the inactivation process. Finally, unlike those of some other regulator of complement activation proteins, the inhibitory effects of CR1 are not restricted by alternative pathway activating surfaces, which makes it particularly suitable for blocking complement activation by nonimmunologic stimuli.[35] Recombinant sCR1 consists of the extracellular amino acid chain of CR1 but lacks the transmembrance and cytoplasmic domains. However, sCR1 retains the inhibitory properties of CR1 and inhibits activation of both the classic and alternative pathways in vitro at concentrations 100 times lower than those of the serum regulator of complement activation protein, factor H.[35]

The data from the control (untreated) circuits corroborate our previous work with both experimental and clinical cardiopulmonary bypass.[18, 29, 36, 37] That is, profound complement activation, marked changes in neutrophil adhesion molecule expression, and an increase in both plasma elastase and plasma IL-8 levels occurred. The apparent dissociation of C3a and TCC generation in the sCR1-treated circuits was an unexpected finding. Inhibition of C3a generation by sCR1 did not alter TCC production. With conventional complement activation the cleavage of C5 releases C5b, which initiates the terminal pathway resulting in the formation of C5b-9. Given that sCR1 is more effective at disassociating the C5 compared with the C3 convertase, for any given reduction in C3a generation one should see an equal if not greater reduction in C5a and C5b and hence C5b-9 (and SC5b-9) generation. This suggests that a novel method of activation of the terminal pathway may be occurring. Vogt and colleagues[38] have previously reported direct activation of C5 by reactive oxygen species with the formation of a novel terminal complex containing oxidized C5 rather than C5b. We have recently demonstrated that the soluble TCC formed in these experiments is not conventional SC5b-9, in that it contains uncleaved C5 (that is, it is SC5-9). We have also shown that the

formation of this neocomplex is not induced by oxidative damage to C5 but by shear stress-induced conformational changes in C5. The full data and detailed discussion of the nature of this neocomplex can found in a parallel report (Moat and associates, submitted for publication). Thus sCR1 partially inhibited conventional complement activation and anaphylatoxin generation in this model, but high circulating levels of a soluble protein that was not classic SC5b-9 but that was recognized by the sandwich enzyme-linked immunosorbent assay for SC5b-9 were unaffected.

What was the effect of sCR1 on neutrophil activation in this model? The marked increase in neutrophil CD11b expression was not inhibited by sCR1. Similarly, the phenomenon of the emergence of an L-selectin–negative population of cells was unaffected. The rate of loss of surface L-selectin by residual L-selectin–positive neutrophils did appear to be reduced by sCR1. However, careful scrutiny of these data reveals that the levels of L-selectin expression in this cell population were the same after 2 hours in the treated and untreated circuits and that the apparent difference was produced by a lower mean initial expression of L-selectin in the neutrophils in the sCR1-treated circuits. The rise in plasma elastase level observed in the untreated circuits was also not affected by sCR1. Given the effect of sCR1 on complement activation in this model, there are three possible explanations for these findings: (1) that the persisting elevation of plasma C3a level (and presumably that of C5a) was sufficient to induce maximal activation of neutrophils; (2) that the generation of the soluble neoterminal complex was able to induce maximal neutrophil activation despite inhibition of anaphylatoxin generation; and (3) that other physical or humoral factors besides complement were responsible for inducing neutrophil activation in this model of cardiopulmonary bypass.

The first hypothesis is possible but we have preliminary unpublished data from experiments using membrane oxygenators in these circuits in which we have been able to abolish C3a generation totally without modifying similar changes in neutrophil adhesion molecule expression and elastase release. On the other hand, in another recent study, dogs with congenital deficiency of C3 undergoing cardiopulmonary bypass demonstrated less upregulation of neutrophil CD11b expression than control animals and had less myeloperoxidase deposition in the lungs.[3] With regard to the second hypothesis, although there is evidence for stimulatory effects of

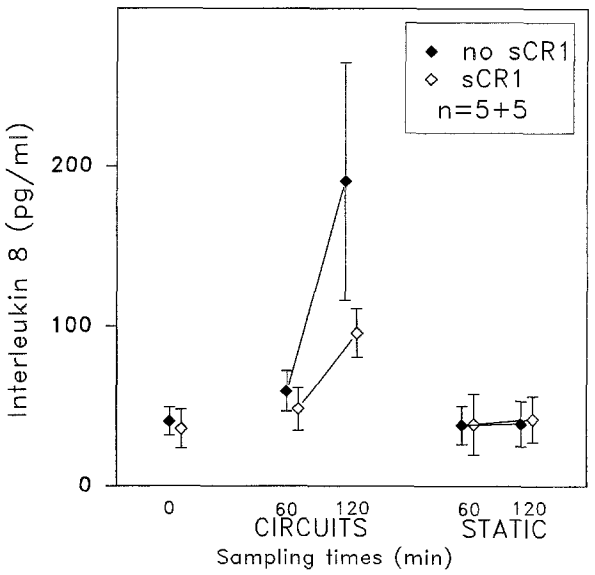

Fig. 6. Plasma concentrations of IL-8 in cardiopulmonary bypass circuits. Samples were taken at 30-minute intervals. Results from control loops of circuit tubing held static are shown on right-hand side. Data are shown as mean plus or minus 2 standard errors. Treatment with sCR1 (50 μg/ml) significantly inhibited IL-8 generation in bubble circuits ($p < 0.01$).

membrane-bound TCC on neutrophils,[39] the biologic activity of the neocomplex generated in these experiments is unknown and requires further study. Finally, other potential neutrophil-activating mediators have been described in clinical and simulated bypass that might explain our results,[40] as might direct physical interaction with the surfaces in the circuit: this is an attractive hypothetical explanation for the total stripping of L-selectin from some neutrophils.

Inhibition of IL-8 generation by sCR1 is an interesting and unexpected finding. IL-8 is transiently detectable in the blood of children and adults undergoing cardiopulmonary bypass.[18, 41] The known cellular sources for IL-8 in these circuits would be mononuclear leukocytes and neutrophils. Established stimuli for the release of IL-8 include IL-1, tumor necrosis factor, and endotoxin.[42, 43] Although C5a desArg did not induce IL-8 release when used as an inflammatory stimulus in a rabbit model of peritonitis,[44] a recent report has shown generation of IL-8 by monocytes stimulated with C5a.[45] IL-8 probably plays an important role in the regulation of transendothelial migration of neutrophils and

thus in neutrophil-mediated endothelial and tissue injury. If this finding of inhibition of IL-8 generation by sCR1 were reaffirmed in vivo, the inhibition could underlie the beneficial effect of sCR1 seen in experimental models of cardiopulmonary bypass.[46] The relationship between complement activation and IL-8 release requires further study.

These results raise the question of the relevance of this model and the observed effects of sCR1 to clinical practice. These experiments only reflect activation of acute inflammatory pathways induced directly and solely by circulation of diluted, heparinized blood through the oxygenator circuit and do not reflect any proinflammatory or antiinflammatory effects of the patient's vasculature; perhaps also for this reason the large fluctuations in neutrophil count we have reported in vivo[18] were not observed. Thus an important advantage of this model was that we were able to study a fixed population of leukocytes over time, which could not have been done in vivo. Furthermore, the conditions we used, a high blood flow rate and no systemic cooling, were chosen to maximize complement activation rather than closely to mimic the situation in vivo, and consequently the plasma levels of C3a were higher than those seen in previous clinical studies.[27] The aim is thus to dissect the relationships between elements of the inflammatory response induced by oxygenator circuitry and allow preliminary evaluation of an agent that could be a candidate for future intervention. In particular, the apparent inhibitory effect of sCR1 on IL-8 generation could prove to be important if this cytokine is shown, in due course, to have a role in the pathophysiologic process of postbypass capillary leak.

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