CARDIOPULMONARY SUPPORT AND PHYSIOLOGY

INTERCELLULAR ADHESION MOLECULE-1 REGULATION IN THE CANINE LUNG AFTER CARDIOPULMONARY BYPASS

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Objective(s): Neutrophil sequestration in the lung after cardiopulmonary bypass has been shown to be dependent on the adhesion molecule CD18. Thus we sought to determine whether endothelial expression of intercellular adhesion molecule-1 (a ligand for CD18) in pulmonary capillaries mediates neutrophil adhesion in this setting. **Methods:** Seven adult mongrel dogs underwent 90 minutes of hypothermic cardiopulmonary bypass with 60 minutes of cardioplegic arrest. After warming, dogs were reperfused for up to 9 hours and lung biopsy specimens were obtained. Lung tissue was examined by Northern and Western blot analysis and by immunohistologic methods. Three sham-operated dogs served as time-matched controls. **Results:** Northern blots demonstrated increased expression of intercellular adhesion molecule-1 messenger ribonucleic acid within 5 minutes of cessation of bypass (or approximately 30 minutes after aortic crossclamp release), which persisted at 9 hours of recovery and was not present in controls. Western blots showed intercellular adhesion molecule-1 protein expression before bypass but a measurable increase in intercellular adhesion molecule-1 protein in four of seven dogs in the bypass group by the ninth hour of recovery. Pulmonary neutrophil accumulation 9 hours after cardiopulmonary bypass was greater in those dogs with an increased intercellular adhesion molecule-1 protein expression. Immunoelectron microscopy demonstrated the pulmonary capillary endothelium capable of increased intercellular adhesion molecule-1 protein expression at the 9-hour time point. **Conclusions:** Cardiopulmonary bypass resulted in intercellular adhesion molecule-1 induction in the canine lung during recovery. An increased expression of intercellular adhesion molecule-1 protein in the lung was associated with an increased accumulation of neutrophils in affected animals. Thus intercellular adhesion molecule-1 expression may serve as a mechanism that predisposes the lungs to inflammatory cell-mediated injury postoperatively. (J Thorac Cardiovasc Surg 1998;115:689-99)

Previous studies by ourselves and others have documented that neutrophils accumulate in the pulmonary capillary bed early after the cessation of

cardiopulmonary bypass (CPB).¹⁻⁵ Furthermore, this accumulation of neutrophils contributes to pulmonary dysfunction and depends on the neutrophil

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surface adhesion molecule CD18.^{3,5} Neutrophil margination and emigration at inflammatory sites depends in part on the cell's ability to adhere to the vascular endothelium. Surface adhesion molecules on both the neutrophil and the endothelial cell modulate this process. One counter receptor or ligand for the leukocyte integrins CD11a/CD18 and CD11b/CD18 found on vascular endothelium is intercellular adhesion molecule-1 (ICAM-1).⁶ This molecule can be upregulated on the endothelial surface after ischemia-reperfusion or after cytokine stimulation.^{7,8} Thus the lungs may be susceptible to increased neutrophil accumulation after bypass caused, in part, by the upregulation of the adhesion molecule ICAM-1 on pulmonary capillary endothelium.

The purpose of this study was to examine the regulation of ICAM-1 in the canine lung after CPB and to determine whether expression of ICAM-1 protein on the pulmonary capillary endothelium correlated with the sequestration of neutrophils in the lung postoperatively. Accordingly, lung biopsy specimens obtained from dogs before and after CPB were examined by Northern blot analysis for ICAM-1 mRNA by Western blot analysis for newly expressed ICAM-1 protein and by immunohistologic examination for neutrophil sequestration and specific tissue localization of ICAM-1 protein.

Methods

Animal preparation. Healthy adult mixed breed dogs (16 to 25 kg) were anesthetized with sodium pentobarbital (30 mg/kg), intubated, and mechanically ventilated with 100% oxygen. After femoral arterial cannulation, ventilatory rate and tidal volume were adjusted to establish a normal pH and carbon dioxide tension as determined by arterial blood gas. After midline thoracotomy, CPB was achieved by selective venous cannulation of the inferior and superior venae cavae with arterial return directed into the distal aortic arch. The bypass circuit used consisted of a cardiectomy reservoir and variable prime membrane oxygenator (Cobe Cardiovascular, Inc., Arvana, Colo.), a heat exchanger, and a roller pump (Sarns/3M Healthcare, Ann Arbor, Mich.). The circuit was primed with 40 to 50 ml/1 kg body weight lactated Ringer's solution. Before CPB, the circuit was demonstrated to be free of endotoxin by *Limulus* ameocyte lysate assay.

Two groups of animals were included in this study. The first group, designated "CPB," consisted of seven animals that underwent CPB with an aortic crossclamp time of 60 minutes and total bypass times of approximately 90 minutes. Before cannulation, animals were anticoagulated with 100 U/kg porcine heparin sodium. After the aorta was crossclamped, the heart was arrested with prograde administration of cold potassium cardioplegia given to effect (Plegisol, Abbott Laboratories, North Chicago, Ill.)

and the animals were cooled to 24° to 28° C. Flow was maintained during bypass at 60 to 70 ml/kg/min at a pressure of 40 to 60 mm Hg. After rewarming and weaning from CPB, animals received protamine sulfate, 1 mg/100 U heparin, delivered for reversal of anticoagulation. Animals were once again ventilated with 100% oxygen. Tidal volume and ventilator rate were set to optimize pH and carbon dioxide tension. Animals were then maintained with the chest open for up to 9 hours while pulmonary tissue samples were obtained. Technique was maintained as constant as possible from one animal to the next.

The second group consisted of three animals designated "sham bypass" controls. These animals served as controls in that they received general anesthesia and mechanical ventilation similar to the animals in group 1. In addition, they underwent midline thoracotomy and cannula placement, but they were not placed on CPB. Also, these animals were not subjected to cooling and rewarming or hemodilution, and there was no reduction of pulmonary blood flow at any time during the procedure. These animals, however, were maintained with the chest open for a similar duration to those animals in group 1. Two animals received heparin anticoagulation and protamine reversal as described for group 1 above, whereas one animal did not. No differences were observed in any of the measured parameters detailed below for the two sham animals receiving heparin and protamine and the single animal that did not. Their results, therefore, were combined.

Approval. The animal studies in this manuscript were reviewed and approved by the Baylor College of Medicine Animal Care and Use Committee. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication No. 86-23, revised 1985).

Northern blot analysis. Lung biopsy material was immediately snap frozen in liquid nitrogen on collection and ribonucleic acid (RNA) extraction was carried out on the same day. RNA was isolated from lung biopsy material using the acid guanidinium phenol chloroform solvent extraction method.⁹ Northern analysis of isolated RNA was subsequently performed with 1% agarose formaldehyde denaturing gels and capillary transfer to nylon membranes using standard procedures.¹⁰ Loading of RNA was monitored using ethidium bromide staining. Membranes were hybridized in Quikhyb rapid hybridization buffer (Stratagene, La Jolla, Calif.) containing $\sim 1 \times 10^6$ dpm/ml random monomer ³²P-labeled canine ICAM-1 cDNA probe and 0.15 mg/ml salmon sperm DNA at 68° C for 2 hours. After washing, filters were exposed to Hyperfilm-MP (Amersham International Ltd., Bucks, UK) as previously described.¹¹⁻¹³

Western blot analysis. Lung tissue was snap frozen in liquid nitrogen and stored at -70° C until use; when thawed, the tissue was extensively flushed with 50 mmol/L Tris, 0.2 mmol/L phenylmethylsulfonyl fluoride buffer, and homogenized with ¼ volume of the same. The

Fig. 1. Northern blots demonstrating the time course of pulmonary ICAM-1 mRNA expression from two representative animals. **A**, In this sham bypass animal, samples time matched to those obtained during a cardiopulmonary bypass experiment showed a minimal ICAM-1 mRNA expression. **B**, In this animal undergoing cardiopulmonary bypass, there was a marked increase in ICAM-1 mRNA expression in the lung 5 minutes after complete cessation of the bypass procedure (or approximately 30 minutes after the release of the aortic crossclamp and lung reperfusion). This expression persisted at 3, 6, and 9 hours of reperfusion. Note the difference in exposure time of the two blots.

homogenate was centrifuged for 3 minutes at 1000g at 4° C, the supernatant was collected and the protein concentration of the supernatant was determined by the Biorad Bradford method. Ten micrograms of supernatant protein was fractionated under reducing conditions (SDS) on 7.5% polyacrylamide and transferred to an Immobilon-P membrane (Millipore Corporation, Bedford, Mass.). After blocking for 1 hour (5% milk, 0.1% Tween-20), primary antibodies (rabbit anti-canine ICAM-1 polyclonal antiserum, 1:20,000 [Scios, Inc., Mountain View, Calif.] and mouse anti-actin, 1:5000 [Amersham]) were incubated overnight at room temperature. The membrane was washed in phosphate-buffered saline solution and incubated 2 hours with secondary antibodies (goat anti-rabbit and goat anti-mouse) conjugated with horseradish peroxidase. The membrane was washed, developed in ECL reagent (Amersham) and exposed to Kodak X-AR film. Blot intensity was measured using a scanning densitometer (AlphaImager 2000, Alpha Innotech, San Leandro, Calif.). Actin detection was included in the Western analysis as an internal standard because actin protein concentration was not expected to change in the lung over the course of the study. ICAM-1 blot intensity was therefore indexed to actin in each lane to control for loading conditions. Within a single blot, changes in ICAM-1 during reperfusion were then expressed as a percentage of the pre-CPB ICAM-1 blot intensity.

Neutrophil sequestration. Canine lung tissue was immersion-fixed in 10% buffered zinc-formalin, embedded in paraffin, and 4 to 5 μ m sections were cut using a rotary microtome. Tissue sections were incubated with SG8H6, an immunoglobulin G1 murine monoclonal antibody to a

canine neutrophil specific antigen,¹⁴ using the Vectastain mouse kit (Vector Laboratories, Inc., Burlingame, Calif.). Antibody detection used the peroxidase-based system with diaminobenzidine (Vector Laboratories) as a substrate. The sections were counterstained with eosin.

Stained tissue sections were analyzed using an image analysis software program (OPTIMAS, Bioscan Inc., Washington, D.C.). Images of lung tissue were captured using a black and white camera (CCD72, Dage MTI) connected to a Leitz Diaplan microscope with frame grabber support with an ALR 486/66 MHz personal computer. Neutrophils in a given 40 $\times$ field were distinguished by their differential gray-scale intensity compared with the eosin-counterstained lung tissue. A mean neutrophil count was determined from a minimum of 20 randomly chosen fields and was expressed as the number of cells per cross-sectional area of alveolar air space.

Immunoelectron microscopy. Canine lung was inflated and fixed with phosphate-buffered saline containing 8% paraformaldehyde for 4 hours at 4° C. Lung tissue was cut into small cubes and cryoprotected overnight at 4° C in phosphate-buffered saline containing a mixture of polyvinylpyrrolidone (PVP, 20%) and sucrose (1.84 mol/L). Cryoprotected lung tissue was frozen in liquid nitrogen and then sectioned on a cryoultramicrotome at -85° C. Ultrathin (80 to 100 nm) cryosections of lung tissue were immunogold labeled with CL18/1 (anti-canine ICAM-1 Mab)¹⁵ followed by secondary antibody conjugated to colloidal gold (10 nm). Immunogold-labeled cryosections were viewed on a JEOL 200 CX transmission electron microscope (JEOL USA, Inc., Peabody, Mass.) and photographed at 10,000 $\times$ magnification. ICAM-1 expression

Fig. 2. Representative Northern blot analysis of ICAM-1 expression in a single cardiopulmonary bypass animal and in a single time-matched sham bypass control at 6 hours of reperfusion. *PRE* samples in both animals demonstrated little or no ICAM-1 mRNA expression. The *POST* sample in the sham animal demonstrated a minimal increase in mRNA expression, whereas the *POST* samples in the dog undergoing bypass demonstrated a much greater increase in ICAM-1 expression. Samples obtained from multiple regions of the lungs in the bypass dog demonstrated the increase in ICAM-1 mRNA expression to be a homogeneous response. *RU*, Right upper lobe, *RM*, right middle lobe, *RL*, right lower lobe, *LU*, left upper lobe.

was subsequently quantitated by counting the number of colloidal gold particles on the cell surface, which was then expressed as gold particles per μm of capillary wall.

Results

Northern blot analysis. Northern blot analysis for pulmonary ICAM-1 expression was performed on tissue samples obtained from all bypass and sham animals included in the study. To determine the time course of ICAM-1 expression, samples were obtained at 5 minutes after the complete cessation of CPB (or approximately 30 minutes after the release of the aortic crossclamp with subsequent myocardial and pulmonary reperfusion), and at 3, 6, and 9 hours of reperfusion in the bypass animals, and at matched time points in the sham animals.

Representative Northern blots demonstrating the time course of ICAM-1 mRNA expression in the lung are presented in Fig. 1. All animals in the study had results consistent with those presented in the figure. Pre-CPB samples had little or no ICAM-1 mRNA present. However, in dogs undergoing CPB (Fig. 1, *B*), a marked increase in ICAM-1 mRNA was seen at the earliest time point sampled after the bypass procedure. Although not as dramatic, a

Fig. 3. Western blots demonstrating the time course of pulmonary ICAM-1 protein expression in two representative animals. *SHAM*, In this time-matched sham bypass animal, there was constitutive expression of ICAM-1 protein in the lung before the procedure but no increase in ICAM-1 protein expression relative to the prebypass amount was detected at any of the later timepoints. *BYPASS*, In this animal undergoing cardiopulmonary bypass, at 3 and 6 hours after the bypass procedure there was no increase in ICAM-1 expression relative to the prebypass amount, but at 9 hours after cardiopulmonary bypass, ICAM-1 protein expression increased appreciably.

sustained increase in ICAM-1 expression at 3, 6, and 9 hours also occurred after bypass. In contrast, sham bypass animals (Fig. 1, *A*) had only a weak ICAM-1 mRNA signal present at matched time points "after" bypass. Furthermore, in the dogs undergoing CPB the observed increase in ICAM-1 message present after bypass appeared to be homogeneous throughout multiple segments of the lung, as demonstrated in Fig. 2.

Western blot analysis. Representative Western blot analyses from a single animal undergoing CPB

with 9 hours of reperfusion and a time-matched “sham bypass” control are presented in Fig. 3. All dogs demonstrated a variable but significant amount of ICAM-1 protein in the tissue samples obtained after thoracotomy and before CPB. Against this background, a detectable increase in ICAM-1 protein expression was apparent only in the CPB animals. The collective results of all the study animals are demonstrated by the plots in Fig. 4. All three sham animals had no increase in ICAM-1 expression throughout 9 hours of recovery (Fig. 4, *A*). In four of seven animals undergoing 9 hours of reperfusion after CPB, however, there was a threefold or greater increase in ICAM-1 protein expression at the 9-hour time point (Fig. 4, *B*). A comparable increase in ICAM-1 protein was not seen at 3 and 6 hours after CPB.

Neutrophil sequestration. On the basis of the observations made in Fig. 4, study animals were divided into three groups including “sham bypass” controls ($n = 3$), CPB animals with a threefold or greater increase in ICAM-1 protein expression at 9 hours of recovery ($n = 4$), and CPB animals with little or no increase in ICAM-1 protein expression at 9 hours ($n = 3$). Pulmonary neutrophil counts were determined by a blinded observer for each group before CPB and at 9 hours of recovery. As shown in Fig. 5, *A*, a similar number of neutrophils was present in the lung in each group before CPB. At 9 hours of reperfusion, however, the group with increased ICAM-1 protein also had a significantly increased number of neutrophils present. Furthermore, when all animals from each group were considered collectively, linear regression analysis demonstrated a positive correlation between the increase in ICAM-1 expression at 9 hours of recovery and the number of neutrophils present in the lung at the same time point with $r = 0.75$ and $p = 0.01$ (Fig. 5, *B*).

Immunoelectron microscopy. Immunoelectron microscopy was used to identify the specific tissue localization of the increased ICAM-1 protein detected by Western blot analysis. Standard immunohistologic studies and light microscopy did not allow for the differentiation of ICAM-1 expression on alveolar epithelial cells versus capillary endothelial cells because of their close approximation, necessitating the ultrastructural detail available by electron microscopy. Antibody recognizing ICAM-1 was recognized by a secondary antibody conjugated to colloidal gold that appears as a black bead on the electron micrograph. As depicted in Fig. 6, *A*, tissue sections taken at 9 hours after bypass from a dog

with an increased neutrophil count showed gold beads present on the luminal surfaces of both type I alveolar epithelial cells and capillary endothelial cells. Similarly treated sections of lung obtained from the same animal before CPB demonstrated ICAM-1 immunoreactivity on alveolar epithelial cells only. On the capillary endothelium, no significant amount of immunoreactivity above background (nonspecific IgG control) was detected. Similarly, one time-matched sham control and two CPB dogs with low pulmonary neutrophil counts failed to show ICAM-1 on the capillary endothelium at the 9-hour time point. Fig. 6, *B*, graphically represents the change in capillary endothelial ICAM-1 expression in the dog depicted in Fig. 6, *A*, and suggests approximately a fourfold increase in gold bead density over the prebypass value.

Discussion

The results of this study clearly demonstrate the presence of the adhesion molecule ICAM-1 in the pulmonary microcirculation after CPB and also suggest that ICAM-1 expression contributes to the capillary sequestration of neutrophils in the lungs postoperatively. Our data showed an induction of the ICAM-1 gene in the lung after CPB, an increased expression of ICAM-1 protein in the lung that correlated with an increase in pulmonary capillary neutrophil sequestration, and ultrastructural evidence that pulmonary capillary endothelial cells express ICAM-1.

To put these results into context, a brief discussion of neutrophil trafficking in the lung is necessary. Unlike the systemic vascular bed, where the site of neutrophil margination, sequestration, and emigration is in the postcapillary venules, neutrophil margination and sequestration in the lung primarily takes place in the alveolar capillaries.^{16, 17} Normally, the lung contains a large number of marginated neutrophils (i.e., unactivated cells that are transiently removed from the circulation but are readily mobilizable to replete the circulating pool) within its capillary bed. Activated neutrophils sequester within the same capillary bed, but sequestration implies an active process and neutrophil retention is likely to be sustained. Both the neutrophil and the capillary endothelium may be involved in the sequestration process. Many of the factors that regulate this process, however, remain unknown. One important mechanism for the initial retention of activated neutrophils in the pulmonary capillary bed is a stimulus-induced decrease in their deformabil-

Fig. 4. Plots representing the collective data for ICAM-1 protein expression in the lung as determined by Western blot analysis. Values for ICAM-1 are expressed as a change from the *PRE* bypass sample as determined by densitometry. **A**, Results from three sham bypass controls demonstrate no appreciable increase in ICAM-1 expression at matched timepoints 3, 6, and 9 hours of recovery. **B**, Results from animals undergoing cardiopulmonary bypass demonstrate a threefold or greater increase in ICAM-1 expression in four of seven animals at 9 hours of recovery but no appreciable increase in ICAM-1 expression at earlier timepoints. (NOTE: Samples were not available for testing in every animal at 3 hours and at 6 hours of recovery.)

ity. Intravascular inflammatory mediators, including complement fragments, can decrease neutrophil deformability within 1 minute of binding to the neutrophil surface and prevent neutrophils from passively conforming to the shape of the narrow capillary lumen.^{18,19} This stiffening of the neutrophil, however, is transient, even if the stimulus is sustained.²⁰ Thus additional mechanisms are necessary to sustain neutrophil sequestration.

Both leukocytes and endothelial cells are capable of expressing adhesion molecules that influence the sequestration process. Neutrophils constitutively express on their surface the adhesion molecule complex CD11/CD18. Three heterodimers, CD11a/CD18, CD11b/CD18, and CD11c/CD18 are present. On neutrophil activation by a chemotactic stimulus, the CD11/CD18 expressed on the neutrophil surface undergoes a conformational change to an activated state capable of enhanced recognition of an endothelial ligand, and an increase in surface expression of CD11b/CD18 from granular stores occurs.^{6,17}

One ligand for neutrophil CD11/CD18 present on endothelial cells is a member of the immunoglobulin

supergene family, ICAM-1. Burns and colleagues²¹ have demonstrated that, in contrast to venular endothelium in the systemic vascular bed that constitutively expresses ICAM-1, in the mouse, the pulmonary capillary endothelium has little or no constitutive ICAM-1 expression. Pulmonary capillary endothelium, however, can produce ICAM-1 in response to specific stimuli.

Previous studies in animals and in patients have documented neutrophil activation in response to CPB.²²⁻²⁷ This activation has included increased expression of surface CD11b/CD18.^{24,26,27} In our canine model of CPB we have documented neutrophil sequestration, associated with pulmonary injury and dependent on expression of neutrophil CD18, within the pulmonary capillary bed 3 hours after CPB.⁵ In light of these results and the preceding discussion, it was logical to examine the canine lung after CPB for ICAM-1 regulation. In vitro comparisons of cultured canine jugular vein endothelial cells and human umbilical vein endothelial cells in our laboratory have previously suggested that cytokine induction of and expression kinetics of ICAM-1

Fig. 5. A, Comparison of neutrophil (PMN) localization in the lung before and 9 hours after cardiopulmonary bypass as determined by immunohistology and computerized image analysis. Number of neutrophils is expressed as PMN per square millimeter alveolar surface area. On the basis of the 9 hour results in Fig. 4, animals were separated into three groups: *SHAM*, sham bypass controls, $n = 3$; *CPB HIGH ICAM-1*, $n = 4$; and *CPB LOW ICAM-1*, $n = 3$. Values are expressed as mean $\pm$ SEM. * $p = 0.03$ vs *SHAM*, determined by t test. **B,** Linear regression analysis demonstrating a significant correlation between the number of neutrophils sequestered in the lung at 9 hours of recovery and the change in ICAM-1 expression at 9 hours of recovery relative to ICAM-1 expression prebypass.

are quite similar in the two species. To our knowledge, however, before this study no attempt to measure pulmonary endothelial ICAM-1 expression has been made in the context of CPB in patients or in animal models.

Our Northern blot data clearly indicated an induction of the ICAM-1 gene in the lung as early after the cessation of CPB as it was feasible to measure. This elevation of ICAM-1 mRNA was sustained throughout the 9-hour course of recovery after bypass. Western blots of lung tissue homogenates demonstrated a significant amount of ICAM-1 protein expression in the lung before CPB. Ultrastructural immunohistologic examination suggested most of this ICAM-1 expression was limited to type I alveolar epithelium, an observation consistent with previous studies in the mouse.²¹ An increase in ICAM-1 protein expression was not detectable by Western blot at 3 and 6 hours of reperfusion, but at 9 hours of reperfusion four of seven animals demonstrated ICAM-1 protein expression to be measurably increased. Ultrastructural immunohistologic studies at this time point confirmed that the pulmonary capillary endothelium was capable of ICAM-1

protein expression after bypass. Furthermore, neutrophil localization at 9 hours of recovery was increased in those dogs with increased ICAM-1 protein expression, lending support to the concept that this increased ICAM-1 expression on capillary endothelium was functionally significant.

Although this study documents the induction of ICAM-1 in the lung after CPB, it also raises other questions. The stimulus causing induction of pulmonary ICAM-1 is unclear. Also, it is unclear why some animals responded to bypass with increased ICAM-1 protein expression and others did not. Horgan and colleagues⁷ have demonstrated that one stimulus that can increase pulmonary ICAM-1 is ischemia/reperfusion. During bypass, the lung is handled uniquely in that after crossclamping the aorta, along with the heart, it is the only organ excluded from flow through the extracorporeal circuit (with a minor contribution of blood flow through the bronchial circulation). Unlike the heart, however, it does not receive cold cardioplegic solution. Thus the lung may suffer an ischemic insult, with subsequent reperfusion at the end of bypass. Ischemia/reperfusion may have a direct effect on the

Fig. 6. **A**, Transmission electron micrograph of ICAM-1 protein tissue localization in the peripheral lung at 9 hours of reperfusion after cardiopulmonary bypass in a dog with increased neutrophil localization. ICAM-1-specific gold particles were detected on the luminal surfaces of both the alveolar epithelium (*open arrows*) and the capillary endothelium (*solid arrows*). Similarly treated sections of lung obtained before CPB in the same animal demonstrated ICAM-1 immunoreactivity on the alveolar epithelium, but on the capillary endothelium only a trace of immunoreactivity was detected. **B**, Plot demonstrating the change in pulmonary capillary endothelial ICAM-1 protein expression in the dog depicted in **A**. ICAM-1 protein density is expressed as gold beads/ μm of capillary wall. Electron micrographs were arranged in montages for the purpose of counting. *PRE* values were based on an examination of 330 μm of capillary wall from 18 separate vessels. *POST* values were based on examination of 127 μm of capillary wall from five separate vessels. Values are expressed as the mean $\pm$ SD based on the number of vessels counted. *N*, Neutrophil; bar = 1 μm .

pulmonary capillary endothelium, or a second messenger, such as tumor necrosis factor- α , produced locally in response to ischemia/reperfusion may act as a stimulus. Alternatively, substances capable of endothelial activation, released into the blood in response to blood exposure to the extracorporeal circuit, may affect the capillary endothelium during reperfusion. The extent to which any of these regulating mechanisms may be active, however, is presently unknown.

Our observations also raise other interesting possibilities. As mentioned above, in our previous studies in this canine model, pulmonary capillary neu-

trophil sequestration was increased three- to fourfold 3 hours after bypass. This sequestration was clearly CD18-dependent, as documented by systemic administration of a blocking anti-CD18 antibody.⁵ Whether ICAM-1 participates in this early process, however, remains unclear. Western blot data from the this study failed to show an increase in ICAM-1 protein at the 3-hour time point, raising the possibility that neutrophil sequestration at 3 hours after bypass, although CD18-dependent, could be independent of ICAM-1 protein expression on the capillary endothelium. Another CD18-dependent mechanism could be active at this early time point

(e.g., homotypic aggregation or CD18 recognition of another endothelial ligand). Alternatively, enough ICAM-1 was expressed on the endothelium at this time point to support adhesion but was below our ability to detect its presence against the background ICAM-1 protein present because of expression on the alveolar epithelium.

What is clear from this study, however, is that at 9 hours of reperfusion, those dogs with increased ICAM-1 protein expression had an increased number of sequestered neutrophils. Animals with lower ICAM-1 expression had much lower numbers of neutrophils present. These data suggest, although do not prove, that the increased expression of pulmonary ICAM-1 played a role in the sustained or continued sequestration of neutrophils at later time points of reperfusion. Alternatively, other adhesion ligands, co-expressed with ICAM-1 but not measured in this study could contribute to the sequestration process. Nevertheless, the variable response in ICAM-1 protein expression observed between individual animals in this study suggests that some, yet not others, may be predisposed to inflammatory cell-mediated pulmonary injury later in recovery. These interesting observations warrant the performance of additional studies, not only to more fully understand the regulation of pulmonary ICAM-1 after CPB but also to determine whether pulmonary ICAM-1 expression is of pathophysiologic significance to the patient undergoing cardiac surgery.

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Commentary

If sufficiently sensitive techniques are used to assess lung function, virtually all patients undergoing cardiopulmonary bypass (CPB) have some post-CPB evidence of pulmonary malfunction.¹ Nevertheless, the number of patients in whom life-threatening lung failure develops is relatively few; full-blown adult respiratory distress syndrome occurs in only 1% to 2% of patients. A clinically relevant pathophysiologic model of pulmonary dysfunction after CPB should therefore optimally help to explain both the cellular and molecular basis responsible for this phenomenon in the majority of patients and also provide leads to the understanding of the marked inter-patient variability observed.

Pulmonary injury in the CPB setting appears largely related to complement activation with resultant formation of terminal complement component soluble mediators, to cytokine generation, to modulation of small molecule mediators such as nitric oxide and prostaglandin/leukotriene intermediates, and to neutrophil sequestration; indeed, all these events may be mechanistically linked, at least in part, both here and in other circumstances in which ischemia-reperfusion physiology predominates.² Although there has been some debate in the literature regarding the importance of neutrophils in lung injury, most of the apparent discrepancies in results can be explained by considering the pulmonary reaction to CPB as a biphasic insult—an initial process occurring rapidly within the first hour that is most dependent on soluble mediators and relatively independent of cellular activation, and a second process (1 to 4 hours later) that critically involves circulating phagocytes.³ This latter phase prominently includes activated neutrophil sequestration in the capillaries and generation of cytotoxic activity by those granulocytes. It is reasonable to postulate that the adhesive moieties responsible for neutrophil-endothelial cell adhesion in this pathologic setting would be the same molecules used by neutrophils during normal physiologic processes and in other pathologic settings.⁴ Normal neutrophil-endothelium interaction includes selectin-mediated rolling of granulocytes along the blood vessel wall, followed by integrin-mediated extravasation processes that are called into play during an inflammatory response. On the neutrophil side, quantitative and, equally important, qualitative modulation of both L-selectin and CD11/CD18 β_2 integrin moieties in response to reperfusion, foreign biomaterial contact, and to the soluble mediator changes induced by CPB itself have been postulated to be the critical adhesive alterations responsible for granulocyte activation/extravasation and con-

sequent lung damage; experimental data lend support to this concept. The current article by Dreyer and associates addresses the equally relevant endothelial side of this adhesive interaction and, in particular, modulation of one of the two major cellular ligands for myeloid CD11a/CD18 and CD11b/CD18, that is, intercellular adhesion molecule-1 (ICAM-1).

In a canine model of CPB, the authors find that ICAM-1 messenger ribonucleic acid (mRNA) in the pulmonary capillary vasculature increases immediately after CPB, although curiously surface expression of protein is not detected in their assay system until approximately 8 hours after the detection of increased message. Although this represents a seemingly long delay for the translation process in the setting of rapid transcriptional up-regulation, the time course for ICAM-1 protein appearance is consistent with some other studies of cytokine-induced alterations in expression. It is indeed possible, as the authors suggest, that both hypothermia (known to delay expression of β_2 integrins on neutrophils) and local tissue hypoxia are responsible for this long delay from RNA to protein. The results are also consistent with prior cardiac studies in man carried out by Kilbridge and colleagues,⁵ which examined the heart and skeletal muscle of pediatric patients undergoing CPB for new expression of mRNA both for ICAM-1 and also for one of the major endothelial cell selectins (specifically, E-selectin) before and after CPB. Those investigators also documented a rapid up-regulation of message for both molecules during CPB using a ribonuclease protection assay. Hence it appears that CPB results in generation of "sticky" blood vessel walls, as well as hyperactive neutrophils, although the significant differences in time course for the two phenomena leave open the question of how these two processes interact, if at all, and whether the endothelial alterations are a primary event in CPB or a secondary one.

Two additional questions are raised by these studies. First, is this vessel wall alteration a uniform finding, or is there significant subject variability? The current article found up-regulation of pulmonary endothelial ICAM-1 to occur in only a subset of canine subjects; Kilbridge's group⁵ described a similar phenomenon in man. Such results could reflect a host of acquired differences in underlying physiology. One interesting alternative is that there could be underlying genetic differences accounting for the variability. It is known that there are a number of different polymorphic alleles for ICAM-1, as well as other important adhesion molecules in the human population. Moreover, some of these alleles are reported to be associated with an increased risk for atherogenesis.⁶ Of course, although such observations are intriguing, any idea that genetic differences in either regulatory or structural sequences for these genes will ultimately explain and/or predict patient response to CPB is purely speculative.

Finally, one must raise the question of whether these observations on the response of the pulmonary vasculature to CPB will have direct clinical therapeutic relevance some day. Support for this notion comes from experiments in radiation-induced lung injury. It has recently been shown that mice that have been engineered to lack the functional gene for ICAM-1 and mice that receive anti-ICAM-1 blocking antibody fail to develop radiation-induced pulmonary inflammation.⁷ Thus it is at least

reasonable to postulate that ICAM-1 could be a future target for ameliorating post-CPB lung injury. Only more basic and clinical research will determine whether an approach to abrogate damage at the end-organ, as opposed to abrogating the effector mediator side of the equation, will ultimately prove clinically useful in CPB.

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