CARDIOPULMONARY SUPPORT AND PHYSIOLOGY

INHIBITION OF INTERLEUKIN-8 BLOCKS MYOCARDIAL ISCHEMIA-REPERFUSION INJURY

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Introduction: **Interleukin-8 is thought to play a role in neutrophil activation and transcapillary migration into the interstitium. Because neutrophils are principal effector cells in acute myocardial ischemia-reperfusion injury, we postulated that the inhibition of interleukin-8 activity with a neutralizing monoclonal antibody directed against rabbit interleukin-8 (ARIL8.2) would attenuate the degree of myocardial injury encountered during reperfusion.** ***Methods:*** **In New Zealand White rabbits, the large branch of the marginal coronary artery supplying most of the left ventricle was occluded for 45 minutes, followed by 2 hours of reperfusion. Fifteen minutes before reperfusion, animals were given an intravenous bolus of either 2 mg/kg of ARIL8.2 or 2 mg/kg anti–glycoprotein-120, an isotype control antibody that does not recognize interleukin-8. At the completion of the 120-minute reperfusion period, infarct size was determined.** ***Results:*** **In the area at risk for infarction, 44.3% ± 4% of the myocardium was infarcted in the anti–glycoprotein-120 group compared with 24.8% ± 9% in the ARIL8.2 group ($p < 0.005$). In control animals, edema and diffuse infiltration of neutrophils were observed predominantly in the infarct zone and the surrounding area at risk. Tissue myeloperoxidase determinations did not differ significantly between groups, indicating that the cardioprotective effect of ARIL8.2 was independent of an effect on neutrophil infiltration.** ***Conclusions:*** **A specific monoclonal antibody that neutralizes interleukin-8 significantly reduces the degree of necrosis in a rabbit model of myocardial ischemia-reperfusion injury. (J Thorac Cardiovasc Surg 1998;116:114-21)**

Myocardial ischemia-reperfusion injury is a major determinant of morbidity and mortality in patients undergoing cardiothoracic operations. Despite the use of such cardioprotective techniques as cardioplegia and hypothermia, a significant degree of cardiac dysfunction is encountered during and after the operation, particularly in patients who sustained significant physiologic deterioration before the operation.[1, 2] Several pathogenic mechanisms involving the activated endothelium mediate this distinct form of tissue injury. Numerous studies show that neutrophils accumulate rapidly in and around the infarcted tissue after reperfusion.[3, 4] In the process of adhering to the endothelium, neutrophils become activated. Activated leukocytes mediate tissue injury through the release of a variety of substances, such as oxygen-derived free radicals and proteases, that are involved in necrotic tissue remodeling.[5] Adjacent tissue that is still viable may also be damaged irreversibly by these neutrophil products, resulting in extension of the infarct size.

Evidence supporting the importance of neutrophils in ischemia-reperfusion injury is provided by studies with monoclonal antibodies against adhesion molecules, which block many of the deleterious effects of reperfusion of ischemic tissue (see refer-

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ence 1 for review). Neutrophil-mediated injury during reperfusion results when flowing neutrophils encounter activated endothelial cells expressing adhesion molecules that mediate an initial rolling and then firm adherence. During this process the neutrophils become activated, promoting chemotaxis into the ischemic tissue.[6-8] A variety of chemotactic factors contribute to neutrophil activation and chemotactic function. It is conceivable that by inhibiting one of the factors that promote activation or chemotaxis it may be possible to attenuate myocardial ischemia-reperfusion injury without globally affecting neutrophil-endothelial cell adhesion.

The chemokine interleukin 8 (IL-8) was recently shown in vitro to be an important mediator of neutrophil activation and transendothelial migration.[9] Reoxygenation of cultured hypoxic endothelial cells and monocytes induces the release of significantly elevated quantities of IL-8.[10, 11] Recent evidence suggests a role for IL-8 in the injury pattern seen after myocardial infarction and cardiopulmonary bypass. Elevated IL-8 levels are detected in the serum of patients after myocardial infarction, and IL-8 is measured in coronary sinus blood after ischemic cardiac arrest during heart operations requiring cardiopulmonary bypass.[12, 13] Because the activation of neutrophils is a known prominent feature of myocardial ischemia-reperfusion injury, we postulated that the inhibition of IL-8 activity with a monoclonal antibody capable of neutralizing IL-8 would attenuate the degree of myocardial injury encountered during reperfusion.

Methods

Monoclonal antibody. A neutralizing antirabbit IL-8 antibody (ARIL8.2) was generated and screened for specificity as described elsewhere in detail.[14, 15] This antibody was selected from a series of antirabbit IL-8 monoclonal antibodies because of its demonstrated ability to bind recombinant rabbit IL-8, inhibit rabbit IL-8 binding to rabbit neutrophils, and finally, to prevent IL-8 mediated neutrophilic chemotaxis and degranulation.[14, 15] A matching idiotypic antibody (anti–gp-120), one that binds the major envelope glycoprotein of human immunodeficiency virus type 1, glycoprotein-120, but does not bind rabbit IL-8, was used as a control.

In situ coronary ligation model. To evaluate the effect of inhibiting IL-8 on injury sustained during ischemia-reperfusion, we used a well-characterized regional ischemia-reperfusion injury model. In this model adult New Zealand White rabbits weighing 3 to 4 kg were used in research protocols approved by the Animal Care Committee of the University of Washington, Seattle. All animals received humane care according to 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). Rabbits were anesthetized with an initial intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg). The rabbits were endotracheally intubated and maintained on inhaled halothane (1% to 2%) anesthesia in 100% oxygen administered with a small-animal respirator. A 20-gauge flexible catheter was placed in the left carotid artery to measure heart rate (HR), measure mean arterial pressure (MAP), and collect blood samples. A double-lumen thermodilution probe and injectate catheter (model 94-011-3F; Baxter Healthcare Corporation, Irvine, Calif.) were placed retrograde through the left femoral artery and vein to the aorta and inferior vena cava to measure core temperature and cardiac output (CO) by thermodilution technique. The heart was exposed through a median sternotomy. A 5F Millar catheter was placed through a small purse-string sutured incision in the left ventricle to allow estimation of the left ventricular peak systolic pressure (LVESP) and left ventricular end-diastolic pressure (LVEDP). A 4.0 Vicryl suture (Ethicon, Inc., Somerville, N.J.) was passed twice around a large arteriolateral branch of the left main coronary artery supplying most of the left ventricle, and the ends of the suture were passed through a small length of polyethylene tubing to form a snare. After a 20- to 30-minute stabilization period, baseline values were recorded. Regional myocardial ischemia was produced by reversibly occluding the artery for 45 minutes, followed by 120 minutes of reperfusion. Fifteen minutes before reperfusion, animals were given intravenously 2 mg/kg anti–gp-120 (the nonbinding control antibody) or 2 mg/kg ARIL8.2. Thermodilution CO, central venous pressure (CVP), HR, MAP, LVESP, and LVEDP were recorded at baseline, 45 minutes of ischemia, and 60 and 120 minutes of reperfusion. The CO determinations were performed in triplicate and the results were averaged. Systemic vascular resistance was calculated as $(MAP - CVP) \times 80/CO \times (dynes \cdot sec \cdot cm^{-5})$. Additional mechanical hemodynamic data calculated during this preparation included maximum positive pressure change, pressure rate index ($LVESP \times HR/1000$), and developed pressure ($LVESP - LVEDP$). After 120 minutes of reperfusion, all animals were killed with an intravenous bolus of concentrated pentobarbital and the myocardial tissue was taken and processed for calculation of infarct size or histologic analysis.

Determination of infarct size. At completion of the 120-minute reperfusion period, the coronary artery was reoccluded and 6 ml 20% Evans blue dye (Sigma Chemical Co., St. Louis, Mo.) was injected into the left atrium and allowed to circulate to stain all perfused tissue. The area supplied by the ligated vessel remained unstained, demarcating the myocardium at risk for infarction. After the heart was arrested with pentobarbital, it was rapidly excised. The left ventricle was isolated from the rest of the heart, weighed, and then cut into 2 mm thick transverse slices. The normal myocardium (stained blue) was separated from the area at risk (unstained). The area at risk was placed in a 37° C solution of 1% triphenyltetrazolium chloride (TTC) for 30 minutes. TTC stains the viable tissue brick red, leaving the necrotic zone pale. The

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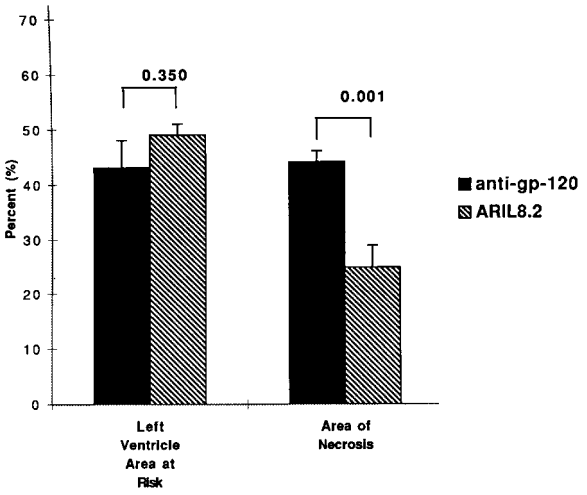

Fig. 1. LVAAR and infarct size, expressed as a percentage of LVAAR divided by LVAN. Values are expressed as mean ± standard error of the mean.

TTC-stained (noninfarcted) tissue was separated from the TTC-unstained (necrotic) tissue and each area was weighed. The left ventricular area at risk (LVAAR) was calculated as the sum of the noninfarcted and necrotic weights of the tissue perfused by the occluded vessel divided by the weight of the left ventricle, expressed as a percentage. The left ventricular area of necrosis (LVAN) was calculated as the weight of necrotic tissue divided by the weight of the left ventricle, expressed as a percentage. The infarct size was calculated by dividing the weight of the TTC-unstained tissue by the weight of the total area at risk (LVAN/LVAAR).

Myeloperoxidase (MPO) assay. Tissue neutrophil accumulation was quantified by MPO assay. Left ventricular area not at risk and LVAAR were isolated as described previously; however, the LVAN was not separated out from the LVAAR. These tissues were homogenized in a solution containing 20 mmol/L potassium phosphate and centrifuged for 30 minutes at 20,000*g* at 4° C. The pellets were frozen on dry ice overnight. After thawing, the pellets were sonicated for 1 minute at 4° C in a buffer solution consisting of 0.5% hexylcyclotrimethylammonium bromide dissolved in 50 mmol/L potassium phosphate buffer (pH 6) containing 30 U/ml aprotinin. The samples were centrifuged for 30 minutes at 40,000*g* at 4° C. An aliquot of the supernatant was allowed to react with a solution of *o*-inosidine dihydrochloride. Activity was defined as the quantity of enzyme degrading 1 μmol peroxide/min at 37° C and expressed in units per gram weight of tissue.

Microvascular blood flow. Regional myocardial blood flow was measured in 11 additional rabbits. Colored (high red, medium red, blue, and violet) polystyrene microspheres (15 ± 0.1 mm in diameter; Triton Technology, San Diego, Calif.) were used to measure flow at baseline, end of ischemia, and 1 and 2 hours after reperfusion, according to previously described methods.[16] Approximately 500,000 microspheres were suspended in 1 ml normal saline solution and then injected through a catheter positioned in the left ventricle. Withdrawal of reference arterial blood flow was begun through a catheter placed in the carotid artery 30 seconds before microsphere injection and continued for 30 seconds after the injection. To distinguish the normally perfused myocardium from the myocardium that was made ischemic by the coronary occlusion, the coronary artery was again ligated immediately after the last microsphere injection, and the animals received 5 ml 10% Evans blue dye, as described previously. Nonischemic tissue from the right ventricle and ischemic tissue from the left ventricle were easily identified and separated by this technique. The microspheres were then recovered from the tissue and blood samples by digestion in a 4 mol/L potassium hydroxide solution at 72° C for 3 hours and subsequent microfiltration. The dyes were recovered from the spheres within a known volume of a solvent (dimethylformamide) and their concentrations were determined by spectrophotometry at the optimal wavelengths for each dye. The composite spectrum of each dye solution was resolved into the spectra of the single constituents by a matrix inversion technique. Blood flow to each myocardial sample was calculated by determining total tissue spheres divided by the tissue weight in grams. This value was then multiplied by the inverse of the spheres per milliliter per minute (total references spheres divided by withdrawal rate).

Statistics. Student's *t* tests were used to compare the mean percentage changes in infarct size and MPO levels between the ARIL8.2 and anti–gp-120 groups. Hemodynamic and microvascular blood flow parameters were compared with baseline values by means of paired *t* tests and between treatment groups by means of independent sample *t* tests at each time point.

Results

Forty-six animals treated with either the ARIL8.2 or anti–gp-120 antibody were included in this analysis. Eleven animals were used to determine infarct size, 20 were examined to assess MPO activity, 10 were used to assess myocardial blood flow at the microvascular level, and five were examined by fixation of tissue and hematoxylin-eosin staining. Three were excluded because of ventricular fibrillation during early ischemia, before antibody infusion. One ARIL8.2-treated animal had intermittent fibrillation during the 45-minute ischemic period and was excluded when it died of refractory fibrillation immediately on reperfusion. One anti–gp-120–treated animal was excluded for refractory ventricular fibrillation at 1 hour of reperfusion.

Effect of ARIL8.2 on myocardial infarct size. Ligation of the proximal marginal artery in the rabbit heart consistently created equivalent LVAARs in the two groups (Fig. 1). In the anti–gp-120–treated animals, LVAAR was 43% ± 5% (n = 5), compared with 49% ± 2% in the ARIL8.2-

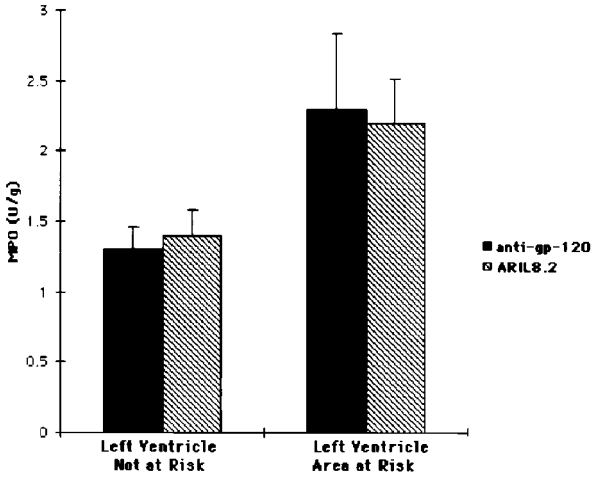

Fig. 2. MPO activity in the left ventricle area not at risk for infarction versus LVAAR in anti–gp-120–treated versus ARIL8.2-treated animals. Data are expressed as mean ± standard error of the mean of units of MPO activity per gram of tissue. There was no statistical difference between the anti–gp-120–treated and ARIL8.2-treated animals in left ventricle area not at risk or LVAAR, suggesting that the reduction in infarct size was not caused by attenuation of the degree of neutrophil infiltration into the LVAAR.

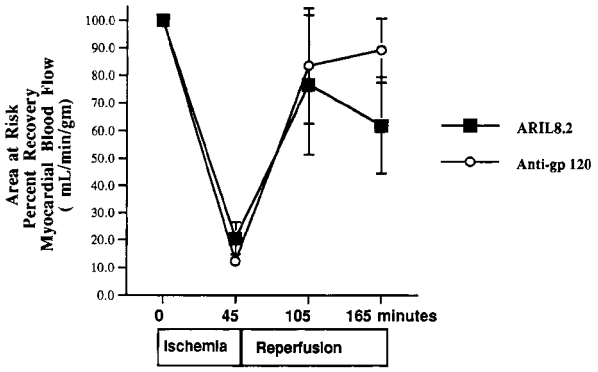

Fig. 3. Microvascular blood flow in the LVAAR in ARIL8.2-treated animals versus anti–gp-120–treated animals. Blood flow at baseline, the end of ischemia, and after 1 and 2 hours of reperfusion is expressed in milliliters per minute per gram, as determined by colored microsphere technique. Mean ± standard error of the mean is shown at each time point.

treated animals ($n = 6$). This difference was not statistically significant. Of the LVAAR, 44.3% ± 2% was infarcted in the anti–gp-120–treated group, compared with 24.8% ± 4% in the ARIL8.2 group (Fig. 1). This represents a 56% reduction in infarct size in the ARIL8.2-treated animals compared with the control group ($p \leq 0.001$).

MPO assay. MPO contents of the nonischemic left ventricle and LVAAR were compared between animals treated with ARIL8.2 ($n = 10$) and anti–gp-120 ($n = 10$) (Fig. 2). There was no statistically significant difference in MPO activity between the areas not at risk for infarction in the ARIL8.2-treated and anti–gp-120–treated animals (1.4 ± 0.18 U/gm vs 1.3 ± 0.16 U/gm; $p = 0.84$). Furthermore, the difference in MPO levels between the ARIL8.2-treated and anti–gp-120–treated animals was not statistically significant (2.2 ± 0.32 vs 2.3 ± 0.54; $p = 0.104$).

Regional microvascular blood flow. The time course of regional myocardial blood flow, expressed as a percentage reduction from baseline, is shown in Fig. 3. In both anti–gp-120–treated ($n = 4$) and ARIL8.2-treated animals ($n = 6$), blood flow was significantly reduced from baseline levels in the LVAAR during ischemia (12% ± 2% vs 20% ± 6% of baseline). Blood flow recovered to 76% ± 25% of baseline in the ARIL8.2-treated animals, compared with 83% ± 21% in anti–gp-120–treated animals at 60 minutes of reperfusion. By 120 minutes of reperfusion, there was still no significant difference between the recovery to baseline in the ARIL8.2-treated animals (62% ± 18%) and the anti–gp-120–treated animals (89% ± 11%). In both groups, myocardial blood flow was reduced with respect to baseline at 60 and 120 minutes of reperfusion; there was no significant difference between treatment groups.

Effect of ARIL8.2 on hemodynamics. MAP, CO, LVESP, and developed pressure were all significantly reduced at the end of ischemia in both groups. Although there were some trends toward improved recovery in the ARIL8.2-treated animals, there were no significant changes in measured global myocardial function in any of these parameters between treatment groups at any time point (Table I).

Discussion

Recent experimental and clinical observations have implicated IL-8 in myocardial ischemia-reperfusion injury. In a canine model of myocardial ischemia-reperfusion injury, IL-8–specific messenger ribonucleic acid is markedly elevated in reperfused segments of myocardium and is limited to both viable and necrotic tissue around the infarct

Table I. *Hemodynamic values in ARIL8.2-treated animals and anti-GP120-treated animals*

	Time 0		*Time 45 min*		*Time 120 min*	
	Anti–GP-20	*ARIL8.2*	*Anti–GP-120*	*ARIL8.2*	*Anti–GP-120*	*ARIL8.2*
HR	100 ± 0	100 ± 0	107 ± 8	96 ± 4.9	97 ± 6.2	101 ± 8
MAP	100 ± 0	100 ± 0	73 ± 4.4	79 ± 6.1	74 ± 6.7	77 ± 8.6
CO	100 ± 0	100 ± 0	69 ± 8.8	72 ± 4.7	75 ± 9.3	75 ± 5.5
CVP	100 ± 0	100 ± 0	97 ± 3.2	103 ± 6.6	94 ± 3.4	108 ± 4.9
SVR	100 ± 0	100 ± 0	114 ± 19	108 ± 5.8	98 ± 3.2	99 ± 7.4
dP/dt	100 ± 0	100 ± 0	76 ± 4.2	79 ± 1.9	68 ± 9.1	81 ± 5.1
LVESP	100 ± 0	100 ± 0	90 ± 12	83 ± 5.5	80 ± 4.4	85 ± 5.5
LVEDP	100 ± 0	100 ± 0	510 ± 187	191 ± 31	375 ± 114	159 ± 29
DP	100 ± 0	100 ± 0	73 ± 2.9	77 ± 5.7	75 ± 5.2	80 ± 5
PRI	100 ± 0	100 ± 0	86 ± 9.7	81 ± 9.5	78 ± 9.3	88 ± 12

All values are expressed as a percentage of baseline value, plus or minus the standard error of the mean. *SVR,* systemic vascular resistance; *+dP/dt,* maximum positive pressure charge; *DP,* developed pressure; *PRI,* pressure rate index.

zone.[17] Clinically, a correlation between serum IL-8 levels and transmural acute myocardial infarction is observed,[12] and atrial IL-8 messenger ribonucleic acid levels increase by several times during cardiopulmonary bypass after ischemic arrest of the heart.[18] In addition, elevated IL-8 levels correlate with the length of cardiopulmonary bypass and coronary sinus myoglobin level, a biochemical marker of myocyte injury.[13, 19] These studies suggest a correlation between IL-8 expression and myocardial injury from oxidative stress. The role of IL-8 as a contributing factor in myocardial ischemia-reperfusion injury, however, was not previously known.

In this study we found that intravenous administration of a monoclonal antibody to IL-8 reduces regional myocardial ischemia-reperfusion injury. This study provides the first evidence that inhibition of IL-8 prevents the extension of myocardial infarction on reperfusion. The protective mechanism of neutralizing IL-8 appears to involve inhibition of neutrophil activation in the secondary zone of at-risk ischemic myocardium, the area most vulnerable to oxidative injury and further tissue necrosis during reperfusion. Although inhibition of IL-8 during regional myocardial ischemia-reperfusion injury reduced the extent of infarction, a trend toward improved ventricular performance in ARIL8.2-treated animals compared with anti–gp-120–treated animals, as assessed by measurements of ventricular contractility, did not reach statistical significance (Table I). Conclusions about ventricular function are limited in this initial study, however, because with this model only a small fraction of the ventricle is infarcted and otherwise healthy rabbits are able to compensate hemodynamically for this degree of injury. Further studies in which regional myocardial function is more specifically studied or in which the heart is subjected to global ischemic injury will more specifically assess the effect of the ARIL8.2 antibody on recovery of ventricular function.

IL-8 plays an important role in both neutrophil activation and chemotaxis. In studies in which IL-8 alone was injected into normal human skin, neutrophils were recruited without the appearance of wheal or flare, suggesting that IL-8 works in cooperation with other inflammatory mediators.[20] Van Zee and colleagues[21] demonstrated that a single-dose infusion of plasma concentrations seen in sepsis of recombinant IL-8 into nonhuman primates resulted in a rapid margination of neutrophils in the lungs and other systemic tissue beds; however, severe neutrophil-mediated injury did not result. In contrast, when IL-8 is neutralized in the presence of severe tissue injury, where other inflammatory mediators are present, a significant degree of injury is averted.[14, 15] These observations suggest that IL-8 works with other inflammatory mediators and that its primary role is in neutrophil activation and chemotaxis.

Our results corroborate previous studies demonstrating that inhibition of neutrophil function is cardioprotective in myocardial ischemia-reperfusion injury. Monoclonal antibodies, peptides, or small molecules such as oligosaccharides that either bind the adhesion molecule directly or interfere with the molecule binding to its ligand have been effective in limiting myocardial injury on reperfusion. For example monoclonal antibodies to P-selectins and L-selectins, CD11a, CD11b, CD18, and intercellular adhesion molecule–1 have all been shown to attenuate myocardial ischemia-reperfusion injury (for a review, see reference 1). Therapies that inhibit

neutrophil adherence to endothelium, however, may carry the potential risk of infectious complications.[22] This may be especially true in clinical situations in which the risk of infection is great. In contrast, neutralizing IL-8 does not directly block neutrophil-endothelial adhesive properties or responsiveness to other neutrophil chemotactic factors, such as platelet-activating factor, complement 5a, or leukotriene B_4. The premise that IL-8 inhibition does not severely suppress nonspecific immunity is supported by studies with IL-8 receptor homolog knockout mice. These animals do not appear to have severe bacterial infections, despite an impairment in extravascular neutrophil recruitment to such stimuli as endotoxin.[23] Approaches that are based on an antibody to IL-8 may be a useful adjunct when infectious risks are high or there are other contraindications to anti–neutrophil-adhesion therapy.

Although ARIL8.2 significantly reduced infarct size in our model, neutrophil accumulation in reperfused tissue, as measured by MPO, was not appreciably reduced by the antibody. IL-8 is both a leukocyte chemoattractant and a potent agent of leukocyte activation, and it is one of a number of chemoattractants that may be activated in response to reperfusion injury. It is possible that other chemoattractants (for example, complement fragment 5a), which would not be affected by ARIL8.2, were generated in our model of myocardial ischemia-reperfusion injury, causing neutrophils to migrate to sites of reperfused tissue. We conjecture that in the presence of ARIL8.2 these leukocytes were not activated and did not release granular contents that damage tissue.[24] Our results thus suggest that IL-8 plays a more important role in ischemia-reperfusion injury as a neutrophil-activating agent than as a chemoattractant.

This study suggests potentially clinical beneficial effects of inhibiting IL-8 in the setting of myocardial ischemia-reperfusion injury. This conclusion has ramifications for the treatment of the reperfused myocardium in a variety of clinical settings, including the treatment of areas of potentially salvageable myocardium in patients after acute myocardial infarction or as an adjunct to current myocardial protection strategies in the setting of cardiopulmonary bypass and heart transplantation. In addition, there is evidence that IL-8 may contribute to inflammatory-mediated pulmonary injury as well. In several models of severe lung injury, the neutralization of IL-8 (with ARIL8.2) has been shown to profoundly inhibit neutrophil recruitment and subsequent neutrophil-mediated lung injury.[14, 15] Treating patients undergoing cardiopulmonary bypass with an agent to neutralize IL-8 therefore not only could have an impact on the degree of myocardial injury but could also reduce some of the whole-body inflammatory response that contributes significantly to morbidity and mortality among patients undergoing heart operations.

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Discussion

Dr. David A. Fullerton *(Winnetka, Ill.).* Dr. Boyle, you and your colleagues are to be sincerely complimented on a rigorous progression of studies trying to iron out some of the mechanisms of ischemia and reperfusion injuries. I know the focus of your laboratory has been on endothelial cell function and interaction with leukocytes, and this is one more step along that path. Most of us do not think much about IL-8—or any other cytokines—on a daily basis, and your presentation was clear.

Having seen some of the IL-8 work that you presented in your background in the literature recently, I viewed it as interesting, but most other cytokines are also elevated when a person is undergoing cardiopulmonary bypass. What prompted you to focus on the role of IL-8 in neutrophil–endothelial cell interaction? Do you believe that this is early enough in the cascade of events to effectively inhibit this interaction?

Dr. Boyle. This is an important distinction. As you point out, a variety of different cytokines and other inflammatory mediators are elevated in patients undergoing cardiopulmonary bypass, but in studying these different cytokines in detail and trying to understand what they do, we were drawn toward IL-8 because of its role in neutrophil activation. Unlike inhibition of interleukin-1 or tumor necrosis factor, which activate endothelial cells in a redundant fashion, it seemed to us that inhibition of IL-8 might be beneficial.

Your second question, whether it is early or late enough in the cascade, reflects an extremely delicate balance. As I pointed out, some recent clinical and experimental evidence suggests that if you wipe out the ability of the neutrophil to attach to the endothelium, laboratory animals and patients in clinical studies may have an increased incidence of infection. What we think is interesting about this particular therapy is that the neutrophil should still bind to the endothelium and should still respond to other chemotactic factors. By targeting IL-8, we may be able to reduce myocardial injury and apparently still allow some of the other inflammatory functions to remain intact.

Dr. Fullerton. Do you think that you can dissociate the injurious effect of the neutrophils from the immunologic, protective effects of the neutrophils by using this [antibody to IL-8] on a therapeutic basis?

Dr. Boyle. I think that the evidence we have provided shows that by targeting IL-8 we can reduce the injurious effects of myocardial reperfusion injury. Whether the neutrophil will still function properly is unknown. There is some evidence, for example, that knockout mice missing the homolog of the IL-8 receptor do not have an increased incidence of infection, unlike people with leukocyte adhesion deficiency who are missing the neutrophil receptors for CD11 and CD18. Perhaps inhibiting IL-8 may be a way to reduce injury without significantly reducing the ability to fight infection.

Dr. Fullerton. It is intuitively attractive, because obviously the neutrophil has been implicated in myocardial infarction studies for some time but it has been a therapeutic obstacle to neutralize their effects. It is cumbersome to perform neutrophil depletion in people or animals and it is potentially risky to completely block neutrophil adhesive functions, so I think one of the things that is particularly attractive about your line of investigation is that it might be able to dissociate the good from the bad. One would like to preserve favorable neutrophil function but prevent their bad effects. Could you speculate on how you might use this strategy therapeutically? When would you have to give this agent? You were able to give it shortly before you reperfused your subjects, but if you could speculate on a clinical basis, would you have to give it in the preoperative medications?

Dr. Boyle. In our model we showed that we could administer the antibody during ischemia, just before reperfusion. This might be important, for example, as an adjunct to thrombolysis in patients with evolving myocardial infarctions. It would be nice to give this agent, as you said, even before the patient is put to sleep. As you know, one of the advantages of taking care of patients undergoing cardiopulmonary bypass is that in many cases we can give a drug like this before the operation, unlike in cardiology or trauma, where one encounters a patient with an ongoing event. The patient population we most commonly see in cardiothoracic surgery is thus ideally suited

for therapies aimed at preventing the inflammatory aspect of ischemia-reperfusion injury.

Dr. Vaughn A. Starnes *(Los Angeles, Calif.).* One of the aspects that I was struck by was the amount of reperfusion that you demonstrated in the two groups, the lack of return to baseline of blood flow into the infarcted area. We know that the size of an infarct is determined by reperfusion injury, so one might suggest that you did not get an increased infarct size because you did not reperfuse it. Could you respond to this hypothesis?

Dr. Boyle. Yes, we calculated infarct size by counterstaining with, in our case, Evans blue dye, with which one can demarcate the area at risk, cut it away, and treat it with 1% TTC. This stains the viable tissue red and the nonviable tissue white, and these tissue amounts are expressed as percentage weights to yield an estimate of infarct size. In terms of your question about myocardial blood flow, I think that this is one of the most interesting aspects of this study. I was surprised to see that the reduction in infarct size was independent of increased recovery of blood flow. That raised a number of fascinating questions that we will be working out in a variety of different ways in the laboratory. One mechanism is that perhaps the neutrophils are still getting into the microvasculature and sticking to the activated endothelium, which is mediated by other mechanisms such as the selectins and the integrins, but are perhaps not becoming activated to release the cytoplasmic granules that are so damaging. We are going to use a variety of techniques to look at this in the future, and it will be interesting to see how this all turns out. In cardiothoracic surgery, as you know, we use a lot of combinations of different drugs in cardioplegia to try to achieve optimal myocardial protection. If it turns out that antibody to IL-8 is beneficial in reducing neutrophil-mediated injury, it could be used in conjunction with an agent that promotes blood flow, such as cyclic guanosine monophosphate analogs or nitric oxide donors.

Dr. Aurelio Chaux *(Los Angeles, Calif.).* You showed us some slides in which the number of neutrophils in the tissues was apparently lower than the control value. Do you have neutrophil counts for both control and treated animals?

Dr. Boyle. This is a critical issue in this study. There are a variety of different ways to quantify neutrophil infiltration in tissue. One is to have a pathologist who is unaware of the protocol individually count the neutrophils. As you saw, there were hundreds on the untreated sections, making this approach difficult. We were relatively convinced by the gross appearance, but we agree with you that this assessment needs to be objectively quantified. We are in the process of performing an MPO assay in our laboratory. This should allow us to put a number on the amount of MPO in both groups of tissues, which in turn is directly related to neutrophil infiltration. Unfortunately, this will not tell us whether the neutrophils are activated; it will only yield a gross estimate of their numbers. This is a critical issue that deserves further attention.