Thromboxane A₂ and Peptidoleukotrienes Contribute to the Myocardial Ischemia and Contractile Dysfunction in Response to Intracoronary Infusion of Complement C5a in Pigs

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Intracoronary infusions of activated complement C5a result in myocardial ischemia, contractile dysfunction, and leukocyte accumulation. The hypothesis was tested that the generation of the coronary vasoconstrictors, thromboxane A₂ and the 5-lipoxygenase leukotrienes (LTC₄ and LTD₄), contributes to the C5a-induced decrease in coronary blood flow and contractile function. The left anterior descending coronary artery in anesthetized swine was cannulated and servo pump–perfused with arterial blood at constant pressure and measured flow. Regional subendocardial contractile function was assessed with sonomicrometry. The interventricular vein was cannulated for sampling of coronary venous blood for leukocyte count. The responses in left anterior descending coronary artery blood flow and percent segment shortening to intracoronary infusions of LTC₄ (1 μg), LTD₄ (1 μg), thromboxane agonist U46619 (7.5 μg), and C5a (500 ng) were assessed before and after 1) LTD₄/LTE₄ receptor blockade with leukotriene receptor blocker LY171883 (10 mg/kg i.v.) (n = 5), 2) thromboxane A₂/prostaglandin H₂ receptor blockade with thromboxane receptor blocker BM13505 (2 mg/kg i.v.) (n = 5), and 3) combined thromboxane and leukotriene receptor blockade (n = 5). In the absence of receptor blockade, intracoronary C5a decreased coronary flow (50–60%) and regional segment function (60–70%) compared with the preinfusion levels. This was accompanied by a fall in coronary venous blood leukocyte levels by 5–6×10⁶ cells/ml in the absence of alterations in arterial blood leukocyte count. Intracoronary injections of LTD₄, LTC₄, or U46619 also resulted in prompt decreases in coronary blood flow (50–60%) and segment function (70–80%) from preinfusion levels. Leukotriene receptor blockade with LY171883 abolished these responses to LTD₄ and LTC₄. Administration of LY171883 also attenuated (p < 0.05) the myocardial response to C5a; coronary flow and segment function decreased by approximately 28% from preinfusion levels. Thromboxane receptor blockade with BM13505 eliminated the response in coronary flow and segment function to intracoronary U46619. Similar to LY171883, administration of BM13505 blunted (p < 0.05) the C5a-induced decreases in coronary flow and contractile function, which fell by approximately 20–25% from the preinfusion level. After the combined LTD₄/LTE₄ receptor and thromboxane A₂/prostaglandin H₂ receptor blockade, intracoronary C5a resulted in little change in both coronary blood flow and segment shortening. In contrast to the flow and function effects, the C5a-induced myocardial leukocyte extraction was not decreased by leukotriene and/or thromboxane receptor blockade. It is concluded that the production of the vasoactive eicosanoids, thromboxane A₂ and the peptidoleukotrienes (LTC₄, LTD₄, and LTE₄), is primarily responsible for the myocardial ischemia and contractile dysfunction in response to activated complement C5a. (Circulation Research 1990;66:596–607)
The serum complement pathway is an essential component of the mammalian host defense system and participates in a wide variety of pathophysiological processes including autoimmunity, hypersensitivity, and inflammation. There is increasing evidence that the complement system is involved in the inflammatory response resulting from acute myocardial ischemia, which is characterized by the local infiltration of leukocytes.1-5 The extent of myocardial infarction due to coronary occlusion is reduced by the prior depletion of complement.6,7 and components of the complement pathway are found in ischemic myocardium and colocalize with areas of granulocyte accumulation.5,6,8 Activation of the complement cascade by either the classical or alternative pathway results in the generation of the biologically active polypeptide fragments, the anaphylatoxins C3a and C5a. The action of these molecules, particularly C5a, on specific membrane receptors that are present on a wide variety of cell types, including smooth muscle cells, mast cells, and blood cells (i.e., leukocytes), can result in the release of secondary mediators, including kinins, histamine, thromboxane A2, and leukotrienes, with profound effects on vascular permeability and vessel caliber. Intracoronary infusions of C5a produce transient increases in coronary vascular resistance, contractile dysfunction, and a myocardial accumulation of leukocytes.9 This myocardial response is associated with sixfold to eightfold increases in coronary venous levels of thromboxane B2,10 which is the breakdown product of thromboxane A2, a potent coronary vasoconstrictor. However, it is not clear whether the C5a-induced generation of thromboxane A2 is directly responsible for the observed decreases in coronary blood flow, since cyclooxygenase blockade failed to blunt the ischemic response.10 Furthermore, in the pulmonary vascular bed and the isolated heart, activated complement can result in an enhanced generation of the major components of slow-reacting substance of anaphylaxis (SRS-A), the 5-lipoxygenase peptidoleukotrienes (LTC4, LTD4, and LTE4).11,12 These leukotrienes are known to have potent coronary vasoconstrictor effects.13,14

The goal of the present study was to determine the contribution of thromboxane A2 and the peptidoleukotrienes, LTC4, LTD4, and LTE4, to the decreases in coronary blood flow and contractile dysfunction induced by activated complement C5a.

Materials and Methods

General Preparation

Domestic pigs (25–30 kg) of either sex were premedicated with ketamine (2.5 mg/kg i.m.) and anesthetized 10–15 minutes later, initially with sodium thiopental (10–15 mg/kg i.v.) followed by α-chloralose (100 mg/kg i.v.) and morphine sulfate (30 mg/hr s.c.). After intubation with a cuffed endotracheal tube, animals were ventilated with a positive-pressure respirator (Harvard Apparatus, South Natick, Massachusetts) with an end-expiratory pressure of 3–5 cm H2O. Arterial blood was regularly sampled for determination of PO2, PCO2, or pH (Corning Medical, Medfield, Massachusetts). Blood gas values were kept within the physiological range by adjustments of the ventilatory rate and oxygen supplementation. The anesthetic level was maintained with bolus doses of α-chloralose (250–500 mg i.v.). Animal core temperature (rectal) was monitored (Yellow Springs Instrument, Yellow Springs, Ohio) and maintained at 37°C with a circulating-water heating pad.

Aortic blood pressure was measured with a strain gauge manometer (Statham P23Db, Gould, Oxnard, California) through a polyethylene catheter placed into the aorta via the right femoral artery. Left ventricular (LV) pressure was obtained using a catheter-tip manometer (model 7F, Millar, Houston, Texas) inserted into the left ventricle via the right carotid artery. This signal was recorded at full scale and high gain for determination of peak LV pressure (LVP) and LV end-diastolic pressure (LVEDP) and differentiated to obtain LV dp/dt.

The heart was exposed through a midline sternotomy and left thoracotomy in the fourth intercostal space and suspended in a pericardial cradle. A section of the left anterior descending coronary artery (LAD) just distal to the bifurcation of the left main artery was dissected free from underlying tissue for cannulation. Anticoagulation was achieved with sodium heparin (500 units/kg i.v.) and maintained by hourly supplemental doses of 250 units/kg. Two extracorporeal circuits were used and are described below (Figure 1).

LAD Perfusion

The LAD was cannulated just distal to the left main bifurcation and perfused from the left common carotid artery, using a silastic and polyethylene extracorporeal tubing circuit. Coronary artery perfusion pressure was measured just proximal to the cannula tip through a side arm (Statham 23dB, Gould). Coronary pressure was held constant at 90–95 mm Hg in the various experiments by use of a low-pulsation servo-controlled roller pump especially designed for coronary artery perfusion.15 Coronary blood flow was measured using a transit-time flow transducer (Transonic Systems, Ithaca, New York) in the perfusion circuit. Oclusive zero-flow records were obtained repeatedly during each experiment, and the flowmeter was calibrated with blood by use of the timed-collection method. At the termination of each experiment, 2 cm3 monastral blue dye (Sigma Chemical, St. Louis, Missouri) was rapidly injected (10 seconds) into the perfusion line, and the heart was arrested with an intravenous bolus of saturated KCl. The weight of the dyed portion was used to estimate the mass of the LAD-perfused myocardium for normalization of measured coronary blood flow per gram of myocardium.
Coronary Venous Blood Sampling

The interventricular vein adjacent to the cannulated LAD was ligated and cannulated distally with a small (16-gauge) Teflon and silastic catheter (8-cm length) to allow sampling of coronary venous blood draining the LAD perfusion region. The catheter end was placed 5–10 cm below heart level, and the blood was continuously drained into a small beaker and was returned to a peripheral vein by use of a roller pump (Cole-Palmer Instrument, Chicago, Illinois). During and after each C5a injection, coronary venous blood samples for complete blood count determination were collected in EDTA and processed using a QBC differential blood cell counter (Clay Adams, Parsippany, New Jersey). To avoid systemic effects of C5a, blood collected during the injection period was discarded.

Sonomicrometry

Pairs of lensed ultrasonic dimension crystals (2-mm diameter) were placed into the inner third of the myocardial wall (6–8 mm deep), approximately 1.0–1.5 cm apart and perpendicular to the base-apex chord, to allow continuous measurement of regional myocardial contractile function. One crystal pair was placed into the region perfused by the cannulated LAD (treatment region). A second pair was placed into the area perfused by the circumflex artery in the basal-lateral region of the ventricular free wall. Dimension signals were calibrated before and after each experiment.

Preparation of Drugs

Porcine C5a was prepared from zymosan-activated porcine serum containing 10 μM 2-mercaptomethyl-3-guanidinoethylthio-propanoic acid (Calbiochem, La Jolla, California) as a carboxypeptidase inhibitor as previously described by Hugli et al. and prepared by Dennis Chenoweth, Baxter-Travenol, Santa Ana, California. The purity and quantity of the polypeptide was established by amino acid analysis after acid hydrolysis. The solutions of C5a were formulated by diluting with 1% gelatin in normal saline. LTC4 and LTD4 in phosphate buffer were provided as a gift from Merck Frosst, Canada, and were diluted with 1% gelatin for administration. The thromboxane A2 analog U46619 in methyl acetate was donated by The Upjohn Company, Kalamazoo, Michigan. A stock solution was prepared by removing the methyl acetate by evaporation and reconstituting the residue in ethanol. Aliquots for injection (7.5 μg) were diluted in 1% gelatin to a final ethanol concentration of less than 0.15%. All vehicles were shown in pilot studies to have no significant effects on coronary blood flow and contractile function. The leukotriene receptor blocker LY171883 (Eli Lilly, Indianapolis, Indiana) and the thromboxane receptor blocker BM13505 (Boehringer-Mannheim, Mannheim, FRG) were dissolved in normal saline before intravenous injection.

Experimental Design

Four series of experiments were performed: 1) In the “leukotriene receptor blockade” group (n=5), control responses to separate intracoronary challenges of LTC4 (1 μg), LTD4 (1 μg), and C5a (500 ng) were obtained. The leukotriene receptor blocker LY171883 (10 mg/kg in saline) was administered intravenously, and the injection protocol was repeated with C5a given first, followed by the LTC4 and LTD4 injections to confirm receptor blockade. 2) In the “thromboxane receptor blockade” group (n=5), responses to separate intracoronary challenges of LTD4 (1 μg), thromboxane analog U46619 (7.5 μg), and C5a (500 ng) were obtained before and after thromboxane receptor blockade with BM13505 (2 mg/kg i.v. in saline). After blockade, C5a was given first, followed by the LTD4 and U46619 challenges. 3) In the “combined receptor blockade” group (n=5), responses to LTC4 or LTD4 (1 μg), U46619 (7.5 μg), and C5a (500 ng) were obtained under control conditions. After 30 minutes, leukotriene receptor blockade was achieved with LY171883 (10 mg/kg i.v.), and a second C5a (500 ng) injection was given. After a 30-minute interval, BM13505 (2 mg/kg i.v.) was administered, and the response to a third injection of C5a (500 ng) was tested. Ten minutes after this last injection, combined thromboxane and leukotriene receptor blockade was confirmed by separate challenges with leukotriene LTC4 or LTD4 (1 μg) and U46619 (7.5 μg). 4) In the “time control” group (n=4), the response to three injections of C5a (500
ng) given 30 minutes apart without receptor blockade was assessed to rule out preparation degradation and tachyphylaxis to C5a.

**Experimental Protocol**

The protocol for an experimental run consisted of a prestimulus period, a 1-minute drug injection period, and a 3-minute recovery period. Coronary venous blood samples were obtained before injection, at 30 and 60 seconds during injection, and 15, 30, 45, 60, and 120 seconds after injection. Arterial blood samples were obtained before injection, at 60 seconds during injection, and 60 and 120 seconds after injection. The C5a injections in the leukotriene receptor blockade group and the thromboxane receptor blockade group occurred 60 minutes apart, with all blocking agents administered approximately 10–15 minutes before the second C5a challenge. This 60-minute interval for C5a challenges was determined in a separate study to be sufficient to avoid problems with tachyphylaxis known to be present with repeated exposure to C5a. In the combined blockade group, the interval between C5a challenges was 30 minutes. Validation of this injection interval is presented in the “time control” group results.

Aortic pressure, LVP, LVEDP, LV dP/dt, ultrasonic segment lengths, and mean coronary blood flow were recorded on an eight-channel oscillographic recorder (Gould-Brush, Cleveland, Ohio). All measurements were obtained with respiration suspended at end expiration to avoid respiratory fluctuations in segment lengths.

**Data Analysis**

Data were read from analog records at the control time point, 15-second intervals during the injection period, 1 minute after injection, and every 30 seconds for 4 minutes thereafter. At each time point, mean coronary blood flow, systolic and diastolic aortic blood pressure, peak LVP, LVEDP, LV dP/dt, and segment lengths were read. Data from three to four consecutive cardiac cycles were averaged for each experimental time point. Regional segment lengths in millimeters were determined at end diastole and end systole. The end-diastolic length (EDL) was measured after the A wave, at the time of the sharp upswing in the high-gain LVP signal. The end-systolic length (ESL) was defined at the point 20 msec before peak negative dP/dt. Regional myocardial function was calculated as percent segment shortening (%SS) defined as \((\text{EDL} - \text{ESL}) / \text{EDL} \times 100\).

The study was designed to have each animal serve as its own control. The preinjection control value for an individual pig for each experimental condition was taken just before the onset of the C5a injection period. For the coronary blood flow and regional function data, all measurements are expressed as a percent of this control value. The averages of these values for all pigs in each experimental group are shown in the figures. The standard errors of the mean given in the figures and text were derived from data sets containing one value from each pig studied in any given experimental group \((n = 1\) degrees of freedom).

Statistical analyses of the difference between the preinjection control point and the response at selected points during and after the injection period were made by a repeated-measures analysis of variance. A paired t test with Bonferroni’s correction was used to compare the statistical differences in the responses after each intervention in all experimental groups and is indicated on the figures.

**Results**

**Leukotriene Receptor Blockade Group**

Average values for five pigs that were subjected to the leukotriene blockade protocol are shown in Figures 2 and 3. Before leukotriene receptor blockade, the intracoronary injection of LTC_4 (1 μg) resulted in a marked decrease in coronary blood flow to a nadir of 41.3±8.1% of the preinjection level of 0.89±0.10 ml/min/g \((p<0.01)\) (Figure 2). This was accompanied by a significant fall \((p<0.01)\) in regional contractile function in the LAD perfusion area to a minimum value of 16.6±9.1% of the preinjection %SS level of 23.4±1.5% of the EDL (11.7±1.0 mm). Changes in segment shortening in the circumflex region were minor, slightly increasing by less than 10% during and after the LTC_4 injection. Heart rate (100±9 beats/min), peak LVP (101.8±5.7 mm Hg), and LVEDP (3.3±0.4 mm Hg) did not significantly change during the injection period. After leukotriene receptor blockade with LY171883 (10 mg/kg), the LTC_4-induced fall in coronary blood flow and LAD regional function were abolished (Figure 2). This occurred in the absence of major effects of LY171883 on either resting coronary blood flow (0.82±0.09 ml/min/g) or segment function (%SS=19.9±1.7% of EDL). Also, heart rate (95±5.2 beats/min), LVP (100.8±6.1 mm Hg), and LVEDP (3.7±0.9 mm Hg) values were similar to those before blockade. Before receptor blockade, the intracoronary injection of LTD_4 (1 μg) resulted in decreases in coronary blood flow and regional segment function similar to that found with LTC_4. Coronary flow fell to a nadir of 47.4±9.1% of the preinjection value of (0.85±0.10 ml/min/g) \((p<0.01)\) accompanied by a reduction in LAD segment shortening to 22.2±13.4% of the preinjection %SS of 22.4±1.1% of EDL \((p<0.01)\). Similar to LTC_4, changes in heart rate, LVP, LVEDP, and circumflex segment function were minor during and after the injection of LTD_4. The LTD_4-induced decreases in coronary flow and LAD segment function were also eliminated by the administration of LY171883 and remained within 5% of the preinjection values throughout the experimental run.

The intracoronary injection of C5a (500 ng) before leukotriene receptor blockade decreased coronary blood flow to a minimum value of 40.5±6.7% of the preinjection level (0.82±0.10 ml/min/g) 30 seconds
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**FIGURE 2.** Graphs showing average responses in five animals (leukotriene blockade group) to the intracoronary infusion of 1 μg leukotriene C₄ (LTC₄) before and after leukotriene receptor blockade with leukotriene receptor blocker LY171883 (10 mg/kg i.v.). Coronary blood flow (top panel) and percent segment shortening (bottom panel) in the left anterior descending coronary artery (LAD) perfusion region at time = 0. Before LY171883, LTC₄ produced a prompt but transient decrease in coronary flow and contractile function. These changes were greatly attenuated by the administration of LY171883. *p<0.01 compared with preinjection levels.

into the injection period (p<0.01) (Figure 3). Segment shortening (LAD) fell to 50.4±9.6% of the preinjection level (p<0.01) in the absence of significant changes in shortening in the circumflex region. The decrease in blood flow was accompanied by a fall in the coronary venous blood leukocyte count to a nadir of 5.9±0.6×10⁴ cells/μl from the preinjection value of 11.6±0.8×10⁴ cells/μl. Arterial blood leukocyte levels were not significantly altered over the experimental run. Changes in heart rate, LV, and LVEDP were minor; these changes remained within 10% of their preinjection values. The administration of LY171883 (10 mg/kg) resulted in a significant attenuation of the myocardial response to C₅a (500 ng) (Figure 3). The fall in coronary blood flow (to 72.3±7.0% of preinjection) was significantly blunted (p<0.05) and short-lived compared with the control C₅a injection before LY171883. At 45 seconds into the injection period, coronary blood flow had returned to and slightly above the preinjection level in contrast to the control C₅a injection, which was still significantly depressed at this time point (p<0.05). The C₅a-induced decrease in segment shortening in the LAD perfusion region was also attenuated (p<0.05) compared with the control injection, falling to only 72.9±5.8% of the preinjection %SS value (20.2±1.5% of the EDL). In contrast to these differences, the C₅a-induced fall in the coronary venous leukocyte count was not significantly altered by leukotriene receptor blockade (p>0.10). After LY171883, coronary venous leukocyte count fell to a nadir of 4.8±0.5×10⁴ cells/μl from the preinjection value of 10.6±0.6×10⁴ cells/μl. Alterations in heart rate, LV, LVEDP, and circumflex segment function were minor and similar to the control C₅a injection.
**Thromboxane Receptor Blockade Group**

Average values for five pigs that were subjected to the thromboxane blockade protocol are shown in Figures 4–6. Before thromboxane receptor blockade, the intracoronary injection of LTD₄ resulted in a decrease in coronary blood flow to a nadir of 55.8±6.9% of the preinjection value of 0.74±0.08 ml/min/g (Figure 4). Segment shortening in the LAD region fell to 40.8±5.4% of the preinjection %SS (23.8±1.8% of EDL [10.7±0.7 mm]). These responses were not attenuated by thromboxane receptor blockade. After the administration of BM13505 (2 mg/kg), the decrease in coronary blood flow in response to LTD₄ was slightly, but not significantly, enhanced, falling to a nadir of 43.1±6.8% of the preinjection level of 0.73±0.08 ml/min/g (Figure 4). This was accompanied by a greater fall in LAD segment shortening to a minimum value of −4.0±1.5% of the preinjection %SS (21.2±1.7% of EDL [10.1±0.5 mm]) compared with the control response before administration of BM13505 (p<0.05). Changes in heart rate, LVP, LVEDP, and segment shortening in the circumflex region during the injections of LTD₄ were minor and similar before and after BM13505. However, absolute heart rates tended to be higher (89±6 vs. 73±4 beats/min), peak LVPs lower (118±4 vs. 105±6 mm Hg), and LVEDPs slightly lower (4.7±0.3 vs. 3.7±0.5 mm Hg) after the administration of BM13505. As shown above, the administration of BM13505 had minimal effects on resting coronary blood flow or regional segment shortening. The myocardial response to intracoronary injections of the thromboxane agonist U46619 (7.5 µg) before and after BM13505 is shown in Figure 5. Before thromboxane receptor blockade, U46619 produced prompt but short-lived decreases in coronary blood flow (53.4±9.9% of preinjection level) and LAD segment shortening (36.2±14.1% of preinjection level) in the absence of significant changes in heart rate, LVP, LVEDP, and circumflex segment shortening. The myocardial response to U46619 was completely blocked (p<0.01) by the administration of 2 mg/kg BM13505 (Figure 5). The myocardial response to intracoronary injections of
C5a (500 ng) before and after thromboxane receptor blockade is depicted in Figure 6. The C5a-induced decrease in coronary blood flow was significantly attenuated (*p<0.05) by the administration of BM13505. Coronary flow fell to 81.8±8.8% of the preinjection level compared with 49.1±9.7% during the control injection before BM13505 (*p<0.05). The administration of BM13505 also altered the C5a-induced fall in LAD segment shortening. The response was slower to develop with the decrease in shortening significantly less (*p<0.01) at 30 seconds of injection. However, the maximal decrease (60.0±11.4% of preinjection level) was only slightly, but not significantly, attenuated compared with the control experimental run (36.5±11.1% of preinjection level) before BM13505. The myocardial extraction of leukocytes occurring in response to the intracoronary injection of C5a was not significantly altered by the administration of BM13505. Before blockade, intracoronary C5a resulted in a peak myocardial extraction (arterial-coronary venous blood count) of 8.7±0.7×10³ cells/µl. In the presence of BM13505, peak myocardial extraction reached a value of 6.8±0.7×10³ cells/µl, not significantly different (*p>0.10) compared with the control C5a experimental run. Alterations in heart rate, LVP, LVEDP, and circumflex segment shortening occurring during and after the injection of C5a were minor and not different before and after thromboxane receptor blockade.

**Combined Receptor Blockade Group**

Average values for five pigs that underwent the combined blockade protocol are depicted in Figures 7 and 8. In these animals, the control injection of C5a significantly decreased coronary blood flow.
to a nadir of 49.6±3.5% of the preinjection level of 0.69±0.13 ml/min/g (p<0.01) (Figure 7). This was accompanied by a fall in LAD segment shortening to 74.8±5.9% of the preinjection value of 28.1±1.3% of EDL (12.2±0.5 mm) (p<0.01). As found in the leukotriene blockade group, the administration of LY171883 significantly blunted (p<0.05) the C5a-induced decrease in coronary flow, with blood flow decreasing to a nadir of 88.1±5.2% of the preinjection level of 0.82±0.17 ml/min/g. The C5a-induced fall in segment shortening in the LAD region to 88.5±5.4% of the preinjection level was slightly (p=0.055) reduced by LY171883 compared with the control run (Figure 7). The addition of thromboxane receptor blocker with BM13505 tended to further attenuate the C5a-induced decreases in coronary blood flow and contractile function. The intracoronary injection of C5a after the addition of BM13505 after LY171883 administration resulted in no significant decrease in coronary blood flow (p>0.10) from the preinjection value of 0.71±0.12 ml/min/g. Similarly, LAD segment shortening remained within 5% of the preinjection level (%SS=26.2±2.5% of EDL) over the entire experimental run. This was a significant reduction (p<0.01) compared with the response observed in the control run before LY171883 and BM13505. The combined leukotriene and thromboxane receptor blockade did not significantly influence the C5a-induced myocardial leukocyte extraction, with coronary venous leukocyte counts falling by approximately 6.0×10³ cells/µl during both the control C5a injection and after LY171883 and BM13505 administration (Figure 8). An effective coronary vascular leukotriene and thromboxane receptor blockade was still present after the last C5a injection. At this time, the injection of leukotriene (LTC4 or LTD4) was ineffective in altering coronary flow and LAD segment shortening with levels remaining within 5% of preinfusion values (coronary flow, 0.71±0.11 ml/min/g; %SS, 25.1±2.3% of EDL) over the experimental run. This was in contrast to the initial leukotriene injection that markedly lowered coronary flow to 42.6±6.1% of preinjection (0.80±0.15 ml/min/g) and LAD segment shortening to 51.2±9.5% of the preinjection %SS (27.2±2.6% of EDL). Similarly, the intracoronary challenge with U46619 after the last C5a injection was without major effect on coronary flow or LAD segment function, which remained within 7% of preinfusion levels. This was in contrast to the initial injection of U46619, which markedly decreased coronary flow and segment function (26±6.7% and 50.8±14%, respectively, of preinjection levels). Heart rates (82±10 vs. 83±10 beats/min), peak LVPs (89±6 vs. 94±7 mm Hg), and LVEDPs (4.2±1.1 vs. 4.4±0.8 mm Hg) were similar after the combined blockade compared with the control experimental run.

**Time Control Group**

There was no detectable effect of time or tachyphylaxis to three injections of C5a given 30 minutes apart (n=4). In these animals, the first injection of C5a resulted in a decrease in coronary blood flow to a value of 62.3±9.9% of the preinfusion level (0.87±0.10 ml/min/g). With the second injection, coronary flow fell to 54.6±11.8% of the preinfusion level (0.93±0.13 ml/min/g). The third injection of C5a decreased coronary flow to 62.0±16.0% of the preinfusion level (0.97±0.17 ml/min/g), not different (p>0.10) compared with the first injection response. Similar results were obtained in LAD %SS, which fell to 62.3±9.9% of the preinjection level during the first C5a injection and to 67.8±11.7% of the preinjection level during the third injection of C5a. There was also no diminution in the C5a-induced myocardial extraction of leukocytes, which was 7.6±1.9×10³ cells/µl with the first injection and 7.4±1.8×10³ cells/µl with the third injection.

**Discussion**

These data demonstrate that the generation of thromboxane A2 and the peptidoleukotrienes (LTC4, LTD4, or LTE4) contribute to the coronary vasoconstriction and myocardial ischemia resulting from intracoronary infusions of C5a. Pharmacological blockade of either the prostaglandin H2/thromboxane A2 receptor with BM13505 (Figure 6) or the LTD4/LTE4 receptor with LY171883 (Figure 3) attenuated the C5a-induced decrease in coronary blood flow and fall in contractile function. The residual myocardial response to C5a after the combined receptor blockade was less than 5% of the preinjection value (Figure 7). This blunting of the decrease in coronary blood flow in response to C5a occurred at constant coronary perfusion pressure and in the absence of major changes in heart rate and LVP. The attenuation of the response to C5a could not be explained by
preparation degradation over time or tachyphylaxis to C5a. The myocardial accumulation of leukocytes associated with the intracoronary infusion of C5a was not influenced by thromboxane and/or leukotriene receptor blockade.

The majority of the information on the effects of activated complement on vascular resistance has been obtained in the pulmonary circulation. Several studies have now shown that systemic activation of the complement cascade occurring with the protamine reversal of heparin or the infusion of complement-activated plasma results in a characteristic pulmonary hypertension, pulmonary leukostasis, and extravasation of plasma proteins. It is now apparent that many of these changes are secondary to the generation of metabolites of arachidonic acid from both the cyclooxygenase and 5-lipoxygenase pathways. The increase in pulmonary vascular resistance is associated with marked increases in plasma thromboxane B2 levels and can be attenuated with cyclooxygenase blockade or with scavengers of hydrogen peroxide. The contribution of components of SRS-A, the 5-lipoxygenase peptidoleukotrienes (LTC4, LTD4, and LTE4), to C5a-mediated pulmonary effects has also been suggested. Incubation of lung fragments with activated complement was shown to release an LTD4-like substance with a chromatographic behavior identical to LTD4. In addition, this substance induced smooth muscle contraction in a bioassay model, which was blocked by the leukotriene receptor blocker FPL55712.

The role of thromboxane A2 and the peptidoleukotrienes in the C5a-induced vascular resistance changes in the myocardial circulation is not as well understood. It has recently been demonstrated that the myocardial ischemia and contractile dysfunction induced by intracoronary C5a is accompanied by an enhanced production of thromboxane A2 as reflected by thromboxane B2 levels in coronary venous blood. However, cyclooxygenase blockade with aspirin or indomethacin was without major effect. In the present study, blockade of the prostaglandin H2/thromboxane A2 receptor with BM13505 (Figure 6) attenuated the C5a-induced decrease in coronary blood flow and contractile function. The degree of receptor blockade achieved with the 2 mg/kg dose of BM13505 was sufficient to virtually eliminate the myocardial response to the thromboxane A2 agonist U46619 (Figure 5). It is unlikely that the attenuation of the C5a response was due to a nonspecific action of the drug on the coronary vascular smooth muscle since the decrease in coronary blood flow elicited by a different receptor-mediated coronary vasoconstrictor (LTD4 or LTC4) was not attenuated by the administration of BM13505 (Figure 4). This drug also had no effect on the C5a-induced myocardial leukocyte extraction, suggesting that the drug does not alter the interaction of C5a with its leukocyte membrane receptor, although this possibility cannot be completely excluded. Thus, these data demonstrate that the enhanced production of thromboxane A2 known to occur in response to C5a does contribute to the increased coronary vascular resistance.

In a previous study, the administration of the cyclooxygenase inhibitors, aspirin or indomethacin, eliminated the production of thromboxane but did not significantly attenuate the myocardial response to C5a, in contrast to the effect of thromboxane receptor blockade demonstrated here. The reason for these apparent contradictory results are presently unclear. Several possibilities seem reasonable: 1) Cyclooxygenase-dependent coronary vasodilators such as prostaglandin I2 might be produced in addition to thromboxane A2. Thus, the effect of cyclooxygenase inhibition would be confounded by the elimination of both a coronary vasoconstrictor and vasodilator, resulting in no net change from the control response. This masking influence would not occur with the blockade of the receptor for the vasoconstrictive component alone (i.e., with BM13505). 2) Cyclooxygenase inhibition could result in the greater production of another coronary vasoconstrictor (i.e., peptidoleukotrienes), offsetting the loss of the thromboxane A2 generation. The known coronary vasoconstrictor properties of the leukotrienes, Figure 2), the parallel nature of their generation from arachidonic acid, and the present data (Figures 3 and 7) indicating their action during the C5a injection, are consistent with this possibility. There is experimental data from in vitro preparations demonstrating that “shunting” of arachidonic acid metabolism to the enhanced generation of the peptidoleukotrienes via the 5-lipoxygenase pathway can occur on cyclooxygenase blockade. The mechanism for this shunting effect is unclear, but a simple redirection of substrate by cyclooxygenase blockade to the 5-lipoxygenase pathway is unlikely. Evaluation of this shunting mechanism in the present in vivo model of intracoronary C5a injections, which uses measurements of coronary venous leukotriene levels before and after cyclooxygenase blockade, although attractive, is hindered by the short half-lives of these compounds in blood.

In the present study, the blockade of the LTD4/LTE4 receptor with LY171883 also attenuated the C5a-induced fall in coronary blood flow and systolic shortening; thus, the involvement of these peptidoleukotrienes is indicated in this response (Figure 3). The degree of receptor blockade was sufficient to eliminate the coronary vasoconstriction in response to intracoronary challenges with either LTC4 or LTD4 (Figure 2). This receptor blocker has been shown to be selective for the LTD4/LTE4 receptor. The inhibitory effect of LY171883 on the coronary vasoconstriction to LTC4 is likely due to the rapid conversion of exogenous LTC4 to LTD4 in blood and its subsequent action on the LTD4/LTE4 receptor. Although there is some evidence for the existence of a separate LTC4 receptor that can be demonstrated in vitro or in the presence of agents that inhibit the
conversion of LTC₄ to LTD₄ via the γ-glutamyltransferase enzyme, its physiological significance is unknown. However, our data suggest that a major role for an LTC₄ receptor-mediated vasoconstriction in porcine coronary arteries in the present model is unlikely, since combined thromboxane A₂ and LTD₄/LTE₄ receptor blockade eliminated most of the C5α-induced vasoconstriction with little residual effect remaining (Figure 7).

It should be noted that the attenuation of the C5α-induced myocardial ischemia by leukotriene and/or thromboxane receptor blockade occurred in the absence of significant changes in the magnitude of the myocardial leukocyte accumulation (Figure 8). In addition, it has been previously shown that similar or even greater degrees of leukocyte accumulation can result from intracoronary infusions of leukotriene B₄ without detectable changes in coronary flow or contractile function. Collectively, these data suggest that, in this model, capillary plugging by leukocytes, in itself, is not responsible for the fall in myocardial perfusion and that coronary vasoconstriction via the production of eicosanoids is more important. However, the presence of blood leukocytes and/or platelets does appear to play a role in the C5α-induced vasoconstriction since their removal greatly attenuates the response. The C5α-induced production of eicosanoids may not be entirely dependent on the presence of blood cellular components. There is evidence that activated complement C3a can result in the release of histamine and cyclooxygenase metabolites in Langendorff isolated hearts perfused with crystalloid solution in the absence of blood cells.

The specific cells that are the source of the C5α-liberated eicosanoids in this model are not known. Activated blood leukocytes are capable of producing both cyclooxygenase and 5-lipoxygenase products. In addition, the release of thromboxane from activated platelets is a well-recognized phenomenon. The role of platelets in the myocardial response to C5α in the present model is unclear. Porcine C5α has been shown to directly activate guinea pig platelets. However, because the porcine C5α used in the present study does not aggregate platelets isolated from farm pigs (unpublished results), this effect may be species dependent. A direct effect of C5α on platelets may not be necessary. An interaction of activated polymorphonuclear leukocytes with platelets as a component of the inflammatory response has been previously described. Platelet deposition in inflamed tissue is greatly attenuated by prior depletion of circulating neutrophils. In the present model, blood cellular components alone do not appear to be solely responsible for the increase in coronary venous blood thromboxane B₂ levels seen with C5α. Incubation of whole blood with C5α does not result in a significant increase in thromboxane A₂ levels. Thus, the release of these eicosanoids may require the interaction of activated leukocytes and/or platelets with the vascular wall. It is also possible that the vascular wall itself is a source of the eicosanoids released in the present model. Although vascular endothelium is not normally considered a rich source of thromboxane, endothelial cells can generate measurable amounts of thromboxane A₂. Furthermore, the production of LTC₄ from endothelial cells via an intercellular transfer of leukotriene A₄ from polymorphonuclear leukocytes has also been described. Oxygen derived free-radical species generated from complement C5α-activated leukocytes and their action on the vascular endothelium or platelets may be an important component of the C5α response. It has been reported that the enhanced arachidonic acid metabolism occurring in the pulmonary vascular bed with complement activation is dependent on the production of oxygen free-radical species or H₂O₂. Furthermore, in the buffer-perfused lung, free-radical generation with xanthine and xanthine oxidase also results in thromboxane release. In an isolated heart preparation, activation of neutrophils with phorbol ester in buffer resulted in coronary vasoconstriction and contractile dysfunction that could be attenuated with free-radical scavengers; thus, polymorphonuclear leukocytes may be the source of these free radicals. The C5α activation of leukocytes, their adherence to the vascular wall, the concomitant production of free radicals, and free-radical–dependent mobilization of membrane arachidonic acid may all be necessary components of the myocardial response to C5α.

Several studies have indicated that the generation of arachidonic acid metabolites, especially the leukotrienes, contributes to the extent of myocardial injury that results from acute coronary occlusion. The mechanism by which these products are formed and subsequently promote further myocardial injury is not clear. However, our data suggest that the production of lipoxygenase- and cyclooxygenase-derived coronary vasoconstrictors secondary to leukocyte activation may be involved.

In conclusion, the coronary vasoconstriction and contractile dysfunction in response to the intracoronary infusion of activated complement C5α is mediated in part by thromboxane A₂ and the 5-lipoxygenase peptidoleukotrienes.

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