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C5a-Induced Hemodynamic and Hematologic Changes in the Rabbit

Role of Cyclooxygenase Products and Polymorphonuclear Leukocytes

CLAES LUNDBERG, FRANCOIS MARCEAU, and TONY E. HUGLI

From the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California

Hemodynamic and hematologic changes occurring after intravascular complement activation have implicated the anaphylatoxins in this response. In this study, the hemodynamic and hematologic effects of purified C5a were investigated in rabbits; and involvement of prostanoids, histamine, and polymorphonuclear leukocytes (PMNs) were examined. The anaphylatoxin C5a induces a reversible systemic arterial hypotension which coincides with an increase in central venous pressure (CVP), decreased cardiac output (CO), increased plasma prostanoid levels, as well as neutropenia. Total peripheral resistance (TPR) remained unchanged. The cyclooxygenase inhibitor indomethacin abolished the C5a-induced hypotension and normalized plasma prostanoid levels without altering the C5a-induced neutropenia. The thromboxane (Tx) A_2 synthetase inhibitor dazoxiben reduced TxB_2 plasma levels and increased 6-keto-prostaglandin $PGF_{1\alpha}$ and PGE_2 levels without altering the hypotensive response. However, with dazoxiben treatment both TPR and CVP decreased. The H_2-receptor antagonist cimetidine reduced C5a-induced hypotension and diminished prostanoid release. Both the hypotensive response and elevated prostanoid release were observed after C5a challenge in animals rendered neutropenic prior to challenge. It is concluded that C5a-induced arterial hypotension in the rabbit is a PMN-independent reaction, mediated through cyclooxygenase products and, to some degree, by histamine. The mechanism producing systemic arterial hypotension does not seem to involve peripheral vasodilation but appears to be a secondary effect of pulmonary vasoconstriction, possibly mediated by TxA_2. (Am J Pathol 1987, 128:471–483)

INTRAVASCULAR complement activation induces hemodynamic and hematologic changes in both man[1–4] and experimental animals.[5–7] These effects have been shown to require the third component of complement, C3, but occur in the absence of the sixth component of complement, C6.[6] The anaphylatoxin C5a is cleaved from the fifth component of complement, C5, during activation of the complement cascade. C5a is rapidly converted by serum carboxypeptidase N to C5a des Arg, which is now known as the "classic anaphylatoxin." This classic anaphylatoxin was previously reported to have systemic hypotensive effects in guinea pigs,[8] cats,[9] and dogs.[10] However, the exact mechanism responsible for the hypotensive response is not clear. Pavek et al[10] suggested that portal venous blood pooling followed by a decrease in venous return and cardiac output (CO) could cause systemic arterial hypotension in dogs.

Alternatively, effects of C5a on peripheral resistance vessels have also been suggested. Rampart et al[11] proposed that C5a-dependent stimulation of prostaglandin (PG) I_2 production may dilate resistance ves-

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Address reprint requests to Tony E. Hugli, Scripps Clinic and Research Foundation, IMM18, 10666 N. Torrey Pines Rd, La Jolla, CA 92037.

sels, thereby increasing local blood flow. Hugli and Marceau[12] reported a C5a-induced relaxation of rabbit blood vessel strips that coincided with increased production of the vasodilator PGI_2 by the blood vessels. Both the C5a-induced relaxation and PGI_2 release were inhibited by indomethacin; consequently, an indirect effect of C5a on smooth muscle was suggested. Bult et al[7] measured PGI_2 and thromboxane (Tx) A_2 levels during intravascular complement activation (ICA) by cobra venom factor (CVF) in rabbits, and these authors suggested a causal relationship between PGI_2 formation and arterial hypotension. Further, Pavek et al[10] suggested that cyclooxygenase products were involved in the C5a-induced hypotension, since indomethacin administered to two dogs inhibited hypotension without altering the decrease in cardiac output. Besides blood pooling and peripheral vasodilation, pulmonary vasoconstriction is reported to occur after anaphylatoxin is injected in dogs[10] or cats,[9] when zymosan-activated plasma (ZAP) is injected in sheep[13-16] or pigs,[17] and after intravascular complement activation (CVF) in rabbits.[18] Pulmonary vasoconstriction may also contribute to peripheral hypotension because of decreased cardiac output.

The neutrophil response to C5a is the best defined cellular interaction for C5a. Cell surface receptors specific for C5a have been demonstrated on these cells,[19-21] and the ligand–receptor interaction promotes chemotaxis, adherence, oxygen burst, and granular enzyme release from the neutrophil.[22] Intravascular complement activation in both man and experimental animals, as well as injection of C5a des Arg in animals, causes a profound neutropenia. Furthermore, C5a causes prostacyclin release from endothelial cells *in vitro*, but the response has been shown to be neutrophil-dependent.[23] Therefore, one might speculate that the neutrophil is a prime target for C5a and that activation of this cell contributes to the hemodynamic responses observed with C5a.

The present investigation was undertaken to elucidate the mechanism for C5a-induced systemic arterial hypotension in rabbits. Involvement of cyclooxygenase and lipoxygenase products, as well as histamine, was studied by measuring plasma prostanoid levels and by treating the animals with pharmacologic agents that interfere with arachidonic acid metabolism and histamine action, respectively. The role of polymorphonuclear leukocytes (PMNs) was assessed by performing experiments in animals that were rendered neutropenic with nitrogen mustard. In addition, the use of radioactive microspheres permitted CO, regional blood flow (RBF), and vascular resistance to be measured after C5a challenge.

Materials and Methods

Animals and Anesthesia

Male New Zealand white rabbits weighing between 1.0 and 1.3 kg were used. The animals were anaesthetized with diazepam (Valium, Roche, Manati, Puerto Rico; 5 mg/kg, intramuscularly) and thereafter with 20% urethane (Sigma, St Louis, Mo; 1.5 g/kg) injected into an ear vein.

Surgical Procedure

The animals were tracheostomized and allowed to breathe spontaneously. Polyethylene catheters (PE-90; Clay Adams, Parsippany, NJ) were inserted into the right common carotid artery and into the left jugular vein for pressure recording (P23-ID; Gould Statham, Gould Inc., Medical Product Div., Oxnard, Calif) and intravenous injections, respectively. Mean arterial blood pressure (MAP) and body temperature were monitored continuously; the latter was maintained at 37–38 C during the course of the experiment.

Experimental Procedure

After a 30-minute stabilization period, an initial 1-ml blood sample (−5 minutes) was drawn from the right common carotid artery and a dose of 10 μg porcine C5a (100 μg/ml) in 1% bovine serum albumin (BSA) was injected intravenously as a bolus at 0 minutes. When the drop in MAP was maximal (typically between 15 and 30 seconds after C5a injection), a second arterial blood sample was drawn. A third arterial blood sample was drawn 30 minutes after the C5a injection. After the third blood sample was taken, the animals were treated intravenously according to one of the following six protocols: Protocol 1: 500 μl of saline was injected into 8 animals serving as controls. Protocol 2: the cyclooxygenase inhibitor indomethacin was given in a dose of 5 mg/kg, dissolved in 0.1 M sodium carbonate (5 mg/ml), to 6 animals. Protocol 3: the TxA_2 synthetase inhibitor dazoxiben (UK-37-248) was injected into 5 animals in a dose of 25 mg/kg (25 mg/ml saline). Protocol 4: the H_1-receptor antagonist pyrilamine was given in a dose of 2.5 mg/kg (10 mg/ml saline) to 4 animals. Protocol 5: the H_2-receptor antagonist cimetidine was given in a dose of 10 mg/kg (150 mg/ml saline) to 6 animals. Protocol 6: the lipoxygenase inhibitor REV 5901 was given in a dose of 10 mg/kg, dissolved in polyethylene glycol (PEG) 200 (20 mg/ml), to 4 animals.

Ten minutes after each protocol was initiated, the procedure was repeated as described above with a

1-ml arterial blood sample, a 10-μg porcine C5a injection 5 minutes later, a blood sample at the point of maximal decrease in MAP, and the last sample collected 30 minutes after C5a injection. Neither sodium carbonate (0.1 M) used to dissolve indomethacin nor PEG 200 used to dissolve REV 5901 influenced the C5a response. In one set of experiments (n = 2) the porcine C5a was replaced by an equal volume (100 μl) of saline, and these animals were treated as the control group. All 1-ml blood samples were collected in tubes containing 1 mg ethylenediamine tetraacetic acid (EDTA) and 10 μg indomethacin. Blood samples were collected for determination of hematocrit, white blood cell (WBC) count, and platelet count. The remainder of the sample was centrifuged and the plasma stored at −70 C until radioimmunoassay determinations of 6-keto-$PGF_{1\alpha}$, TxB_2, and PGE_2 were performed. WBCs were stained with gentian violet for counting, and smears were stained with May–Grunewald–Giemsa for differential leukocyte counting. Platelets were counted in 1% ammonium oxalate.

In one set of experiments (n = 5) a PE-90 catheter was also inserted into the right jugular vein and positioned close to the right atrium of the heart for central venous pressure (CVP) measurements. After a 30-minute stabilization period, one injection of 10 μg C5a was given intravenously, and MAP and CVP were monitored. In another set of experiments the animals were pretreated with dazoxiben (25 mg/kg) and the experiment performed as described above (n = 4).

Neutropenic Animals

Six rabbits were rendered neutropenic by a single intravenous dose of nitrogen mustard (Mustargen, MSD, West Point, Pa; 1.7 mg/kg).[24] After 3 days the PMNs of the rabbits were reduced by 90–95% to $0.2 + 0.1 \times 10^6$/ml, and experiments were performed as described above, except that only one C5a injection was used and 3 blood samples were drawn.

Pulmonary Water Content

The left lung of the rabbits was removed *post mortem*, weighed (wet weight), lyophilized, and reweighed (dry weight), and water content was expressed as wet weight/dry weight.

Radioimmunoassays

Six-keto-$PGF_{1\alpha}$, TxB_2 and PGE_2 were determined in the rabbit plasma without prior extraction using 125-I radioimmunoassay (RIA) kits (New England Nuclear, Boston, Mass). Iodinated analogs of 6-keto-$PGF_{1\alpha}$, TxA_2 and PGE_2 were used as tracers, and specific antibodies (rabbit) to the three respective prostanoids were used as antisera. Separation of the antibody–antigen complexes from the free antigen was achieved by precipitation of the antibody-bound tracer with polyethylene glycol. The pellet containing the antibody–antigen complex was counted. Major cross-reacting prostaglandins were $PGF_{2\alpha}$ (2.6%), PGE_1 (1.9%), TxB_2 (1.4%), and PGE_2 (1.1%) for the 6-keto-$PGF_{1\alpha}$ assay; PGD_2 (3.9%) for the TxB_2 assay; and PGE_1 (3.7%) for the PGE_2 assay. Detection limits of the 6-keto-$PGF_{1\alpha}$, TxB_2, and PGE_2 RIA kits were 20 pg/ml, 10 pg/ml, and 2.5 pg/ml, respectively. The RIAs were performed according to the manufacturer's suggested protocol.

Measurement of RBF, CO, and Vascular Resistance Using Radioactive Microspheres

Rabbits were tracheostomized and allowed to breath spontaneously. A polyethylene catheter (PE-50) was inserted into the left ventricle of the heart via the right common carotid artery for microsphere injections. The position of this catheter was confirmed from the pressure tracing and postmortem examination. Further, polyethylene catheters (PE-90) were inserted into the left jugular vein for intravenous injections and into the left and right femoral artery for pressure recording and reference sample withdrawal, respectively. The radioactive microsphere technique was described in detail previously.[25] Briefly, 15-μ microspheres (3M, New Brighton, Minn), labeled with 57-cobolt or 65-zinc, were injected into the left ventricle. Approximately 1×10^6 spheres, suspended in 10% dextran, were injected each time. The reference sample was drawn at a rate of 1.31 ml/min. The first microsphere injection was given 5 minutes before injecting 1% BSA intravenously (control;n = 7) or 10 μg C5a intravenously (n = 7). The second injection was made 20 seconds after the BSA injection in the control group, respectively, at the time point when MAP following C5a injection was maximally reduced (typically 15–30 seconds) in the C5a group. The order of injection for the 2 isotopes was varied systematically. In one set of experiments (n = 6) the TxA_2 synthetase inhibitor dazoxiben (25 mg/kg) was injected intravenously 10 minutes prior to the C5a injection, and microsphere injections were performed as described above. After the second microsphere injection, the animals were killed by an overdose of urethane, and the following organs were removed in a uniform manner: stomach, duodenum, jejunum, ileum, colon, right and left kidney, liver, skeletal

muscle (right and left ileopsoas), right lung, heart, brain (right and left cerebral hemisphere), and abdominal skin. The tissue samples were weighed and, together with the reference samples, measured in a gamma counter. Vascular resistance was calculated by dividing the MAP by the RBFs, and in the case of total peripheral resistance (TPR) the MAP was divided by CO.

Preparation of C5a

Porcine C5a was prepared according to Gerard and Hugli,[26] modified by using high performance liquid chromatography (HPLC) and a Pharmacia Mono S column as the final purification step. The purity of the C5a was confirmed by cellulose acetate electrophoresis and amino acid analysis. Bioassay (guinea pig ileum) was used for determining activity between individual C5a preparations. Absence of endotoxin in the C5a preparations was confirmed by the Limulus test in accordance with E-Toxate kit instructions (Sigma, Bulletin 210).

Materials

BSA, indomethacin, and PEG were purchased from Sigma, St Louis, Mo. Cimetidine (Tagamet) was from Smith, Kline and French Laboratories, Carolina, Puerto Rico. REV 5901 was obtained as a kind gift from Dr. Pruss, Revlon Health Care Group, Tuckahoe, New York. Dazoxiben was a kind gift from Pfizer Inc., Groton, Conn.

Statistics

C5a-induced alterations were compared with baseline values obtained immediately prior to intervention by paired analysis. Furthermore, the influence of various drugs on C5a-induced alterations were analyzed in that same animal and thus avoided interanimal variations. For calculation of statistical significance, the Student paired (or unpaired when applicable) t test was used, a P value < 0.05 being considered significant. Data in text, tables and figures are given as means ± standard error (SE).

Results

Control Response to C5a

As shown in Figure 1A (Panel I), two successive (45 minutes apart) intravenous injections of 10 μg porcine C5a into rabbits (the control group) each caused a mean arterial blood pressure drop of 41% and 33%, respectively (not statistically different from one another). The hypotensive response occurred after a latent period of 15–30 seconds, and MAP returned to control levels after 5–10 minutes. In parallel with the hypotension, there was a sharp increase in plasma levels of both 6-keto-$PGF_{1\alpha}$ (426% and 365%, respectively) and TxB_2 (629% and 384%, respectively), and to a lesser extent of PGE_2 (162% and 137%, respectively) (Figure 1B, Panel I). Elevations in plasma prostanoid levels following successive C5a injections were not statistically different from one another.

As shown in Figure 1C (panel I), C5a also caused a fall in peripheral white blood cell count. PMNs were almost completely removed from the peripheral blood (−97% and −98%, respectively) after each C5a injection. The lymphocyte count dropped 55% and 44%, respectively. There was no statistically significant change in either hematocrit or platelet count following C5a challenge (Figure 2). No change in heart rate or contactility was observed after C5a challenge. The latter was determined in 3 animals where the left ventricle pressure was monitored. Panel II of Figure 1 shows the variables measured in nontreated rabbits given saline instead of C5a.

In 5 animals the CVP was also recorded. In parallel with the arterial hypotension that followed C5a challenge (−62%), a simultaneous increase in CVP of 314% was observed. This increase in CVP was reversible and of the same duration as the arterial hypotension (Figure 3).

Effect of Cyclooxygenase Inhibition on the C5a Response

Treatment of animals with the cyclooxygenase inhibitor indomethacin abolished the increase of 6-keto-$PGF_{1\alpha}$, TxB_2, and PGE_2 as observed after C5a challenge prior to treatment (Figure 4B, Panel I). The C5a-induced hypotension was absent in animals treated with indomethacin (Figure 4A, Panel I). However, the C5a-induced leukopenia was not affected by this treatment (Figure 4C, Panel I).

Effect of Thromboxane Synthetase Inhibition on the C5a Response

C5a challenge in animals prior to dazoxiben treatment caused a 372% increase in plasma TxB_2 levels, whereas C5a challenge after dazoxiben treatment caused a 90% increase of this prostanoid (Figure 4B, Panel II). Due to large variations between the data points, plasma TxB_2 levels before and after treatment were not statistically different from one another immediately after C5a challenge (15–30 seconds). After 30 minutes, however, the TxB_2 levels were signifi-

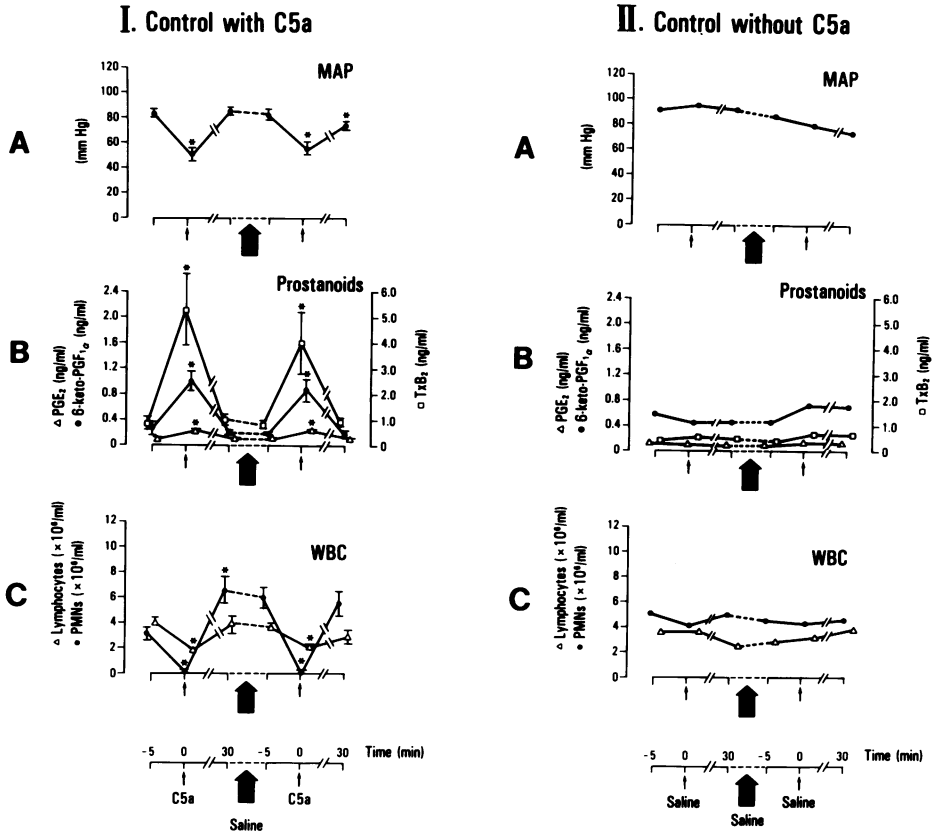

Figure 1—Panel I shows the MAP (**A**), the 6-keto-$PGF_{1\alpha}$, TxB_2, and PGE_2 plasma levels (**B**), and the WBC, ie, PMN and lymphocyte, counts in peripheral blood (**C**). Measurements were made prior to (−5 minutes), immediately after (15–30 seconds), and 30 minutes after each of two consecutive intravenous injections of 10 μg porcine C5a (*small arrows*). The second C5a injection was performed approximately 45 minutes after the first one, and 15 minutes after the rabbits had been treated with 500 μl of saline intravenously (*thick arrow*). Values are given as the mean ± SE of 8 animals. *Asterisks* indicate significant differences, compared with the respective initial values (−5-minute values). Panel II shows the same variables as Panel I (mean for 2 rabbits) where the C5a was replaced by an equivalent volume (100 μl) of saline (*small arrows*). Otherwise, these animals were treated as the control group shown in Panel I.

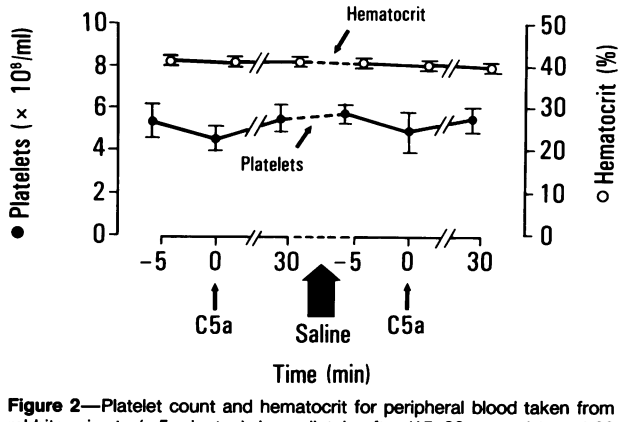

Figure 2—Platelet count and hematocrit for peripheral blood taken from rabbits prior to (−5 minutes), immediately after (15–30 seconds), and 30 minutes after each of two consecutive intravenous injections of 10 μg porcine C5a (*small arrows*). The second C5a injection was performed approximately 45 minutes after the first one and 15 minutes after the rabbits had been treated with 500 μl saline intravenously (*thick arrow*). Values are given as mean ± SE of 8 animals.

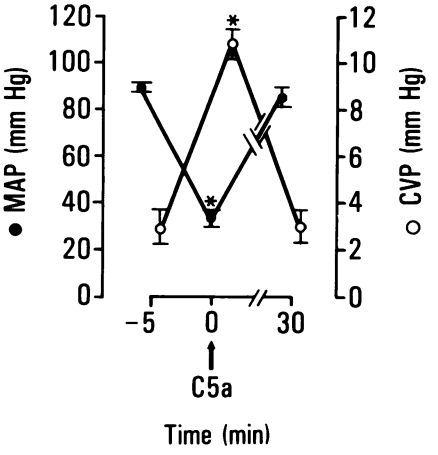

Figure 3—MAP and CVP in rabbits prior to (−5 minutes), immediately after (15–30 seconds), and 30 minutes after an intravenous injection of 10 μg porcine C5a (*small arrow*). Values are given as the mean ± SE of 5 animals. *Asterisks* indicate significant differences, compared with the initial values (−5 minute values).

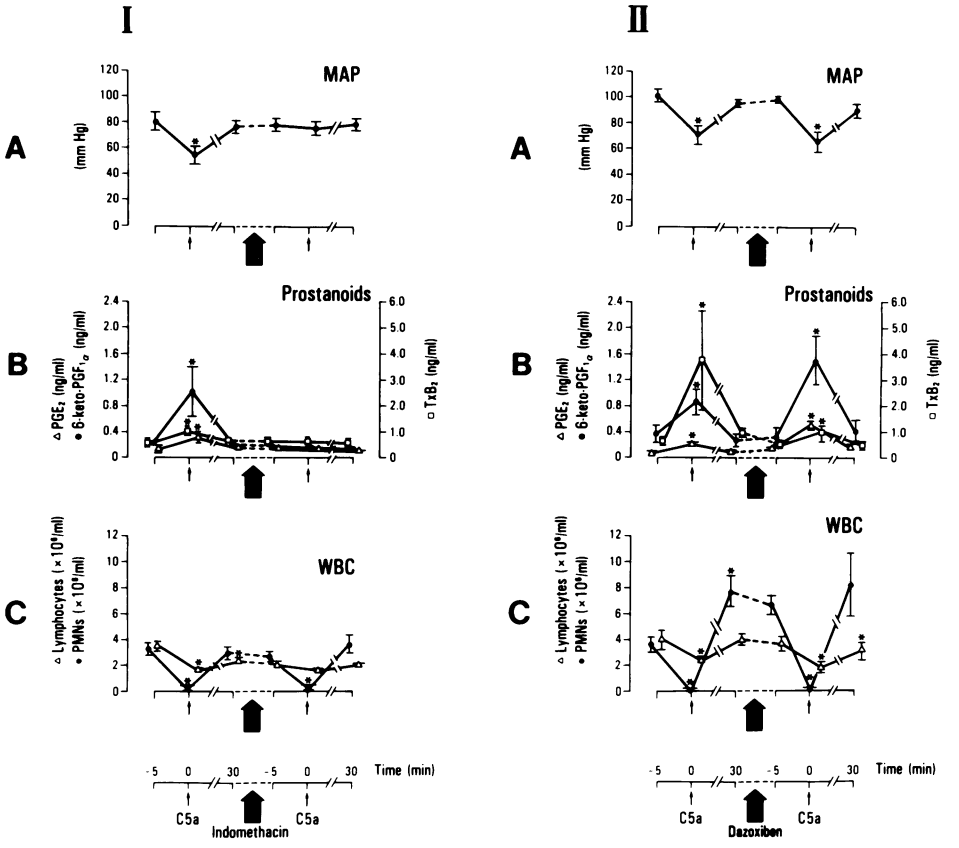

Figure 4—Changes in the MAP (**A**), the 6-keto-$PGF_{1\alpha}$, TxB_2, and PGE_2 plasma levels (**B**), and the WBC, ie, PMN and lymphocyte, counts in peripheral blood (**C**) in rabbits treated with indomethacin (Panel I) or dazoxiben (Panel II). Measurements were made prior to (−5 minutes), immediately after (15–30 seconds), and 30 minutes after each of two consecutive intravenous injections of 10 μg porcine C5a (*small arrows*). The second C5a injection was performed approximately 45 minutes after the first one and 15 minutes after the rabbits had been treated with indomethacin (5 mg/kg) (Panel I) or dazoxiben (25 mg/kg) (Panel II) intravenously (*thick arrows*). Values are given as mean ± SE of 6 animals (indomethacin group) and 5 animals (dazoxiben group), respectively. *Asterisks* indicate significant differences, compared with the respective initial values (−5-minute values).

cantly reduced as compared with the same time point before dazoxiben treatment, and 6-keto-$PGF_{1\alpha}$ and PGE_2 plasma levels were also elevated. However, effects on neither the C5a-induced hypotensive response nor leukopenia were observed (Figure 4A and C, Panel II). The MAP and CVP in 4 animals pretreated with dazoxiben dropped 39% ± 2% and 46% ± 12% from their initial baseline values, respectively, following C5a challenge.

Effect of Histamine Antagonists on the C5a Response

After treatment with the H_1-receptor antagonist pyrilamine and H_2-receptor antagonist cimetidine, C5a still caused systemic arterial hypotension, increased plasma prostanoid levels and induced leukopenia. However, the hypotensive response caused by C5a after cimetidine treatment was significantly smaller, 22%, as compared with a 32% drop in MAP after the first C5a injection (Figure 5A). Furthermore, increases in plasma prostanoid levels following C5a injection with cimetidine present were statistically less than prostanoid level increases following C5a without cimetidine treatment (Figure 5B).

Effect of Lipoxygenase Inhibition on the C5a Response

The lipoxygenase inhibitor REV 5901 was ineffective in altering the C5a-induced hypotension, increase in plasma prostanoid levels, or leukopenia in rabbits. A 25% increase in the plasma LTB_4 level, as assessed by RIA after extraction,[27] was observed in 3 rabbits after a 10-μg injection of porcine C5a. This LTB_4 release by C5a was not observed in REV 5901-treated rabbits (data not shown).

Effect of Neutropenia on the C5a Response

A single dose of nitrogen mustard resulted 3 days later in a peripheral blood PMN count of 0.20 ±

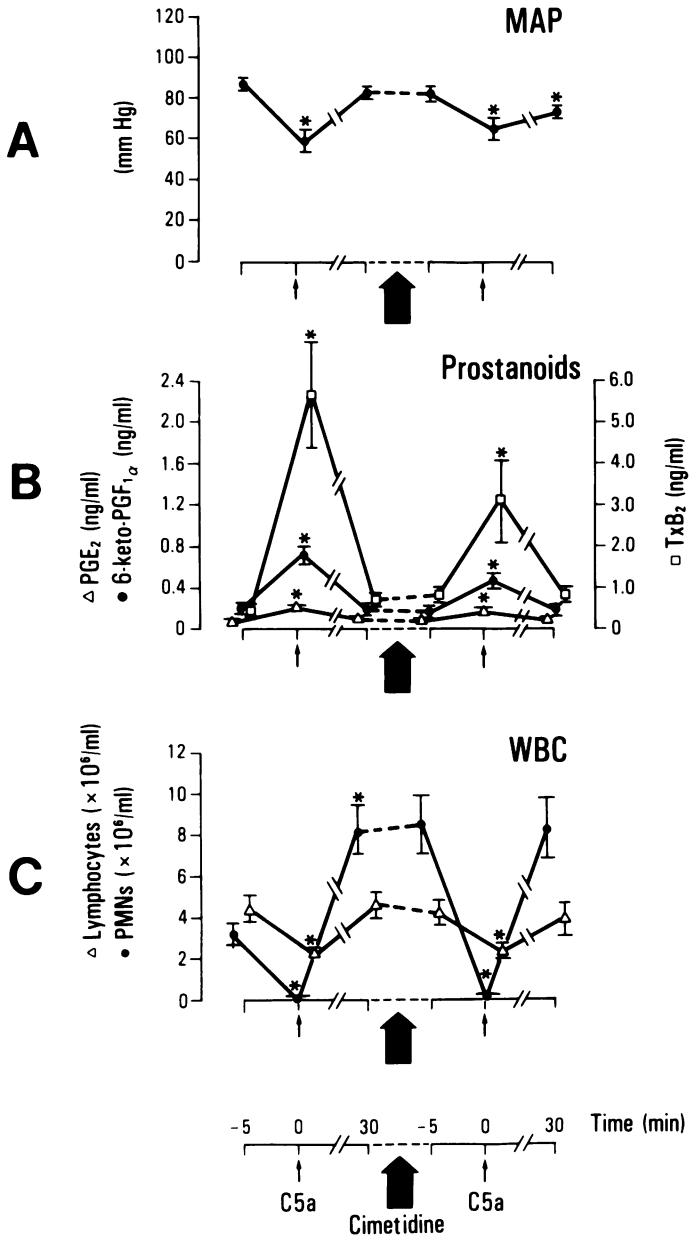

Figure 5—Changes in the MAP (**A**), the 6-keto-$PGF_{1\alpha}$, TxB_2, and PGE_2 plasma levels (**B**), and the WBC, ie, PMN and lymphocyte counts, in peripheral blood (**C**). Measurements were made prior to (−5 minutes), immediately after (15–30 seconds), and 30 minutes after each of two consecutive intravenous injections of 10 μg porcine C5a (*small arrows*). The second C5a injection was performed approximately 45 minutes after the first one and 15 minutes after the rabbits had been treated with cimetidine (10 mg/kg) intravenously (*thick arrow*). Values are given as mean ± SE of 6 animals. *Asterisks* indicate a significant differences compared to the respective initial values (−5-minute values).

0.08×10^6/ml and a lymphocyte count of $1.27 \pm 0.16 \times 10^6$/ml. When compared with the control group, these white blood cell levels represent a 94% reduction in PMNs and a 68% reduction in lymphocyte count. No change in platelet levels were observed. This neutropenia had no impact on C5a-induced hypotension or increase in plasma prostanoid levels, which were similar to the values of the control group (Figure 6). A statistically significant drop in peripheral blood platelet count of 15% was observed after C5a challenge in this group.

Pulmonary Water Content

The wet weight/dry weight of lungs from animals in the control group that received a total of 20 μg of C5a was 5.22 ± 0.19. The wet weight/dry weight of lungs from the two control non-C5a-challenged rabbits were 4.84 and 5.45, respectively, and lungs from normal rabbits possessed a wet weight/dry weight of 5.12 ± 0.09 (n = 4). No statistically significant difference in the pulmonary water content was observed between C5a-challenged and non-C5a-challenged an-

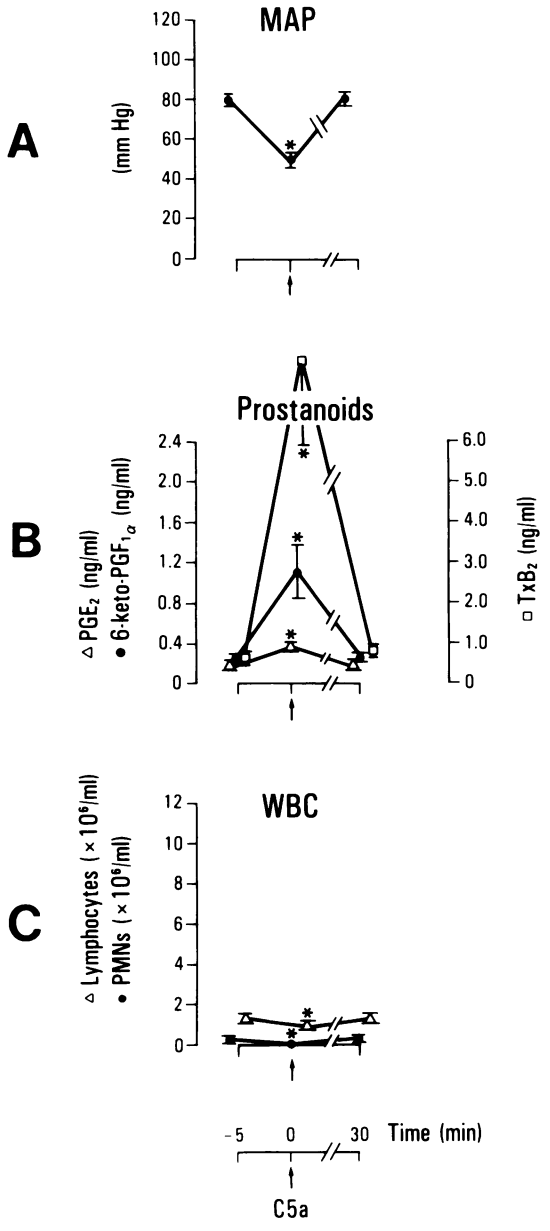

Figure 6—Changes in the MAP (**A**), the 6-keto-$PGF_{1\alpha}$, TxB_2, and PGE_2 plasma levels (**B**), and the WBC, ie, PMN and lymphocyte counts, in peripheral blood (**C**) in neutropenic rabbits. Measurements were made prior to (−5 minutes), immediately after (15–30 seconds), and 30 minutes after an intravenous injection of 10 μg porcine C5a (*arrow*). These rabbits had been rendered neutropenic with a single dose of nitrogen mustard 3 days earlier. Values are given as mean ± SE of 6 animals. *Asterisks* indicate significant differences, compared with the initial value (−5-minute value).

imals. Furthermore, the various treatments used in this study did not statistically affect the pulmonary water content (Student unpaired *t* test).

Effect of C5a on Cardiac Output (CO), Regional Blood Flow (RBF), and Vascular Resistance

In Table 1 MAP is given for the 3 groups used in the microsphere experiments, before and after BSA (control) or C5a injection, respectively. C5a caused a drop in MAP of 34% in the nontreated animals and 44% in the dazoxiben-treated rabbits, whereas injection of BSA caused no change in MAP.

Table 1—Mean Arterial Pressure Before and After BSA or C5a Injection.

	Mean arterial pressure	
	Before (−5 minutes)	After (15–30 seconds)
BSA (CONTROL) (7)	86.4 ± 3.7	85.4 ± 4.4
C5a (7)	95.4 ± 5.1	63.0 ± 5.8*
C5a + dazoxiben (6)	85.5 ± 5.3	47.5 ± 2.7*

Results are shown for the three groups of animals used in the microsphere experiments: one control group received a BSA injection intravenously; a second group received a C5a injection intravenously; and a third group was pretreated with dazoxiben and received a C5a injection intravenously. The number of animals used is given in parenthesis. Values are given as mean ± SE.

*A significant difference compared with the initial values.

Table 2 shows CO and RBF measurements before and after an intravenous injection of 1% BSA in the control group. No difference was observed between the 2 measurements. However, C5a caused a 25% ± 9% drop in CO as compared with the initial value, and RBFs in skin and liver were also reduced by 42% ± 12% and 25% ± 9%, respectively. In dazoxiben-treated animals the CO decreased (14% ± 2%), and reduction of the blood flow was observed in skin (−42% ± 9%) and in both cerebral hemispheres (−29% ± 9% and −31% ± 8%).

From the microsphere data and the MAP, vascular

Table 2—Cardiac Output and Regional Blood Flows for Various Organs in the Control Rabbits

	Flow Measurements	
	Before (−5 minutes)	After (20 seconds)
Cardiac output (ml/min/kg)	266.7 ± 24.0	264.6 ± 22.2
Regional blood flow (ml/min/100 g)		
Skin	11.4 ± 2.0	11.0 ± 1.5
Liver	13.7 ± 1.6	12.4 ± 1.4
Stomach	180.0 ± 30.8	184.8 ± 26.5
Duodenum	239.7 ± 54.3	245.1 ± 37.4
Jejunum	201.6 ± 41.7	229.5 ± 53.4
Ileum	175.1 ± 44.2	192.9 ± 65.6
Colon	65.0 ± 9.5	68.0 ± 10.2
Kidney, left	269.0 ± 22.1	298.2 ± 17.2
Kidney, right	266.9 ± 21.9	297.8 ± 18.4
Ileopsoas, left	7.7 ± 1.7	5.5 ± 1.0
Ileopsoas, right	7.9 ± 1.4	5.6 ± 1.2
Heart	418.7 ± 79.8	446.4 ± 84.4
Cerebral hemisphere, left	61.7 ± 5.7	72.4 ± 5.1
Cerebral hemisphere, right	59.8 ± 6.1	68.5 ± 7.0

Measurements are reported before (−5 minutes) and after (20 seconds) 1% BSA given intravenously. Values are given as the mean ± SE of 7 animals.

resistance was calculated. In the control group neither TPR nor any of the organ vascular resistances changed as compared with the initial value (Figure 7A). TPR did not change after C5a injection, but C5a caused a reduction in the vascular resistance of the left and right ileopsoas of 49% and 45%, respectively (Figure 7B). Vasodilation in skeletal muscle after C5a was confirmed in four experiments performed together with Dr. P. Borgstrom, using intravital microscopy of the rabbit tenuissimus muscle according to the method of Lindbom et al.[28] In dazoxiben-treated rabbits, however, C5a caused a 31% decrease in TPR, as well as a 48% reduction in the peripheral resistance of the colon and a 38% and 39% decrease in the left and right kidney, respectively (Figure 7C).

Discussion

In this study, it was found that purified porcine C5a injected intravenously into rabbits induced a reversible systemic arterial hypotensive response. This hypotension was found to parallel and depend on the release of cyclooxygenase products. However, it occurred without involvement of PMNs, despite the severe neutropenia that followed C5a challenge. Furthermore, histamine appears to be involved to some degree in both C5a-induced hypotension and C5a-induced prostanoid release via the H_2-receptor. The major cause of this C5a-induced hypotension appears to be a pulmonary vasoconstriction, possibly TxA_2-mediated, with decreased CO as an immediate consequence.

The hypotensive effect of classical anaphylatoxin C5a des Arg in guinea pigs, cats, and dogs has been previously reported.[8–10] Systemic arterial hypotension is also induced by intravascular complement activation (CVF) in rabbits[6–7] and by ZAP injection in sheep.[16] A similar hypotensive response was confirmed in rabbits in the present study by means of purified porcine C5a and was found to be reversible and nontachyphylactic. The hypotension occurred simultaneous with a 25% drop in CO and a threefold increase in CVP, but without alterations in TPR. Furthermore, no effect on heart rate or contractility was observed. Thus, these results suggest that C5a causes pulmonary vasoconstriction with a decrease in CO and systemic arterial hypotension resulting as a consequence. Pulmonary vasoconstriction has previously been reported to occur in dogs after injection of C5a des Arg,[10] after ZAP injection in sheep[13–16] and

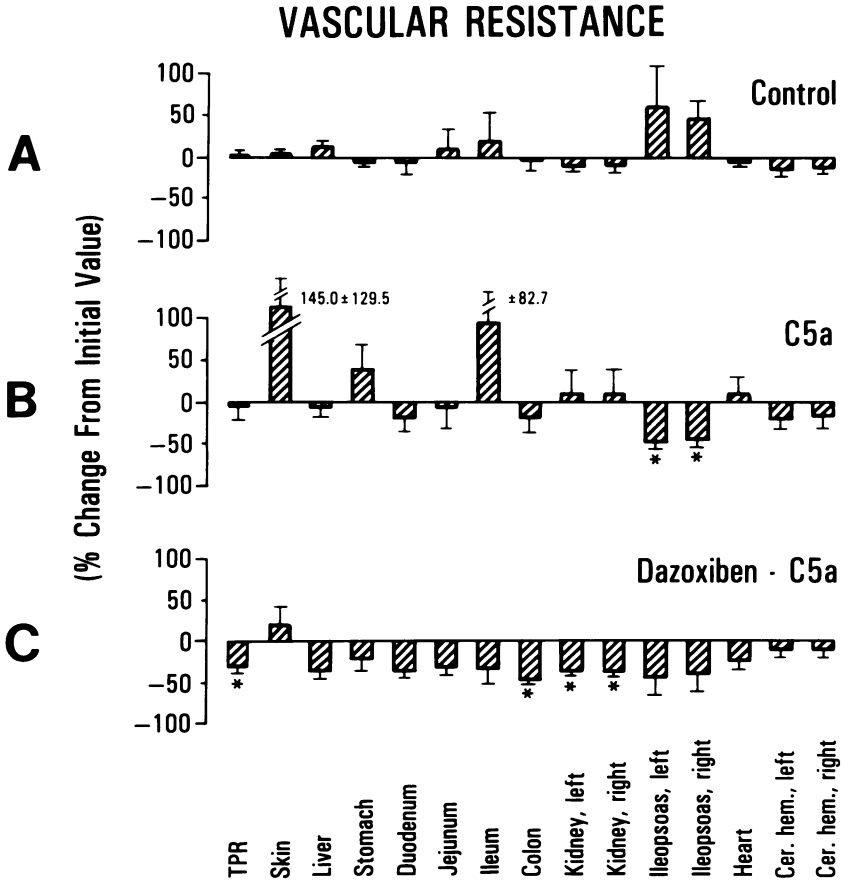

Figure 7—Changes in TPR and organ vascular resistances in rabbits after an intravenous injection of 100 μl saline (n = 7) (**A**); of 10 μg porcine C5a (n = 7) (**B**); and of 10 μg porcine C5a in dazoxyben-treated animals (n = 6) (**C**). Values are given as mean ± SE. *Asterisks* indicate significant changes from the initial value. *Cer.hem.*, cerebral hemisphere.

pigs[17] and in rabbits after intravascular complement activation (CVF).[18]

Purified C5a caused elevation of the prostacyclin (PGI_2) metabolite 6-keto-$PGF_{1\alpha}$, the TxA_2 metabolite TxB_2, and PGE_2 in the circulation. Indomethacin eliminated the observed depression in MAP following C5a injection and prevented prostanoid release, which suggests an important role for prostanoids in C5a-induced hypotension. However, no correlation between prostanoid levels and extent of hypotensive response could be observed in the control group in this study. This might be explained by interanimal variations, eg, an individual animal's ability to compensate for reduced cardiac output and hypotension. However, within the same animal the hypotensive response and the TxB_2 levels seemed to parallel one another; ie, a more severe second C5a-induced hypotension was always associated with a higher level of TxB_2 (as compared with the level following the first C5a injection), and vice versa. Bult et al[7] reported that 6-keto-$PGF_{1\alpha}$ and TxB_2 paralleled the arterial hypotension induced by selective complement activation in rabbits initiated by intravenous injection of CVF. These authors suggested a causal relationship between prostacyclin formation and arterial hypotension due to peripheral vasodilation. We did not observe that 6-keto-$PGE_{1\alpha}$ or PGE_2 levels in our animals paralleled hypotension to the degree that Bult et al[7] reported. Furthermore, we believe that the cause of hypotension is not peripheral vasodilation, but vasoconstriction of selected vessels. Pavek et al[10] reported that two dogs premedicated with indomethacin did not show the usual hypotension after injection of anaphylatoxin, similar to the effects we observed in rabbits.

In our study, dazoxiben reduced C5a-induced TxA_2 release, the most likely prostanoid to cause pulmonary vasoconstriction, but failed to reduce C5a-induced hypotension. However, CO was not decreased to the same extent by C5a when dazoxiben was present. Furthermore, CVP was reduced with dazoxiben present, rather than increased as expected, and the TPR decreased by 31% as compared with the control situation in which C5a failed to affect TPR. Therefore, we conclude that C5a-induced systemic arterial hypotension in untreated and dazoxiben-treated animals may have two different underlying mechanisms: 1) pulmonary vasoconstriction, possibly mediated by thromboxane in untreated animals, and 2) peripheral vasodilation, apparently mediated by vasodilatory prostaglandins in animals given dazoxiben. The pathophysiologic consequences of either mechanism is the same—systemic arterial hypotension.

After C5a challenge in untreated animals, the vasoconstrictory TxA_2 and the vasodilatory prostaglandins in the peripheral circulation appear to be in balance (although in skeletal muscle vasodilatory prostaglandins appear to prevail). However, in pulmonary circulation the TxA_2 vasoconstrictory effect prevails. When this balance is affected by use of the TxA_2 synthetase inhibitor dazoxiben, the vasodilatory PGs prevail, resulting in pheripheral vasodilation. Dazoxiben treatment, while lowering TxA_2, increases the levels of 6-keto-$PGF_{1\alpha}$ and PGE_2 after C5a injection, a condition that might magnify vasodilation. A redirection of endoperoxides into 6-keto-$PGF_{1\alpha}$ by thromboxane synthetase inhibitors like dazoxiben has been suggested by Maguire and Wallis.[29]

Cooper et al[13] reported that ZAP injection in sheep was followed by increased plasma TxB_2 levels and TxB_2 levels paralleled exactly the rise and fall in pulmonary arterial pressure. Indomethacin treatment eliminated both the pulmonary arterial hypertension as well as the increased plasma TxB_2 levels in these animals. The authors concluded that the pulmonary vascular response to autologous ZAP is mediated by stimulation of prostaglandin synthesis. However, C5a-induced thromboxane-dependent pulmonary vasoconstriction is not in agreement with the findings of Gee et al.[15] These authors reported that repeated injections of ZAP into sheep caused a thromboxane-independent pulmonary hypertension.

Activation of the complement system during endotoxin shock is believed to contribute to the hemodynamic changes observed.[30,31] It is well documented that pretreatment with cyclooxygenase inhibitors or selective inhibitors of thromboxane synthetase counteracts the increased synthesis of prostanoids and resultant pulmonary vasoconstriction induced by endotoxin.[32,33]

The H_2-receptor antagonist cimetidine reduced the arterial hypotensive response observed after C5a injection, whereas the H_1-receptor antagonist pyrilamine was without effect. Similarly, Ulevitch and Cochrane[6] reported that the H_2-antagonist burimamide markedly reduced CVF-induced hypotension, whereas H_1-receptor antagonists did not. In our study cimetidine also reduced prostanoid levels that follow C5a challenge, which further supports an important role for these arachidonic acid metabolites in the C5a-induced hypotensive response. We have previously reported that endothelial cells in culture do not respond to C5a. Nor does C5a bind to these cells.[23] However, blood vessel strips *in vitro* have been reported to release prostacyclin in response to C5a.[11,12] This difference in the response to C5a between isolated endothelial cells and blood vessels could be ex-

plained by C5a stimulation of mast cells. Mast cells may release histamine and TxA_2 as well as other mediators as a consequence of C5a activation. Histamine is known to stimulate the release of PGI_2, and to a lesser extent of TxA_2, from endothelial cells.[23] Also, LTC_4 is released by mast cells upon stimulation, and is, like histamine, a potent endothelial cell stimulator.[23] However, lipoxygenase inhibition by REV 5901 had no impact on C5a-induced hemodynamic or hematologic changes in this study. Serotonin and platelet activating factor (PAF; acetyl glyceryl ether phosphorylcholine), are two additional known mediators that can be released from mast cells during activation.

Although C5a induced a complete and reversible neutropenia, the PMN appears not to be involved in the hypotension or release of prostanoids, as judged from experiments using neutropenic animals.

Indomethacin completely blocked the C5a-induced hypotension but did not affect the WBC response. These results support our contention that the hemodynamic changes induced by C5a are PMN-independent. A PMN-independent effect of C5a on circulation is in general agreement with *in vitro* findings by Marceau and Hugli[34] and Hugli and Marceau.[12] They observed both vasoconstrictor and vasodilator responses to C5a in vascular strips in a blood-free environment. Therefore, neutropenia and hemodynamic changes appear to be two independent C5a-induced phenomena.

The lymphocyte count fell following C5a injection and, like the fall in PMNs, was reversible. Less is known about C5a–lymphocyte interaction. However, Bottazzi et al[35] reported that large granular lymphocytes migrated in the Boyden chamber in response to partially purified C5a des Arg. These lymphocytes represent only 2–5% of human peripheral blood mononuclear cells and can therefore not explain the reduced lymphocyte count observed here, provided that the percentage of these large granular lymphocytes is the same in rabbits as it is in man. However, lymphocytes, like PMNs, do not appear to be involved directly in the hemodynamic response to C5a.

None of the experimental groups in this study developed pulmonary edema after C5a injection. This is in agreement with Bodammer and Vogt,[8] who reported that guinea pigs injected with anaphylatoxin showed no signs of lung edema. The role of intravascular complement activation in lung edema and injury is debated, but it appears that complement activation, as an isolated event, fails to cause injury/permeability increases in the lung.[17,36] C5a must be combined with some other factor, like hypoxia, in order to cause lung permeability changes.[18] Furthermore, arachidonic acid has been shown to raise vascular resistance but not permeability in lungs of unanesthetized sheep. This pulmonary hypertension was inhibited by indomethacin, which suggests that increased prostanoid levels *per se* do not alter lung permeability.[37] However, prolonged or repeated infusions of ZAP into the sheep has been reported to cause lung injury that is related to the release of toxic oxygen metabolites.[15] The hematocrit of animals in our study did not change after two C5a injections, which indicates that gross losses of plasma due to permeability changes did not occur.

Purified C5a caused only a minor effect on platelet counts in our animals. This contrasts with many other studies where intravascular complement activation has been studied and a marked, but reversible, thrombocytopenia has been observed. However, as discussed by Ulevitch and Cochrane,[7] the thrombocytopenia that follows intravascular complement activation is possibly attributed to the effect of C3 and C3 degradation products and not C5a.

We chose to use purified porcine C5a in this study because this anaphylatoxin is well characterized and the isolation and purification procedures are well established.[26,38] Furthermore, porcine C5a is a potent spasmogen capable of eliciting a contractile response in guinea pig ileal smooth muscle at concentrations as low as 5×10^{-10} M.[26] In 10 rabbits we also studied the effect of purified human C5a, which produced a reaction very similar to that of porcine C5a, ie, a PMN-independent reversible systemic arterial hypotension, increase in plasma prostanoid levels, and neutropenia. All of these variables were of the same magnitude as observed with porcine C5a (data not shown), and the release of prostanoids, as well as the hypotensive response, was inhibited by indomethacin. In addition, it was found that the hemodynamic and hematologic changes that follow injection of human C5a are observed whether C5a is injected intravenously or intraarterially, and the hypotensive response to C5a was dose-dependent. Further, we observed in preliminary experiments that zymosan-activated rabbit plasma injected intravenously into rabbits elicited changes in the animals similar to those of purified human and porcine C5a (data not shown). Consequently, the source of the anaphylatoxin appears to have little influence in qualitative terms.

In conclusion, this study shows that purified C5a induces hemodynamic and hematologic changes similar to those observed after ICA, ZAP, and endotoxin. It is suggested that C5a stimulates a nonbloodborne cell (eg, the mast cell) to release histamine, TxA_2 and perhaps other mediators (eg, PAF). These mediators in turn stimulate smooth muscle cells directly and/or

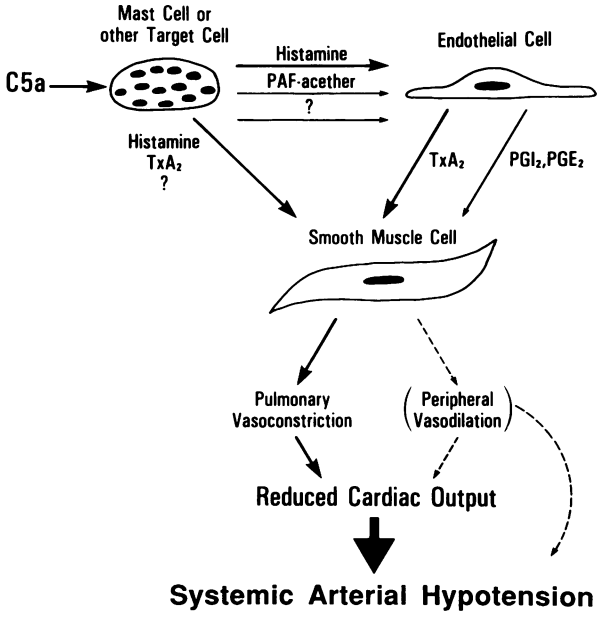

Figure 8—Schematic outline of interactions that are proposed for C5a-induced systemic arterial hypotension *in vivo*. C5a may activate resident tissue cells like the mast cell, which in turn may release numerous mediators (eg, histamine, TxA_2, PAF). These factors are capable of acting on smooth muscle cells directly or indirectly via the endothelial or other cells. Stimulation of endothelial cells may cause the release of smooth muscle contracting substances such as TxA_2 or smooth muscle relaxing factors like PGI_2 and PGE_2. Vasoconstriction is dominant in the lung response to C5a resulting in decreased cardiac output and systemic arterial hypotension.

activate endothelial cells (or other cells in the blood vessel wall) to produce and release prostanoids. The net effect of this prostanoid release on vascular resistance appears to vary in different organs. However, the mechanism for C5a-induced systemic arterial hypotension appears to result from pulmonary vasoconstriction, possibly mediated by thromboxane, followed by decreased cardiac output (see Figure 8). Furthermore, the C5a-induced neutrophil response appears to be unrelated to and independent from the hemodynamic response.

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