Role of circulating blood components and thromboxane in anaphylactic vasoconstriction in isolated canine lungs

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Miyahara, Takashige, Toshishige Shibamoto, Hong-Gang Wang, and Shozo Koyama. Role of circulating blood components and thromboxane in anaphylactic vasoconstriction in isolated canine lungs. J. Appl. Physiol. 83(5): 1508–1516, 1997.—We determined the roles of circulating blood components and chemical mediators in anaphylactic vasoconstriction and microvascular permeability during Ascaris suum antigen-induced anaphylaxis in isolated canine lungs. Either the right or left lower lobe served as the control lung, which was perfused with autologous blood, and the contralateral lobe from the same dog was examined for the effect of albumin Krebs-Henseleit solution (Krebs) or of blockers of various vasoconstrictors with blood perfusion. Pulmonary vasoconstriction occurred after injection of the antigen (15 mg) in both the blood- and Krebs-perfused lungs. However, the percent change of peak pulmonary vascular resistance in the Krebs-perfused lungs tended to be greater than that in the blood-perfused lungs (689.9 ± 289.3 and 389.3 ± 171.9%, respectively). This increased peak pulmonary vascular resistance was attenuated similarly by pretreatment with indomethacin (1.1 × 10⁻⁴ M; cyclooxygenase inhibitor), AA-2414 (10⁻⁵ M; thromboxane-receptor antagonist), or a combination of TCV-309 (10⁻⁵ M; platelet-activating factor-receptor antagonist), diphenhydramine (1.7 × 10⁻⁴ M, histamine H₂-receptor antagonist), and indomethacin but not by pretreatment with TCV-309 or diphenhydramine alone. The filtration coefficient, an index of vascular permeability, did not change significantly at 15 or 60 min after the antigen in all groups. These findings suggest that anaphylactic vasoconstriction in the isolated canine lung is independent of circulating blood components. Thromboxane is the major mediator for the anaphylactic vasoconstriction. Anaphylaxis does not increase pulmonary vascular permeability in isolated canine lungs.

Ascaris suum antigen; filtration coefficient; indomethacin; platelet-activating factor; histamine

PULMONARY ANAPHYLACTIC VASOCONSTRICTION has been induced in isolated perfused sensitized lungs (6, 26, 28). We have reported that an injection of Ascaris suum antigen causes potent pulmonary vasoconstriction in the isolated blood-perfused canine lungs (28). A. suum anaphylaxis is the classic well-established immunoglobulin (Ig) E anaphylactic reaction (22). Various inflammatory mediators, including histamine, platelet-activating factor (PAF), and eicosanoids such as leukotrienes (LTs), thromboxane (Tx) A₂, and prostaglandin D₂, were generated and released from pulmonary tissues or migrating-inflammatory cells in the initiation and propagation of the pulmonary anaphylactic responses (21, 23, 26). Indeed, these chemical mediators induce pulmonary vasoconstriction in dog lungs (24, 30, 31). However, it is not known which mediator is mainly involved in the A. suum-induced anaphylactic pulmonary vasoconstriction.

In the isolated lungs perfused with blood-free perfusate, an antigen challenge induces pulmonary anaphylactic vasoconstriction (6, 26). Enzan et al. (6) showed that pulmonary vasoconstriction was observed after injection of human erythrocytes in the isolated, IgG-immunized rabbit lungs perfused with Krebs-Henseleit solution. Additionally, Selig et al. (26) demonstrated that ovalbumin caused anaphylactic pulmonary hypertension in the isolated, Ringer-perfused, sensitized guinea pig lungs. Mast cells and alveolar macrophages could be potential tissue sources of these inflammatory mediators (7, 9, 25). Granulocytes and platelets also could be important blood-borne sources of various vasoactive substances (7, 19). However, the precise roles of these effector cells in the antigen-induced anaphylaxis still remain to be elucidated. Furthermore, there are no reports that determined whether blood components such as leukocytes, platelets, and complements quantitatively modulate the pulmonary anaphylactic vasoconstriction.

It is known that pulmonary edema can occur in patients with anaphylactic shock (3), although the development of pulmonary edema is not uniformly observed in systemic anaphylaxis. Clinical investigation revealed that this type of pulmonary edema develops within 1 h after exposure to allergens such as hydrochlorothiazide (3). However, it remains unclear whether anaphylaxis-induced pulmonary edema is due to an increase in vascular permeability or to an increase in vascular hydrostatic pressure. Carlson et al. (3) reported that the protein content of edema fluid from a patient with fulminant pulmonary edema during anaphylactic shock was rich compared with that of plasma. On the other hand, in a previous experimental study we (28) failed to detect an increase in pulmonary microvascular permeability during pulmonary anaphylaxis. We previously demonstrated (28) that capillary filtration coefficient (Kf,c), a sensitive indicator of the microvascular permeability, tended to increase, but not significantly, at 10 min after administration of A. suum antigen in the isolated, blood-perfused canine lungs. However, in that previous study, the Kf,c measured at 10 min could not detect conclusively pulmonary microvascular permeability because the lung just before the Kf,c measurement was perfused at zone 2 conditions, in which the pulmonary vascular bed was not fully opened (29). Therefore, Kf,c measurement at zone 3 conditions, in which the pulmonary microvascular vessels are patent, is required to clarify more accurately the change in pulmonary microvascular permeability during anaphylaxis.
of pulmonary microvascular permeability at the early stage of anaphylactic reaction. The first purpose of the present study was to determine whether circulating blood components contribute to anaphylactic vasoconstriction induced by the antigen of A. suum in the isolated canine lungs. Second, we determined the role of histamine, eicosanoids, and PAF in the pulmonary hemodynamic responses by using the corresponding receptor antagonist or enzyme-synthesis inhibitor. Finally, we measured $K_{f,c}$ at zone 3 conditions at the early stage of anaphylactic reaction. It is well known that immunological responses induced by antigen and antibody reaction are highly sensitization dependent. Therefore, in this study, we treated either the right or left lower lobe with agents while the other lobe excised from the same dog served as the paired control lung to eliminate the influence of individual variability of sensitization. The experiments were performed in adherence to the guidelines of both the National Institutes of Health and the Physiological Society of Japan for the use of experimental animals.

METHODS

Isolated Lung Preparation

Thirty-six mongrel dogs (6–15 kg) of either sex were anesthetized with pentobarbital sodium (25 mg/kg iv). They were intubated and mechanically ventilated at a tidal volume of 15–20 ml/kg with room air. The isolated lung preparation and perfusion system have been described previously (28–31). Catheters were placed in the left jugular vein and in the left carotid artery. Ten minutes after treatment with heparin sodium (500 U/kg iv) to avoid coagulation, the dog was bled rapidly through the carotid artery catheter. After left thoracotomy, the left lower lobe and right lower lobe were excised and weighed. Plastic cannulas were secured in the pulmonary artery and vein and the lobar bronchus of each right and left lower lobe. Thereafter, perfusion was begun within 10 min after excision of each lung.

Lung Perfusion

The cannulated lobe was suspended from an electric balance (model LF-600, Murakami Koki) and perfused at constant flow with 200 ml of shed blood or 5% albumin Krebs-Henseleit solution that was pumped from the venous reservoir through a heat exchanger (37°C) into the pulmonary artery. Airway pressure (Paw) was maintained at a constant level of 3 cmH$_2$O. The pump speed and the height of the venous reservoir could be adjusted to maintain the pulmonary arterial and venous pressures at any steady level. The perfused blood or Krebs solution was oxygenated in the venous reservoir by continuous bubbling with 95% O$_2$-5% CO$_2$.

Ppa and Ppv were measured by using pressure transducers that were placed on the reference points at the level of the hilum of the lung. Blood flow (Q) was measured with an electromagnetic flowmeter (model MFV 1200, Nihon Koden, Tokyo, Japan), and the flow probe was positioned in the venous outflow line. Lung weight (W) was continuously monitored and displayed on the physiograph. Ppa and Ppv were initially adjusted to a level within the normal perfusion range in zone 3 conditions (Ppa > Ppv > Paw) and to obtain an isogravimetric state (no weight gain or loss).

Measurements of Pulmonary Vascular Permeability

$K_{f,c}$: The $K_{f,c}$ was used as an index of microvascular permeability (5) and assessed by simultaneously increasing Ppa and Ppv by 6–8 mmHg from an isogravimetric state and observing the increase in W. The sudden increase in vascular pressure caused a rapid weight gain of the lobe due to an increase in perfusate volume of the lung. This was followed by a gradual and prolonged weight gain that was attributed to transcapillary fluid filtration (5). The weight gain rate ($\Delta W/\Delta t$) at each minute after the increase in pressure ($t = 0$) was plotted as a semilogarithmic function with time, and the slow phase of the weight transient was extrapolated to time 0. When the lung was not isogravimetric but was gaining weight, this extrapolated rate of weight gain ($\Delta W/\Delta t_0 = 0$) was subtracted from the weight gain rate 1 min just before $K_{f,c}$ determination. ($\Delta W/\Delta t_0 = 0$) was then divided by the increase in pulmonary capillary pressure ($\Delta Pc$). $P_c$ was measured before and after the increase in vascular pressure by using the double vascular occlusion technique (37). $K_{f,c}$ was normalized to the initial W of 100 g to yield $K_{f,c}$ (in ml·min$^{-1}$·cmH$_2$O$^{-1}$·100 g wet lung wt$^{-1}$).

$$K_{f,c} = \frac{\Delta W}{\Delta t_0 = 0}{\Delta Pc}.$$  

The total vascular (RT), arterial (Ra), and venous (Rv) resistances were calculated as follows:

$$R_T = (Ppa - Ppv)/Q$$  

$$Ra = (Ppa - P_c)/Q$$  

$$Rv = (P_c - Ppv)/Q$$

Experimental Protocol

Hemodynamic parameters of the perfused lung were observed for at least 20 min after the start of perfusion to reach an isogravimetric state at Ppa of 10–19 mmHg, Ppv of 2.5–4 mmHg, and a flow >0.7 l·min$^{-1}$·100 g$^{-1}$. All lobes with a baseline flow of <0.7 l·min$^{-1}$·100 g$^{-1}$ were excluded from the present study.

Role of circulating blood components in anaphylactic response. To investigate the exact role of circulating blood components in pulmonary anaphylactic vasoconstriction, either the right or left lower lobe was perfused with 5% albumin Krebs-Henseleit solution while the other lobe excised from the same dog was perfused with heparinized autologous blood.

ANAPHYLAXIS GROUP WITH AUTOLOGOUS BLOOD (BLOOD GROUP) (n = 7). In these experiments, the lung was perfused with blood, and A. suum antigen (15 mg) (12, 28) was injected into the pulmonary artery.

ANAPHYLAXIS GROUP WITH 5% BOVINE SERUM ALBUMIN KREBS-HENSELEIT SOLUTION (KREBS GROUP) (n = 7). The lung was flushed with Krebs-Henseleit solution (composition in mM: 118 NaCl, 5.9 KCl, 1.2 MgSO$_4$, 2.5 CaCl$_2$, 1.2 Na$_2$HPO$_4$, 25.5 NaHCO$_3$, and 5.6 glucose) until the venous effluent was clear of blood. Then, the closed recirculating system was established with Krebs-Henseleit solution containing 5% bovine serum albumin (Sigma Chemical). In these experiments, the same amount of A. suum antigen (15 mg) as used in the blood group was injected into the pulmonary artery.

In addition, to determine whether there is a difference in the responsiveness to a vasoconstrictor between the lung perfused with blood or 5% Krebs-Henseleit solution, histamine (10 mg) was injected similarly into the right and left
lobe, which was perfused with either 5% albumin Krebs-Henseleit solution or autologous blood in the same manner as described above. We used histamine because it is a vasoconstrictive mediator of anaphylaxis (26, 34).

HISTAMINE GROUP WITH AUTOLOGOUS BLOOD (HISTAMINE BLOOD GROUP) (n = 5). In these experiments, the lung was perfused with blood, and histamine (10 mg) was injected into the pulmonary artery.

HISTAMINE GROUP WITH 5% BOVINE SERUM ALBUMIN KREBS-HENSELEIT SOLUTION (HISTAMINE KREBS GROUP) (n = 5). The lung was flushed with Krebs-Henseleit solution and then perfused with the albumin-Krebs solution in the same manner as in the Krebs group. In these experiments, the same amount of histamine (10 mg) as used in the Histamine Blood group was injected into the pulmonary artery.

Circulating leukocytes were counted with a microcell counter (model F-520, Toa, Kobe, J apan) in the Blood and Krebs groups.

The lungs in the Blood and Krebs groups were prepared for the counting of the numbers of neutrophils by fixation in 10% neutral-buffered Formalin at the end of protocol. Samples were embedded in paraffin, sectioned into 6-µm pieces, and stained with hematoxylin and eosin. The numbers of alveoli and neutrophils were counted at a magnification of ×400, averaging a total 100 alveoli for each specimen.

Role of chemical mediators in anaphylactic response. In these groups, all lobes were perfused with 200 ml of heparinized autologous blood in the closed recirculating system. However, either the right or left lower lobe was pretreated with the receptor antagonists or inhibitor while the other contralateral lower lobe from the same dog was pretreated with the corresponding paired vehicle-treated lung. The effects of each inhibitor or antagonist were evaluated by comparing the response of the agent-treated lung with that of the corresponding paired vehicle-treated lung.

TCV-309 PRETREATED GROUP (TCV-309 GROUP) (n = 4). Ten minutes after an injection of TCV-309 (1.3 mg; [3-bromo-5-[N-phenyl-N-2-[2-(1,2,3,4-tetrahydro-2-isoquinolin-1-ylcarboxyloxy)-ethyl]-carbamoyl]ethyl]carbamoyl]-1-propylpyridinium nitrate), which is a PAF-receptor antagonist (36) and should yield the final concentration of 10 M, A. suum antigen (15 mg) was injected into the pulmonary artery. In the paired control lung, 1-ml saline was injected into the pulmonary artery as a vehicle, followed by the A. suum antigen injection.

DIPHENDYDRAMINE- PRETREATED GROUP (DIPHENDYDRAMINE GROUP) (n = 5). Ten minutes after an injection of diphendydramine-HCl (10 mg), which is a histamine H1-receptor antagonist and should yield a final concentration of 1.7 × 10 M, A. suum antigen (15 mg) was injected into the pulmonary artery. In the paired control lung, 1 ml saline was injected into the pulmonary artery as a vehicle, followed by the A. suum antigen injection.

INDOMETHACIN-PRETREATED GROUP (INDOMETHACIN GROUP) (n = 5). Thirty minutes after an injection of indomethacin (8 mg), which is a cyclooxygenase inhibitor and should yield a final concentration of 1.1 × 10 M, A. suum antigen (15 mg) was injected into the pulmonary artery. In the paired control lung, 2 ml saline and 2 ml 7% NaHCO3 were injected into the pulmonary artery as vehicles, followed by the A. suum antigen injection.

AA-2414-PRETREATED GROUP (AA-2414 GROUP) (n = 5). Ten minutes after an injection of AA-2414 (0.7 mg; [(±)-7-(3,5,6-trimethyl-1,4-benzoquinon-2-yl)-1-phenylheptanoic acid]), which is a selective Tx-receptor antagonist (14) and should yield a final concentration of 10 M, A. suum antigen (15 mg) was injected into the pulmonary artery. In the paired control lung, 2 ml dimethylsulfoxide (DMSO) was injected into the pulmonary artery as a vehicle, followed by the A. suum antigen injection.

COMBINATION OF TCV-309, DIPHENDYDRAMINE, AND INDOMETHACIN GROUP (COMBINATION GROUP) (n = 5). After these three drugs were injected as described above, A. suum antigen (15 mg) was injected into the pulmonary artery. In the paired control lung, 2 ml saline and 2 ml 7% NaHCO3 were injected into the pulmonary artery as vehicles, followed by the A. suum antigen injection.

The concentration of TCV-309 (10 M) or diphendydramine (1.7 × 10 M) employed in this study completely blocked a submaximal increase in Rm induced by 10 µg PAF or 10 mg histamine (52.4 ± 23.0 or 37.8 ± 7.3 mm Hg·1·min·100 g, respectively) in the isolated canine lungs perfused with autologous blood in our preliminary studies. The dose of indomethacin or AA-2414 was chosen as previously described (2, 14).

Kf,c was measured at baseline and at 15 and 60 min after the antigen injection in each group. In some lungs, anaphylactic vasoconstriction was so strong that we reduced the perfusate flow rate to 50% of baseline flow rate to prevent excessive edema formation. The reduction of perfusate flow caused a decrease in Ppv below Paw, resulting in induction of zone 2 conditions (Ppa > Paw > Ppv). In such lungs, Kf,c at 15 min was measured at zone 3 conditions after an increase in Ppv by 3 mm Hg for 3 min by raising the height of the reservoir because Kf,c depends on vascular surface area (29). This last 1-min weight gain was subtracted from the extrapolated weight gain rate for calculation of Kf,c.

Statistical Analysis

All values are expressed as means ± SE. Differences between the agent-treated group and the corresponding vehicle control group were analyzed at each time point by the unpaired t-test. Changes within a group were analyzed by one-way analysis of variance (ANOVA) followed by Fisher’s test. Among the different groups, differences were analyzed by one-way ANOVA followed by Fisher’s test. P values < 0.05 were considered significant.

RESULTS

To prevent excessive edema formation during anaphylactic vasoconstriction, Ppa was kept below 50 mm Hg by reducing the perfusate flow rate. Therefore, the magnitude of pulmonary vasoconstriction was evaluated by the changes in Rm.

Figure 1 shows the time course changes in Rm in both the Blood and Krebs groups. In the Blood group, Rm increased and reached a peak level of 34.5 ± 15.2 mm Hg·1·min·100 g at 2 min after the A. suum
antigen injection from the baseline value of 9.1 ± 0.9 mmHg·l⁻¹·min⁻¹·100 g. Thereafter, Rₜ gradually decreased toward the baseline level. In the Krebs group, Rₜ increased from the baseline value of 4.2 ± 0.4 to 32.6 ± 16.2 mmHg·l⁻¹·min⁻¹·100 g at 2 min after the antigen injection. The peak Rₜ in the Krebs group was not significantly different from that in the Blood group. However, the baseline Rₜ in the Krebs group was significantly smaller than that in the Blood group. The low viscosity of 5% albumin-Krebs solution compared with blood accounts for the smaller Rₜ in the Krebs group during the baseline period. Therefore, the percent change of the peak value of Rₜ in the Krebs group, 689.9 ± 289.3% of baseline, tended to be greater than that in the Blood group, 389.3 ± 171.9%. However, this difference did not reach statistical significance because of much variability.

Figure 2 shows changes in Ra and Rv in the Blood and Krebs groups. In both groups, Ra did not change significantly throughout the experimental period. In contrast, Rv was increased significantly from 4.0 ± 0.3 to 27.7 ± 14.4 mmHg·l⁻¹·min⁻¹·100 g in the Blood group and from 1.6 ± 0.1 to 27.2 ± 14.8 mmHg·l⁻¹·min⁻¹·100 g in the Krebs group at 2 min after the antigen injection.

Figure 3 shows the time course changes in Rₜ in the Indomethacin and receptor-antagonist groups. In the TCV-309 and Diphenhydramine groups, Rₜ increased significantly at 5 min after the antigen injection and then gradually decreased toward the baseline level. The peak Rₜ of the TCV-309 or Diphenhydramine group was similar to that of the corresponding paired control lung. In contrast, the increase in Rₜ in response to the antigen was significantly inhibited by indomethacin or AA-2414. Similarly, the combination of TCV-309, diphenhydramine, and indomethacin significantly attenuated the increased Rₜ compared with that of the paired control lung.

Figure 4 summarizes the effects of blockers on pulmonary vasoconstriction in all blocker groups; the peak level of Rₜ in each blocker group was expressed as a percentage of that in the paired control group. Treatment with TCV-309 and diphenhydramine did not affect the vasoconstriction induced by A. suum antigen. Indomethacin, AA-2414, and combination of TCV-309, diphenhydramine, and indomethacin significantly attenuated the peak levels of Rₜ that were observed in the corresponding control groups (51.6 ± 7.7, 36.8 ± 4.5, and 34.6 ± 9.0%, respectively). There were no significant differences among these three groups.
Figure 5 shows the time course changes in $K_{fc}$ in all groups except the Histamine Blood and Histamine Krebs groups. There were no significant differences in the baseline $K_{fc}$ among any groups studied. In the Blood group, $K_{fc}$ at 15 min after the antigen ($0.204 \pm 0.038 \text{ ml} \cdot \text{min}^{-1} \cdot \text{cmH}_2\text{O}^{-1} \cdot 100 \text{ g}^{-1}$) was not significantly different from that of the baseline value ($0.174 \pm 0.029 \text{ ml} \cdot \text{min}^{-1} \cdot \text{cmH}_2\text{O}^{-1} \cdot 100 \text{ g}^{-1}$). In addition, there were no significant changes in the $K_{fc}$ measured at 15 or 60 min after the antigen injection in all groups studied, although $K_{fc}$ in the Krebs group tended to be greater, but not significantly so, than that of any other blood-perfused groups.

**DISCUSSION**

In the present study, we determined the roles of circulating blood components and various vasoconstrictors in the pulmonary vascular responses to anaphylaxis induced by *A. suum* antigen by using isolated canine lungs perfused at a constant flow. Either the right or left lower lobe served as the control, and the contralateral lower lobe excised from the same dog was used as the experimental one. We have obtained two major findings in the present study. The first finding was that anaphylactic pulmonary vasoconstriction, characterized by predominant venoconstriction, was definitely observed in 5% albumin Krebs-Henseleit solution-perfused lung, in which circulating blood components were washed out. In addition, the increased peak $R_T$ and $R_v$ in response to the antigen in the Krebs group were identical to those in the Blood group. These findings suggest that components of circulating blood, such as leukocytes, platelets, and complements, are not essential for the pulmonary anaphylactic vasoconstriction in the classic well-established IgE anaphylactic reaction of *A. suum* anaphylaxis (22).

The anaphylactic vasoconstriction tended to be greater in magnitude when the lung lobes were perfused with 5% albumin-Krebs solution than when the lobes were perfused with blood, although the peak absolute values of $R_T$ and $R_v$ were identical in both the Blood and Krebs groups. The $R_T$ increased 3.9-fold in the Blood group, whereas it increased 6.9-fold in the Krebs group. The reason for the tendency of this
greater response in the Krebs group is not known. Several explanations could be provided. First, chemical mediators, probably TxA2, might have been released in greater amount in the Krebs group than in the Blood group. In this respect, one possibility was that if some A. suum antigens had been nonspecifically bound to blood components such as large proteins of globulins and circulating cells in the perfusing blood, a smaller amount of the antigen might have reached the effector cells in the Blood group than in the Krebs group. Another possibility may be related to the IgG4 antibody, which was reported to inhibit the anaphylactic reaction in humans (35). It was reported that the IgG4 antibody could inhibit competitively the binding of IgE to mast cells or act as a blocking antibody, preventing antigen from reaching the cell-bound IgE in humans (35). Therefore, IgG4, if present, in the circulating blood might have exerted blocking effects during anaphylaxis in the Blood group (16). However, it is not known whether this interaction between IgG4 and IgE on mast cells exists in dogs (18). Second, the responsiveness to chemical mediators might be greater in the Krebs group than that in the Blood group. However, this possibility is unlikely because, in the present study, histamine caused vasoconstriction to the same degree in the lungs perfused with blood or Krebs. Finally, in addition to endothelial cells, the circulating blood components, such as neutrophils and/or platelets, might have released vasodilators of prostacyclin (21) and/or nitric oxide (NO) (20) in response to anaphylaxis. The vasodilators released in greater amount in the Blood group might have attenuated the vasoconstriction in a greater magnitude than in the Krebs group, in which few blood components except the small amount of marginalized neutrophils were found (20, 21). However, we found that a NO synthase inhibitor of N6-nitro-L-arginine methyl ester (10^{-3} M) did not augment the anaphylactic vasoconstriction in the isolated blood-perfused canine lungs (data not shown). This suggests that NO does not affect the anaphylactic vasoconstriction in the present study. However, further examinations are required in the future.

The total leukocyte counts in the perfusing blood did not change at the early stage of anaphylactic reaction in the present study. This finding contrasts with the anaphylactic response found in in vivo experiments in sheep (21) and rabbits (15), in which transient leukocytopenia occurred during anaphylactic responses. The discrepancy between the present study and the latter two studies might be ascribed to heparin, which was used in the present study, but not in the latter in vivo studies. Actually, heparin has been recently shown to inhibit adhesion of leukocytes in the endothelium by preventing neutrophil carbohydrate ligands from binding to P-selectin, an adhesion molecule expressed on endothelial cells (17).

The second main finding of this study was that pulmonary vasoconstriction induced by A. suum antigen was significantly attenuated by indomethacin, a cyclooxygenase inhibitor, or AA-2414, a Tx-receptor antagonist, but not by TCV-309, a PAF-receptor antagonist, or diphenhydramine, a histamine H1-receptor antagonist. These findings strongly suggest that TxA2 plays a major role in the A. suum-induced pulmonary vasoconstriction. Indeed, in isolated canine lungs, STA2, a stable TxA2 analog, induces pulmonary vasoconstriction but not increased vascular permeability (30), which is the characteristic response of canine pulmonary anaphylaxis (28). In addition, the blood or perfusate concentrations of TxB2, the metabolite of TxA2, were reported to be elevated during systemic and pulmonary anaphylaxis (21, 26). Morel et al. (21) demonstrated that TxA2 was the principal mediator of anaphylactic pulmonary vasoconstriction, based on the finding that the first cardiovascular manifestation of a profound and transient pulmonary vasoconstriction was coincident with the increase in plasma TxB2 levels in sheep when systemic anaphylaxis was induced by bovine
serum albumin. Moreover, in the isolated IgG-immu-
nized rabbit lungs sensitized with human O-N type
erythrocytes, indomethacin or KT2-962, a Tx-receptor
antagonist, significantly inhibited the increase in pul-
monary arterial pressure after the antigen challenge
(6). Thus the present study has also demonstrated the
crucial role of TxA2 in the classic well-established IgE
anaphylactic reaction of A. suum anaphylaxis in the
isolated canine lungs (22).

The cellular source of TxA2, generated in response to
the antigen challenge in canine lungs is not known in
the present study. One potential cell is the mast cell
(25). Mast cells are found in the submucosa of the
pulmonary bronchi and bronchi in close association
with blood vessels and may influence the pulmonary
vasculature by promoting an inflammatory response.

Immunochemical stimulation of mast cells results in
release of TxA2 (25). On the other hand, Holgate et al.
(9) reported that the macrophage-monocyte series might
contribute to the IgE-dependent TxA2 generation. Hen-
derson (7) also reported that TxA2 is released from
alveolar macrophages, monocytes, and platelets in re-
sonse to pulmonary inflammation. Additionally, in the
present study, small numbers of neutrophils still re-
ained in the lungs perfused with Krebs-Henseleit
solution. This indicates that although the lung was
flushed out with the Krebs solution, the marginated
leukocytes could not be depleted in these lungs. Neutro-
phils could be a source of TxA2 in response to anaphy-
laxis (8, 19). Taken together, mast cells and/or alveolar
macrophages and, possibly, the marginated leukocytes
might release TxA2 in the canine pulmonary anaphy-
laxis of the present study.

There are some reports that indomethacin potenti-
ated the antigen-induced pulmonary hypertension by
increasing LT release in guinea pig lungs (4, 26). Selig
et al. (26) showed that although LTs were not detected
in the venous effluent perfusate after the antigen
challenge alone, indomethacin potentiated ovalbumin-
induced pulmonary hypertension associated with in-
creased perfusate concentrations of lipoxygenase me-
tabolite in the isolated guinea pig lung. In the present
study, however, indomethacin significantly attenuated
antigen-induced pulmonary hypertension. The reasons
for the different results of the previous studies (4, 26)
compared with the present study are not known, but
two possible explanations could be provided. One possi-
bility is that LT-induced pulmonary vasoconstriction
may be mediated by the secondary production of Tx as
seen in sheep lungs (21). Even if LT had been synthe-
sized in the A. suum-treated canine lungs, the sub-
sequent generation of TxB2 might have been inhibited by
indomethacin in the present study. As another possibil-
ity, pulmonary vascular reactivity to LT may be differ-
ent among species. Shapiro et al. (27) showed that in six
anesthetized dogs administered intravenous LTD4, sig-
nificant cardiopulmonary responses occurred in four
dogs but that the remaining two dogs did not respond to
LTD4. This finding supports the idea that canine pulmo-
nary vascular reactivity to LT might be variable and
weaker than that of guinea pig lung.

In the present study, diphenhydramine, a histamine
H1-receptor antagonist, did not attenuate vasoconstric-
tion induced by A. suum antigen. Histamine has been
shown to be released during systemic anaphylaxis in
humans (34) and in animals (13, 21, 32). However,
histamine seems not to play a significant role in
systemic anaphylaxis in dogs (32). Silverman et al. (32)
showed that both histamine H1-receptor and H2-re-
ceptor antagonists were ineffective in altering the
physiological responses observed during A. suum-
induced systemic anaphylactic shock. Furthermore,
Krell (13) demonstrated that antigen-induced broncho-
constriction was not inhibited by histamine H1-receptor
antagonist in the A. suum-hypersensitive dog. These
findings are consistent with the present results. More-
over, in the isolated Krebs-perfused rabbit lungs, histo-
mine H1-receptor antagonist also did not attenuate
pulmonary vasoconstriction (6). In contrast, Selig et al.
(26) showed, by using isolated Ringer-perfused lungs
of guinea pig that were sensitized with ovalbumin, that
the histamine H1-receptor antagonist prevented the
antigen-induced changes in pulmonary hemodynamics,
intra-tracheal pressure, and edemagenic responses. Spe-
cies difference may account for the different results of
the guinea pig study of Selig et al. compared with results
of studies using rabbits (6) and the dogs used in the
present study. Failure to prevent the physiological
changes after the antigen administration in the present
study suggests a minor role for histamine in anaphylac-
tic vasoconstriction in canine lungs.

Concentrations of TxB2 in bronchoalveolar lavage
fluid obtained from canine lungs with aerosolized A.
suum antigen challenge were significantly increased
(11), whereas histamine concentrations in bronchoalve-
olar lavage were variable and not significantly in-
creased (10). In these studies, Kleeberger et al. (10, 11)
demonstrated that the increased airway resistance
assessed by collateral system resistance was signifi-
cantly attenuated by an indomethacin or Tx synthase
inhibitor but not by a histamine H1-receptor antago-
nist. These results indicate that, regardless of different
routes of administration of antigen, TxA2, but not
histamine, is released and may influence the diameters
of both blood vessels and airways after antigen chal-
lenge in canine lungs.

PAF, a phospholipid mediator synthesized in various
cell types such as alveolar macrophages and mast cells,
having implicated in human asthma (33) and experi-
mental anaphylactic animal models (15, 26). Pretreat-
ment with TCV-309, a PAF receptor antagonist, did not
reduce antigen-induced pulmonary vasoconstriction in
the present study, suggesting that PAF may not be
involved in pulmonary anaphylactic vasoconstriction in
canine lungs. One possible explanation for the absence
of PAF, such as diphenhydramine or TCV-309 on vaso-
constriction was that more than one mediator is released
at supramaximal concentrations, and thus inhibition of
a single mediator has no effect on the magnitude of the
constriction. For instance, even if the response to
histamine or PAF is completely blocked, the response to
Pulmonary microvascular permeability assessed by $K_{f,c}$ did not change significantly at 15 or 60 min after A. suum antigen administration in the present study. We (28) previously reported that any changes in pulmonary microvascular permeability did not occur within 3 h after anaphylaxis induced in the same manner as in the present study. However, in that previous study (28), the $K_{f,c}$ measured at 10 min after the antigen injection might underestimate the permeability change, because the lung just before the $K_{f,c}$ measurement was perfused under zone 2 conditions (29). One of the purposes of the present study was to reexamine the changes in pulmonary microvascular permeability at the early stage of pulmonary anaphylaxis by keeping the lung perfusion at zone 3 conditions. Actually, in the present study, anaphylactic vasoconstriction in eight lobes was so strong that we inevitably reduced increased Ppa by decreasing the perfusate flow rate, which resulted in perfusion at zone 2 conditions. In such lungs, 3 min before the 15-min $K_{f,c}$ measurement, we made zone 3 conditions by raising the reservoir a little, and then we measured $K_{f,c}$. These accurate $K_{f,c}$ measurement procedures definitely revealed that microvascular permeability does not increase as early as 15 min after the antigen challenge. Thus we conclude that pulmonary anaphylaxis induced by A. suum antigen does not increase pulmonary microvascular permeability in canine lungs.

In summary, pulmonary anaphylactic vasoconstriction induced by A. suum antigen in isolated canine lung is independent of circulating blood components. Presumably, perivascular mast cells, alveolar macrophages, and/or margined leukocytes may be involved in this reaction. Tx rather than PAF or histamine is a major mediator for the pulmonary vasoconstrictive response. It should be noted that the characteristics of antigen-induced mediator release are highly sensitization dependent. Therefore, the bilaterally prepared isolated lung lobes excised from the same dog in this study appear to be a useful model for exploring the relationship between pathophysiological mechanisms, pharmacological modulation, and patterns of mediator release during pulmonary anaphylaxis.

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