研究発表リスト

英文原著

1. Takai D, Nagase T, Shimizu T. New therapeutic key for cystic fibrosis: a role for lipoxins. **Nature Immunol** 2004; 5: 357-8. (News and Views) (論文解説)
2. Nagase T, Uozumi N, Aoki-Nagase T, Terawaki K, Ishii S, Tomita T, Yamamoto H, Hashizume K, Ouchi Y, Shimizu T. A potent inhibitor of cytosolic phospholipase A_2, arachidonyl trifluoromethyl ketone, attenuates LPS-induced lung injury in mice. **Am J Physiol Lung Cell Mol Physiol** 2003; 284: L720-L726.
3. Ohga E, Tomita T, Wada H, Yamamoto H, Nagase T, Ouchi Y. The effects of obstructive sleep apnea on circulating ICAM-1, IL-8 and MCP-1. **J Appl Physiol** 2003; 94: 179-184.
4. Aoki-Nagase T, Nagase T, Oh-hashi Y, Shindo T, Kurihara Y, Yamaguchi Y, Yamamoto H, Tomita T, Ohga E, Nagai R, Kurihara H, Ouchi Y. Attenuation of antigen-induced airway hyperresponsiveness in CGRP-deficient mice. **Am J Physiol Lung Cell Mol Physiol** 2002; 283: L963-L970.
5. Yamaguchi Y, Nagase T, Makita R, Fukuhara S, Tomita T, Tominaga T, Kurihara H, Ouchi Y. Identification of multiple novel epididymis-specific beta-defensin isoforms in the humans and mice. **J Immunol** 2002; 169: 2516-2523.
6. Tomita T, Nagase T, Ohga E, Yamaguchi Y, Yoshizumi M, Ouchi Y. Molecular mechanisms underlying human beta-defensin-2 gene expression in a human airway cell line (LC2/ad). **Respirology** 2002; 7: 305-310.
7. Nagase T, Uozumi N, Ishii S, Kita Y, Yamamoto H, Ohga E, Ouchi Y, Shimizu T. A pivotal role of cytosolic phospholipase A_2 in bleomycin-induced pulmonary fibrosis. **Nature Medicine** 2002; 8: 480-484. (新聞各紙誌上にて紹介される)
8. Nagase T, Ishii S, Shindou H, Ouchi Y, Shimizu T. Airway hyperresponsiveness in transgenic mice overexpressing platelet-activating factor receptor is mediated by an atropine sensitive pathway. **Am J Respir Crit Care Med** 2002; 165: 200-205.
9. Imai Y, Shindo T, Maemura K, Sata M, Saito Y, Kurihara Y, Akishita M, Osuga J, Ishibashi S, Tobe K, Morita H, Oh-hashi Y, Suzuki T, Maekawa H, Kangawa K, Minamino N, Yazaki Y, Nagai R, Kurihara H. Resistance to neointimal hyperplasia and fatty streak formation in mice with adrenomedullin overexpression. **Arterioscler Thromb Vasc Biol** 2002; 22: 1310-1315.
10. Kato N, Ikeda K, Nabika T, Morita H, Sugiyama T, Gotoda T, Kurihara H, Kobayashi S, Yazaki Y, Yamori Y. Evaluation of the atrial natriuretic peptide gene in stroke. **Atherosclerosis** 2002; 163: 279-286.
11. Nishimatsu H, Hirata Y, Shindo T, Kurihara H, Kakoki M, Nagata D, Hayakawa H, Satonaka H, Sata M, Tojo A, Suzuki E, Kangawa K, Matsuo H, Kitamura T, Nagai R. Role of endogenous adrenomedullin in the regulation of vascular tone and ischemic renal injury: studies on transgenic/knockout mice of adrenomedullin gene. **Circ Res** 2002; 90:657-663.
12. Shindo T, Kurihara H, Maemura K, Kurihara Y, Ueda O, Suzuki H, Kuwaki T, Ju KH,

Wang Y, Ebihara A, Nishimatsu H, Moriyama N, Fukuda M, Akimoto Y, Hirano H, Morita H, Kumada M, Yazaki Y, Nagai R, Kimura K. Renal damage and salt-dependent hypertension in aged transgenic mice overexpressing endothelin-1. **J Mol Med** 2002; 80:105-116.

13. Soares AC, Pinho VS, Souza DG, Shimizu T, Ishii S, Nicoli JR, Teixeira MM. Role of the platelet-activating factor (PAF) receptor during pulmonary infection with gram negative bacteria. **Brit J Pharmacol** 2002;137: 621-628.
14. Ishii S, Nagase T, Shimizu T. Platelet-activating factor receptor. **Prostaglandins Other Lipid Mediat** 2002; 68: 599-609.
15. Stromgaard K, Saito DR, Shindou H, Ishii S, Shimizu T, Nakanishi K. Ginkgolide derivatives for photolabeling studies: preparation and pharmacological evaluation. **J Med Chem** 2002; 45: 4038-4046.
16. Barbuti A, Ishii S, Shimizu T, Robinson RB, Feinmark SJ. Block of the background K^+ channel, TASK-1, contributes to the arrhythmogenic effects of platelet-activating factor. **Am J Physiol Heart Circ Physiol** 2002; 282: H2024-H2030.
17. Ogasawara H, Ishii S, Yokomizo T, Kakinuma T, Komine M, Tamaki K, Shimizu T, Izumi T. Characterization of mouse cysteinyl leukotriene receptors, mCysLT1 and mCysLT2: Differential pharamacological properties and tissue distribution. **J Biol Chem** 2002; 277: 18763-18768.
18. Klein A, Pinho V, Alessandrini AL, Shimizu T, Ishii S, Teixeira MM. Platelet-activating factor drives eotaxin production in an allergic pleurisy in mice. **Brit J Pharmacol** 2002;135:1213-8.
19. Ohshima N, Ishii S, Izumi T, Shimizu T. Receptor-dependent metabolism of platelet-activating factor in murine macrophages. **J Biol Chem** 2002; 277: 9722-9727.
20. Honda Z, Ishii S, Shimizu T. Platelet-activating factor receptor. **J Biochem** 2002; 131: 773-779.
21. Weijer S, Leemans JC, Florquin S, Shimizu T, Ishii S, Van der Poll T. Host response of platelet-activating factor receptor-deficient mice during pulmonary tuberculosis. **Immunology** 2003; 109: 552-556.
22. Souza DG, Pinho V, Soares AC, Shimizu T, Ishii S, Teixeira MM. Role of PAF receptors during intestinal ischemia and reperfusion injury. A comparative study between PAF receptor-deficient mice and PAF receptor antagonist treatment. **Brit J Pharmacol** 2003; 139: 733-740.
23. Talvani A, Santana G, Barcelos LS, Ishii S, Shimizu T, Romanha J, Silva JS, Soares MBP, Teixeira MM. Experimental Trypanosoma cruzi infection in platelet-activating factor -deficient mice. **Microb Infect** 2003; 5: 789-796.
24. Tokuoka SM, Ishii S, Kawamura N, Satoh M, Shimada A, Sasaki S, Hirotsune S, Wynshaw-Boris A, Shimizu T. Involvement of Platelet-Activating Factor and LIS1 in Neuronal Migration. **Eur J Neurosci** 2003; 18: 563-570.
25. Noguchi K, Ishii S, Shimizu T. Identification of p2y9/GPR23 as a Novel G Protein-Coupled Receptor for Lysophosphatidic Acid, Structurally Distant from the Edg Family. **J Biol Chem** 2003; 278: 25600-25606.
26. Rijneveld AW, Weijer S, Florquin S, Speelman P, Shimizu T, Ishii S, Van der Poll T.

Improved host defense against pneumococcal pneumonia in platelet-activating factor receptor-deficient mice. **J Infect Dis** 2004; 189: 711-716.

27. Ishii S, Nagase T, Shindou H, Takizawa H, Ouchi Y, Shimizu T. Platelet-activating factor receptor develops airway hyperresponsiveness independently of airway inflammation in a murine asthma model. **J Immunol** 2004; 172: 7095-7102.

和文総説

1. 山本寛, 長瀬隆英. 老年病に合併する感染症の病像と治療のキーポイント. 化学療法の領域 18: 230-234, 2002.
2. 長瀬隆英. 脂質メディエーターと気道炎症. 分子呼吸器病 6: 37-39, 2002.
3. 長瀬隆英. 肺線維症における $cPLA_2$ の役割. 実験医学 20: 1901-1903, 2002.
4. 長瀬隆英. 脂質メディエーターと肺疾患. 呼吸 21: 875-879, 2002.
5. 長瀬隆英. CGRP ノックアウトマウスと気管支喘息. アレルギー科 16: 53-59, 2003.
6. 長瀬隆英. ロイコトリエン研究：最近の進歩. アレルギー 53: 1184-1186, 2004.

New therapeutic key for cystic fibrosis: a role for lipoxins

Daiya Takai, Takahide Nagase & Takao Shimizu

One of the hallmarks of cystic fibrosis is the propensity of patients to develop lung infections with *Pseudomonas aeruginosa*, which eventually compromises lung function. New data suggest loss of CFTR impairs lipoxin production, thus preventing resolution of lung inflammation and creating an environment susceptible to further infection.

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Cystic fibrosis (CF) is an autosomal recessive disorder characterized by progressive lung disease and multiple organ dysfunction caused by abnormal electrolyte transport. CF patients show pancreatic insufficiency, increased sweat electrolyte concentrations and lung pathologies with recurrent bacterial infections. The CF transmembrane conductance regulator (*CFTR*) was identified as the causal gene[1]. Soon after the identification of *CFTR*, the molecule encoded was found to be a cyclic AMP–activated chloride ion transporter[2] whose altered function after *CFTR* mutation [AUTHOR: CORRECT?] can account for the dysfunction of most organs affected in CF. However, this specific molecular defect [AUTHOR: OK? OR WHICH DEFECT?] cannot explain the lung pathology, as CF patients maintain normal chloride ion concentrations in their lungs[3]. No medication can complement the functional loss of CFTR molecule. Several trials of gene therapy to transfer wild-type *CFTR* nucleotide sequences into the airway epithelium have faced many difficulties, resulting in insufficient *CFTR* expression to achieve clinical benefit[4]. In this issue of *Nature Immunology*, Karp *et al.*[5] provide insight into another CF therapeutic approach from the viewpoint of the lipid mediators that regulate inflammatory responses.

CFTR maps to the long arm of chromosome 7, where it spans 250 kilobases (kb), and consists of 27 exons. The gene is transcribed into a 6.5-kb mRNA that encodes a 1,480–amino acid membrane protein. Although the clinical course of the disease varies with the type of mutation in *CFTR* (800 independent mutations have been identified so far), the estimated median survival age of patients of CF was still only 33.4 years in the US in 2001 (ref. 2). More than 70% of CF patients have alleles with deletion of the three base pairs encoding phenylalanine at position 508 (ΔF508)[2] [AUTHORS: PLEASE CLARIFY THE FOLLOWING STATEMENT; ARE YOU DISCUSSING THE MUTATION ON THE OTHER ALLELE IN THESE PATIENTS?] and ones but 22 mutations include ΔF508 are rare, with a frequency of less than 0.1 % of known alleles. In the ΔF508 mutation, mutant CFTR protein is misfolded, leading to rapid ubiquitinylation and protein degradation. Thus, affected cells completely lack CFTR molecules. In some types of mutations (for example, R347P), mutant molecules can reach the cell membrane and show partial chloride conductance. Hence, the clinical manifestations of CF are thought to be related to the type of mutation in *CFTR*.

That CF patients maintain normal chloride concentrations in their lungs indicates another mechanism must exist to explain how the functional loss of CFTR in affected cells results in the pathophysiology leading to progressive lung disease. CF patients are especially prone to chronic airway bacterial infection with pathogens such as *Pseudomonas aeruginosa* and neutrophilic bronchitis and brochiolitis.

To understand the new findings from Karp *et al.*[5], a discussion of how the onset and resolution of inflammation is regulated by lipid mediators is required. A group of eicosonoid lipid mediators known as lipoxins are anti-inflammatory molecules similar to prostaglandin E2 that are synthesized during multicellular responses such as inflammation, atherosclerosis and thrombosis (reviewed in ref. 6). In the airway system, activated epithelial cells generate 15*S*-hydroxyeicosatetraenoic acid (HETE) from arachidonic acid with 15-lipoxygenase and release 15*S*-HETE[7]. Polymorphonuclear neutrophils (PMNs) quickly take up 15*S*-HETE and convert it to 15*S*-epoxytetraene with 5-lipoxygenase. The 15*S*-epoxytetraene is then hydrated into 5*S*,6*R*,15*S*-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid (LXA_4) and its positional isomer 5*S*,14*R*,15*S*-trihydroxy-6,10,12-*trans*-8-*cis*-eicosatetraenoic acid (LXB_4). In addition to these native lipoxins, aspirin (acetylsalicylic acid) triggers the endogenous generation of 15-epimeric lipoxins (15-epi-LXA_4 and 15-epi-LXB_4) by the enzyme cyclooxygenase-2.

LXA_4 and LXB_4 have an inhibitory effect on

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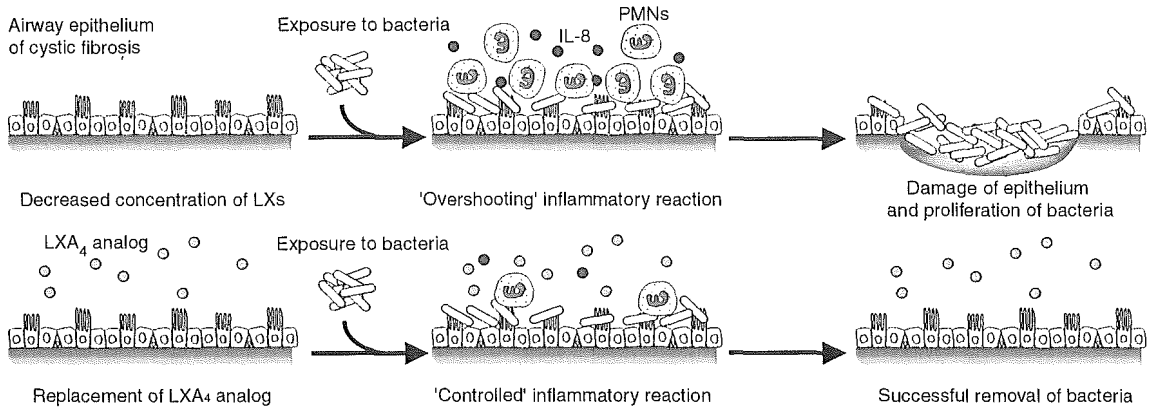

Figure 1 The pathophysiology of CF-affected airway systems and bacterial infection. Top: In the CF airway, because of decreased concentrations of lipoxins, bacterial infection results in an 'overshooting' of inflammation, with excessive numbers of PMNs and interleukin 8 (IL-8), tissue damage and failure to remove bacteria. Bottom: After supplementation with LXA_4 analog, bacterial infection causes 'controlled' inflammation with a moderate number of PMNs, and successful removal of the bacteria results.

PNMs and eosinophils and also activate monocytes and macrophages. Thus, these lipoxins are proposed to be involved in resolving inflammation. LXA_4 and LXB_4 avoid unwanted effects elicited by PMNs such as PMN-dependent vascular injury and further release of chemoattractants after the initial steps of inflammation. The 15-epi-lipoxins have also inhibitory effect on PNMs. The lipoxin receptor was identified as a seven-transmembrane receptor [AUTHOR: PLEASE SPECIFY BY NAME.] that belongs to the G_i-coupled chemoattractant receptor family.

Recently, another class of anti-inflammatory lipid mediators, resolvins, which are produced from ω-3 polyunsaturated fatty acids such as eicosapentaenoic acid or docosahexaenoic acid, was identified[8]. Although the production of resolvins seem to be regulated through the availability of ω-3 polyunsaturated fatty acids, thus using a different mechanism than the tightly regulated production of lipoxins, competition with 'pro-anti-inflammatory' mediators among chemicals sharing structural similarity indicates that inflammatory reaction is highly regulated and balanced by interactions among cells participating in the inflammation process at each stage. A similar well-known example of such balancing interaction occurs between prostacyclin and thromboxane: both are produced from the same precursor but have diametrically opposite functions in platelet activation, vasoconstriction and possibly in immunological reactions[9,10].

The enzyme phospholipase A_2 (PLA_2) is essential in the production of proinflammatory mediators, which also include various eicosanoids. Although many distinct types of PLA_2 have been reported, cytosolic PLA_2 is thought to be particularly important. Cytosolic PLA_2 preferentially hydrolyzes phospholipids containing arachidonic acid and is activated by submicromolar concentration of calcium and by phosphorylation of serine residues. Lung inflammatory diseases, including acute lung injury and pulmonary fibrosis, are attenuated in mice with disruptions in the gene encoding cytosolic PLA_2 (refs. 11,12). Given that lipoxins belong to the 'downstream' mediators of cytosolicPLA_2, these observations may indicate the complexity of the regulation of lipid mediators in the lung. Is the chronic airway infection and inflammation of CF due to disruption of this regulation?

Karp *et al.*[5] found decreased concentrations of LXA_4 in airway fluids of CF patients. In a mouse airway infection model, supplementation with LXA_4 analogs attenuates PMN accumulation in airway and improves resolution of *P. aeruginosa* infections[5], although those [AUTHOR: WHICH IS "those"?] authors used C57BL/6 mice, not *Cftr*$^{-/-}$ mice, which are susceptible to *P. aeruginosa*–induced inflammation. These results together demonstrate a previously unknown pathophysiology in the airway system in CF: because of decreased concentrations of lipoxins, bacterial infection results in 'overshooting' of inflammation, tissue damage and failure to remove bacteria (**Fig. 1**, top). However, after supplementation with LXA_4 analogs, bacterial infection causes inflammation but is well controlled and bacteria are successfully removed [AUTHOR: CORRECT?] (**Fig. 1**, bottom). Karp *et al.* also demonstrated increased lymphocyte recruitment by LXA_4 analogs[5]. This result is consistent with the hypothesis that lipoxins and resolvins make regional inflammation mature from the 'acute' phase to the 'chronic' phase[8]. Ibuprofen, a nonsteroidal anti-inflammatory drug like aspirin, has been used clinically for the management of CF. There was a substantial reduction in the rate of decrease in lung function in children with CF who were treated with high-dose ibuprofen[13], although this drug does not produce 15-epi-LXA_4.

Although lipoxins' antagonistic effects on cysteinyl leukotriene receptors have been clearly demonstrated, a complete view of the anti-inflammatory mechanisms of lipoxins and resolvins has not been achieved. Do they regulate small GTPase proteins, protein kinase A or transcription factor NF-κB in inflammatory cells? Karp *et al.*[5] did not demonstrate such an 'exorbitant' connection of an ion channel to a lipid mediator in this study. However, electrolyte and/or pH imbalance might affect the production, export and uptake of lipid mediators. The catalytic activities of various enzymes (such as leukotriene A4 hydrolase–aminopeptidase, a zinc-containing chloride-sensitive enzyme) might be affected by local electrolyte concentrations. Alternatively, mutant CFTR molecules might directly regulate the expression of 5-lipoxygenase and/or 15-lipoxygenase, which are essential for production of lipoxins. Nevertheless, the present observations and the authors' interpretation of CF-associated lung disease as a deficiency of maturation of regional inflammation might produce new possibilities for therapeutic intervention in this intractable disease. Furthermore, in a more fundamental and broader sense, this study provides a hint for the elucidation of

the molecular 'switch' governing how acute inflammation matures and finally resolves. Clearly, these findings bear clinical relevance for the modulation of lipid mediators in CF-affected airways and for our understanding of the relationship between CFTR deficiency and selective inhibition of anti-inflammatory lipid mediators.

1. Riordan, J.R. *et al. Science* **245**, 1066–1073 (1989).
2. Gibson, R.L., Burns, J.L. & Ramsey, B.W. *Am. J. Respir. Crit. Care Med.* **168**, 918–951 (2003).
3. Verkman, A.S., Song, Y. & Thiagarajah, J.R. *Am. J. Physiol. Cell Physiol.* **284**, C2–15 (2003).
4. Griesenbach, U., Ferrari, S., Geddes, D.M. & Alton, E.W. *Gene Ther.* **9**, 1344–1350 (2002).
5. Karp, C.L. *Nat. Immunol.* 5, '–' (2004). **[ED: PLEASE PROVIDE PAGE NUMBRS (KARP).]**
6. Serhan, C.N. *Prostaglandins Other Lipid Mediat.* **68–69**, 433–455 (2002).
7. Nagase, T. *et al. Biochem. Biophys. Res. Commun.* **168**, 485–489 (1990).
8. Serhan, C.N. *et al. J. Exp. Med.* **196**, 1025–1037 (2002).
9. Samuelsson, B., Dahlen, S.E., Lindgren, J.A., Rouzer, C.A. & Serhan, C.N. *Science* **237**, 1171–1176 (1987).
10. Shimizu, T. & Wolfe, L.S. *J. Neurochem.* **55**, 1–15 (1990).
11. Nagase, T. *et al. Nat. Immunol.* **1**, 42–46 (2000).
12. Nagase, T. *et al. Nat. Med.* **8**, 480–484 (2002).
13. Konstan, M.W., Byard, P.J., Hoppel, C.L. & Davis, P.B. *N. Engl. J. Med.* **332**, 848–854 (1995).

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A potent inhibitor of cytosolic phospholipase A_2, arachidonyl trifluoromethyl ketone, attenuates LPS-induced lung injury in mice

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Nagase, Takahide, Naonori Uozumi, Tomoko Aoki-Nagase, Kan Terawaki, Satoshi Ishii, Tetsuji Tomita, Hiroshi Yamamoto, Kohei Hashizume, Yasuyoshi Ouchi, and Takao Shimizu. A potent inhibitor of cytosolic phospholipase A_2, arachidonyl trifluoromethyl ketone, attenuates LPS-induced lung injury in mice. *Am J Physiol Lung Cell Mol Physiol* 284: L720–L726, 2003. First published December 27, 2002; 10.1152/ajplung.00396.2002.—Acute respiratory distress syndrome (ARDS) is an acute lung injury of high mortality rate, and sepsis syndrome is one of the most frequent causes of ARDS. Metabolites of arachidonic acid, including thromboxanes and leukotrienes, are proinflammatory mediators and potentially involved in the development of ARDS. A key enzyme for the production of these inflammatory mediators is cytosolic phospholipase A_2 ($cPLA_2$). Recently, it has been reported that arachidonyl trifluoromethyl ketone (ATK) is a potent inhibitor of $cPLA_2$. In the present study, we hypothesized that pharmacological intervention of $cPLA_2$ could affect acute lung injury. To test this hypothesis, we examined the effects of ATK in a murine model of acute lung injury induced by septic syndrome. The treatment with ATK significantly attenuated lung injury, polymorphonuclear neutrophil sequestration, and deterioration of gas exchange caused by lipopolysaccharide and zymosan administration. The current observations suggest that pharmacological intervention of $cPLA_2$ could be a novel therapeutic approach to acute lung injury caused by sepsis syndrome.

acute respiratory distress syndrome; lipopolysaccharide; sepsis; eicosanoid; leukotriene

ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) is characterized by acute lung injury, and severe sepsis is one of the most important causes of ARDS (9, 10, 31). Although patients with ARDS are intensively treated with currently available drugs, the mortality rate for ARDS remains high, and it ranges from 40 to 70%. Potential mechanisms that cause ARDS include damage to the alveolar-capillary membrane and polymorphonuclear neutrophil (PMN) adhesion, activation, and sequestration, leading to respiratory failure (9, 10, 31).

Platelet-activating factor (PAF) and metabolites of arachidonic acid are potentially involved in the development of ARDS (23, 30, 32). PAF is a proinflammatory mediator produced from phospholipids (12–14). Thromboxanes (TXs) and leukotrienes (LTs) are potent mediators generated from arachidonic acid by cyclooxygenase and 5-lipoxygenase (7), respectively. TXA_2 may increase lung permeability, whereas LTB_4 is a potent neutrophil chemoattractant. Phospholipase A_2 (PLA_2) is a key enzyme for the production of proinflammatory mediators, including eicosanoids and PAF. Although a number of distinct types of PLA_2 have been reported to be characterized, cytosolic PLA_2 ($cPLA_2$) is thought to be particularly important (29, 30, 34, 36, 37). The $cPLA_2$ preferentially hydrolyzes phospholipids containing arachidonic acid and is activated by submicromolar concentration of Ca^{2+} and by phosphorylation of a serine residue (5, 16, 18, 33). Recently, it has been reported that an analog of arachidonic acid in which the $-COOH$ functionality is replaced by $-COCF_3$, named arachidonyl trifluoromethyl ketone (ATK), is a potent and selective slow-binding inhibitor of $cPLA_2$ (33, 35).

In the present study, we hypothesized that pharmacological intervention of $cPLA_2$ could affect acute lung injury. To test this hypothesis, we chose to use ATK as an inhibitor of $cPLA_2$ and examined the effects of ATK in a murine model of acute lung injury induced by lipopolysaccharide (LPS) and zymosan administration.

METHODS

Animal preparation. We used male C57BL/6 mice (7–8 wk old). Animals were anesthetized with pentobarbital sodium (25 mg/kg ip) and ketamine hydrochloride (25 mg/kg ip) in combination and then paralyzed with pancuronium bromide (0.3 mg/kg ip). Anesthesia and paralysis were maintained by supplemental administration of 10% of the initial dose every hour. After tracheostomy, a metal endotracheal tube (inside diameter 1 mm, length 8 mm) was inserted in the trachea. Animals were mechanically ventilated (model 683; Harvard Apparatus, South Natick, MA) with tidal volumes of 10 ml/kg and frequencies of 2.5 Hz. We opened the thorax widely by means of midline sternotomy and applied a positive end

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expiratory pressure of 2 cmH_2O by placing the expired line underwater. During the experiments, oxygen gas was continuously supplied to the ventilatory system ($F_{I_{O_2}} = 1.0$). A heating pad was used to maintain the body temperature of animals. To assess the development of lung injury physiologically, we measured lung elastance (E_L) and resistance (R_L) as previously described (1, 21–27). Briefly, we measured the tracheal pressure (Ptr), flow, and volume (V). We calculated E_L and R_L by adjusting the equation of motion: $Ptr = E_L \cdot V + R_L(dV/dt) + K$, where K is a constant. Changes in E_L and R_L reflect lung parenchymal alterations and stiffening of the lungs.

Experimental acute lung injury induced by LPS/zymosan administration. One minute before intravenous administration, two deep inhalations (three times tidal volume) were delivered to standardize volume history and measurements were made as baseline. In the physiological study, mice were divided into four experimental groups, i.e., saline-treated (n = 6), ATK/saline-treated (n = 4), LPS/zymosan-treated (n = 7), and ATK/LPS/zymosan-treated groups (n = 5). In the LPS/zymosan-treated group, mice received 3 mg/kg LPS from *Escherichia coli* O111:B4 (Sigma Chemical, St. Louis, MO) intravenously. Two hours later, 10 mg/kg of zymosan A from *Saccharomyces cerevisiae* (Sigma) were intravenously administered (19, 30). In the saline-treated group, animals received saline instead of LPS and zymosan in the same manner and served as controls. In the ATK-treated group, 20 mg/kg ATK (Cayman Chemical, Ann Arbor, MI) were administered intraperitoneally 30 min before saline or LPS administration. The current dose of ATK and timing of ATK administration were applied on the basis of previous reports (11, 20) and our preliminary experiments. In all groups, measurements were made at 30-min intervals for 4 h.

Assessment of respiratory failure. At the end of experiment, blood samples for gas analysis were obtained from the left ventricle. We then measured Pa_{O_2}, Pa_{CO_2}, and pH to assess the extent of respiratory failure (blood gas analyzer; AVL Medical Systems, Schaffhausen, Switzerland).

Bronchoalveolar lavage fluid. At the end of the experiment, bronchoalveolar lavage (BAL) was performed (1 ml of phosphate-buffered saline five times) in saline-treated (n = 6), LPS/zymosan-treated (n = 7), and ATK/LPS/zymosan-treated groups (n = 5). In each animal, 90% (4.5 ml) of the total injected volume was consistently recovered. After BAL fluid (BALF) was centrifuged at 450 g for 10 min, total and differential cell counts of the BALF were determined from the cell fraction (29, 30). The supernatant was stored at −70°C until measurement of protein was performed. The concentration of protein was measured by Lowry's method with bovine serum albumin as a standard.

TX and LT assay. TXA_2 (measured as TXB_2), LTB_4, and $LTC_4/D_4/E_4$ in the BALF were determined by enzyme immunoassay (EIA) kits (Amersham Pharmacia Biotech, Piscataway, NJ). The detection limits of the EIA assays for TXB_2, LTB_4, and $LTC_4/D_4/E_4$ were 3.6, 6, and 10 pg/ml, respectively.

Myeloperoxidase activity assay. At the end of experiments, the left lungs were removed from mice of each group (n = 4, respectively). Myeloperoxidase (MPO) activity was measured as previously reported (3, 15). Briefly, the frozen lungs were weighed and homogenized in hexadecyltrimethylammonium bromide (HTAB) buffer (0.5% HTAB in 50 mM phosphate buffer, pH 6.0). The homogenates were sonicated and centrifuged at 40,000 g for 15 min. The supernatant was mixed with assay buffer containing potassium phosphate buffer, H_2O_2, and o-dianisidine hydrochloride. Then, the supernatant was placed in a spectrophotometer for reading at 460 nm as previously described (3, 15).

Histological study. At the end of experiments, the right lungs of the mice were removed and fixed with 10% formalin. After fixation, the tissue blocks obtained from midsagittal slices of the lungs were embedded in paraffin. Blocks were cut 4 μm thick with a microtome, and then hematoxylin-eosin staining was performed.

Data analysis. Comparisons of data among each experimental group were carried out with analysis of variance. If statistical significances were detected, a Scheffé test was then applied as a post hoc test. Data are expressed as means ± SE. P values <0.05 were taken as significant.

RESULTS

Physiological data following LPS/zymosan or saline administration. There were no significant differences in baseline E_L and R_L among each group. Fig. 1 and Table 1 demonstrate the physiological data after LPS/zymosan or saline administration. As shown, E_L and R_L in LPS/zymosan-treated group were significantly increased compared with saline-treated group, which

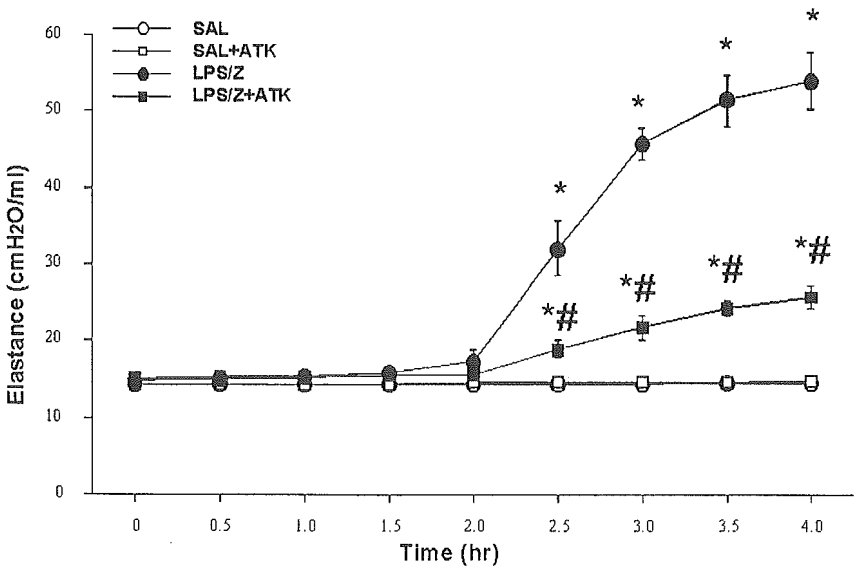

Fig. 1. The time course of response in lung elastance in saline-treated (SAL, n = 6), arachidonyl trifluoromethyl ketone (ATK)/saline-treated (SAL+ATK, n = 4), LPS/zymosan-treated (LPS/Z, n = 7), and ATK/LPS/zymosan-treated groups (LPS/Z+ATK, n = 5). In LPS/zymosan-treated groups, zymosan was administered 2 h after LPS treatment, whereas saline was treated in the same fashion in the saline-treated groups. *P < 0.001 vs. saline-treated group; #P < 0.001 vs. LPS/zymosan-treated group.

Table 1. *Physiological results*

	pH	$PaCO_2$, mmHg	R_L, $cmH_2O \cdot ml^{-1} \cdot s^{-1}$
Saline, ip Saline, iv	7.492 ± 0.041	27.7 ± 3.7	0.412 ± 0.004
ATK, ip Saline, iv	7.455 ± 0.049	29.5 ± 3.3	0.418 ± 0.007
Saline, ip LPS/zymosan, iv	7.134 ± 0.050*	48.6 ± 4.6*	0.539 ± 0.020*
ATK, ip LPS/zymosan, iv	7.444 ± 0.041‡	32.2 ± 3.4‡	0.459 ± 0.014†‡

Values are means ± SE. R_L, lung resistance; ATK, arachidonyl trifluoromethyl ketone. *P < 0.01 vs. saline-treated group. †P < 0.05, ‡P < 0.01 vs. LPS/zymosan-treated group.

Table 2. *Total cell counts and cell fractions in BALF*

	Total cell counts × 10^5	Macrophages, %	PMNs, %	Lymphocytes, %
Saline, ip Saline, iv	1.12 ± 0.05	95.7 ± 0.3	0.4 ± 0.1	3.9 ± 0.3
Saline, ip LPS/zymosan, iv	3.56 ± 0.13*	93.4 ± 0.6	3.3 ± 0.5*	3.3 ± 0.5
ATK, ip LPS/zymosan, iv	2.28 ± 0.32†‡	94.7 ± 0.5	1.1 ± 0.1†‡	4.2 ± 0.4

Values are means ± SE. BALF, bronchoalveolar lavage fluid; PMN, polymorphonuclear neutrophil. *P < 0.01 vs. saline-treated group. †P < 0.05, ‡P < 0.05 vs. LPS/zymosan-treated group.

reflects physiological alterations in lung parenchyma. The administration of ATK significantly reduced LPS/zymosan-induced responses in E_L and R_L, whereas there were significant differences between saline-treated and ATK/LPS/zymosan-treated groups.

Administration of LPS/zymosan elicited respiratory failure, which was not observed in saline-treated groups. Hypoxemia was prominent in LPS/zymosan-treated mice, whereas ATK administration reduced LPS/zymosan-induced hypoxemia (Fig. 2). After LPS/zymosan treatment, increases in Pa_{CO_2} and decreases in pH were observed, although there were no differences in Pa_{CO_2} or pH levels between saline-treated and ATK/LPS/zymosan-treated groups. As shown, ATK had little effect on physiological data in saline-treated groups.

Analyses of BALF. Table 2 and Figs. 3 and 4 summarize the analyzed data of BALF. As shown, LPS/zymosan administration increased protein amount and number of PMN in BALF, indicating LPS/zymosan induced protein leakage and PMN infiltration. The protein leakage and PMN sequestration were significantly attenuated by the treatment of ATK. Meanwhile, there were significant differences in BALF protein amount and number of PMN between saline-treated and ATK/LPS/zymosan-treated groups.

TX and LT assay. To assess the biosynthesis of $cPLA_2$ products, we performed TXA_2 (measured as TXB_2), LTB_4, and $LTC_4/D_4/E_4$ assay of the BALF. Figures 5–7 summarize the results of BALF TXB_2, LTB_4, and $LTC_4/D_4/E_4$ assay in each experimental group. LPS/zymosan administration markedly increased TXB_2, LTB_4, and $LTC_4/D_4/E_4$ levels in BALF compared with the saline-treated group, whereas the levels of these eicosanoids were significantly reduced in the ATK/LPS/zymosan-treated group. However, there were significant differences in BALF TXB_2, LTB_4, and $LTC_4/D_4/E_4$ levels between saline-treated and ATK/LPS/zymosan-treated groups.

MPO activity assay. To assess the PMN infiltration in the lung, we performed MPO activity assay. Figure 8 shows the results of MPO activity in lung tissue. LPS/zymosan administration markedly increased MPO activity in lungs compared with the saline-treated group, whereas the MPO activity was significantly attenuated in the ATK/LPS/zymosan-treated group. However, no significant difference in lung MPO activity was observed between saline-treated and ATK/LPS/zymosan-treated groups.

Histological study. Figure 9 represents lung histology following LPS/zymosan administration. As shown, LPS/zymosan administration induced prominent lesions, as well as alveolar thickening, distortion, and cellular infiltration. In contrast, the alveolar architecture is well preserved and histological changes are minimal in ATK-treated animals.

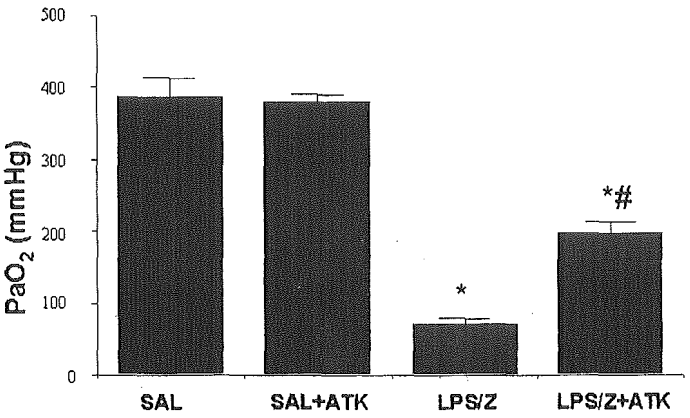

Fig. 2. Effects of cytosolic phospholipase A_2 ($cPLA_2$) inhibitor ATK in hypoxemia induced by LPS/zymosan treatment. *P < 0.001 vs. saline-treated group; #P < 0.001 vs. LPS/zymosan-treated group.

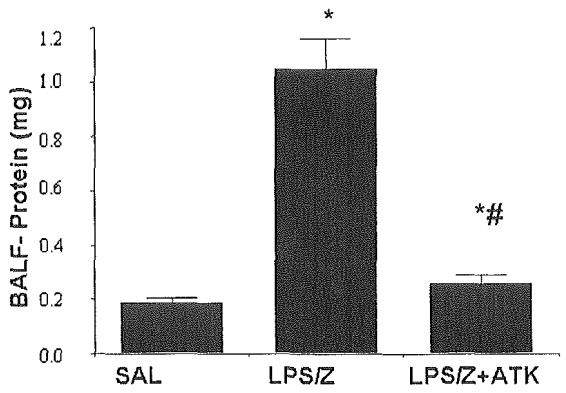

Fig. 3. Effects of $cPLA_2$ inhibitor ATK in protein leakage induced by LPS/zymosan treatment. BALF, bronchoalveolar lavage fluid. *P < 0.01 vs. saline-treated group; #P < 0.01 vs. LPS/zymosan-treated group.

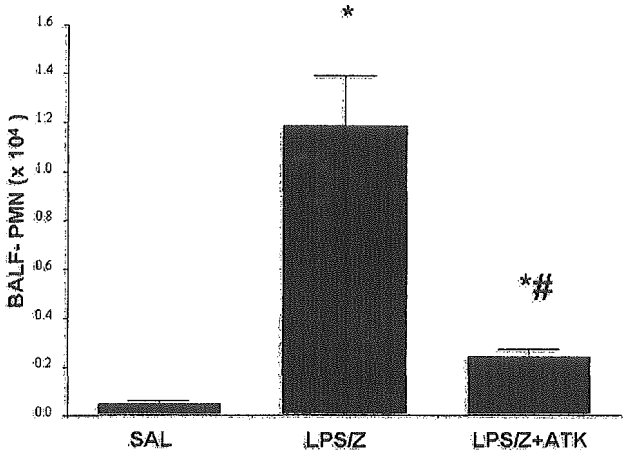

Fig. 4. Effects of $cPLA_2$ inhibitor ATK in neutrophil infiltration induced by LPS/zymosan treatment. PMN, polymorphonuclear neutrophil. *$P < 0.001$ vs. saline-treated group; #$P < 0.001$ vs. LPS/zymosan-treated group.

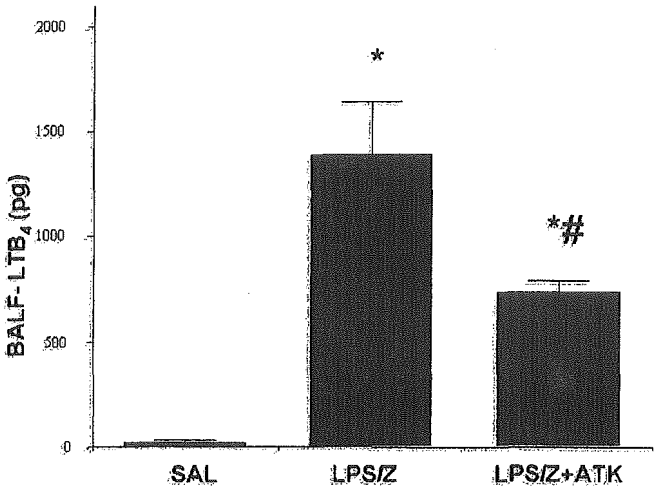

Fig. 6. Effects of $cPLA_2$ inhibitor ATK in LTB_4 production induced by LPS/zymosan treatment. *$P < 0.001$ vs. saline-treated group; #$P < 0.05$ vs. LPS/zymosan-treated group.

DISCUSSION

The results of the current study show that $cPLA_2$ is important in the pathogenesis of acute lung injury. Inhibition of $cPLA_2$ significantly attenuated acute lung injury induced by endotoxemia. These observations indicate that pharmacological inhibition of $cPLA_2$ may be an effective treatment for acute lung injury, probably because it inhibits production of inflammatory mediators including TXs and LTs.

The sepsis syndrome is the most frequent cause of ARDS and is associated with 35–45% incidence of ARDS development (9, 10). It is postulated that both endotoxemia and phagocytosis of bacteria are involved in the pathogenesis of ARDS associated with septic syndrome (6). Therefore, we used the current model of acute lung injury induced by combined administration of LPS and zymosan (19). In this model, circulating LPS and phagocytosis of bacterial particles by LPS-primed PMN elicit acute lung injury, which may mimic sepsis-associated acute lung injury.

After LPS/zymosan administration, we observed increases in E_L, protein leakage, and PMN infiltration and severe exacerbation of gas exchange. PMN infiltration in the lung was confirmed by MPO activity assay and histology. Consistently, marked increases in TXs and LTs were detected in the BALF. These findings were significantly attenuated by the treatment of $cPLA_2$ inhibitor ATK. Potential mechanisms by which $cPLA_2$ mediates sepsis-induced acute lung injury include the release of proinflammatory mediators. The present results also suggest that the major mediator of PMN infiltration is a $cPLA_2$ product, most probably LTB_4 (38). Recent evidence using lung injury models overexpressing the LTB_4 receptor shows that LTB_4 is an important mediator of neutrophil-mediated lung injury (4). It is suggested that not only infiltration but also activation of PMN in lungs may be essential to induce the development of acute lung injury. The $cPLA_2$-initiated pathways may mediate both infiltration and activation of PMN triggered by septic syndrome, resulting in sepsis-associated ARDS. In human

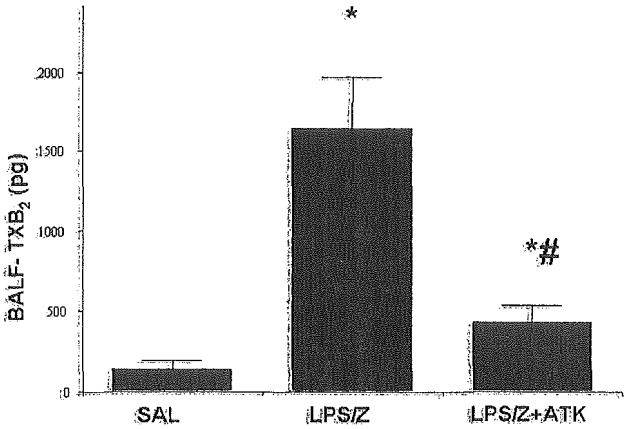

Fig. 5. Effects of $cPLA_2$ inhibitor ATK in thromboxane (TX) B_2 production induced by LPS/zymosan treatment. *$P < 0.05$ vs. saline-treated group; #$P < 0.01$ vs. LPS/zymosan-treated group.

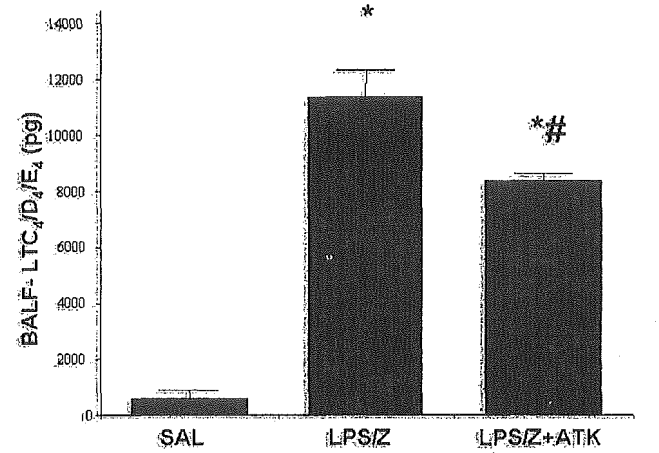

Fig. 7. Effects of $cPLA_2$ inhibitor ATK in leukotriene (LT) $C_4/D_4/E_4$ production induced by LPS/zymosan treatment. *$P < 0.001$ vs. saline-treated group; #$P < 0.05$ vs. LPS/zymosan-treated group.

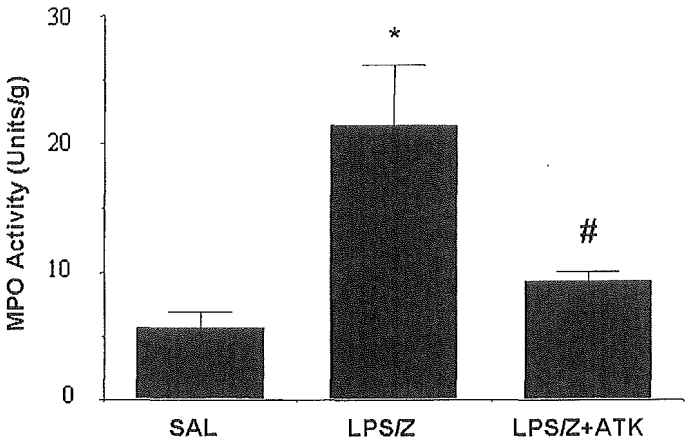

Fig. 8. Effects of $cPLA_2$ inhibitor ATK in lung myeloperoxidase (MPO) activity induced by LPS/zymosan treatment ($n = 4$ for each group). *$P < 0.05$ vs. saline-treated group; #$P < 0.05$ vs. LPS/zymosan-treated group.

neutrophils during sepsis, elevated $cPLA_2$ expression and activity have been recently reported, suggesting that $cPLA_2$ plays a major role in neutrophil function in septic syndrome (17).

Of note, it has been recently shown that acute lung injury induced by LPS/zymosan administration is attenuated in $cPLA_2$ gene-disrupted mice (30). It seems that the effects of ATK administration are similar to those of $cPLA_2$ gene disruption in terms of inhibiting lung injury. This observation may further confirm that the intervention of $cPLA_2$ could be an effective approach to treat acute lung injury. However, differences were also found between these two studies. In this study, we measured TXB_2, LTB_4, and cysteinyl LTs ($LTC_4/D_4/E_4$) in BALF to confirm the generation of $cPLA_2$ products. Although the ATK administration significantly attenuated LPS/zymosan-induced production of TXB_2, LTB_4, and cysteinyl LTs, the ATK administration reduced each eicosanoid by 73, 47, and 27%, respectively, compared with LPS/zymosan administration. In contrast, $cPLA_2$ gene disruption reduced each eicosanoid by >90% in this model, compared with LPS/zymosan administration in wild-type mice. This finding suggests that the present manner of ATK administration may still be insufficient to inhibit $cPLA_2$ completely. Because it is postulated that pharmacological intervention of $cPLA_2$ could be useful in the manage-

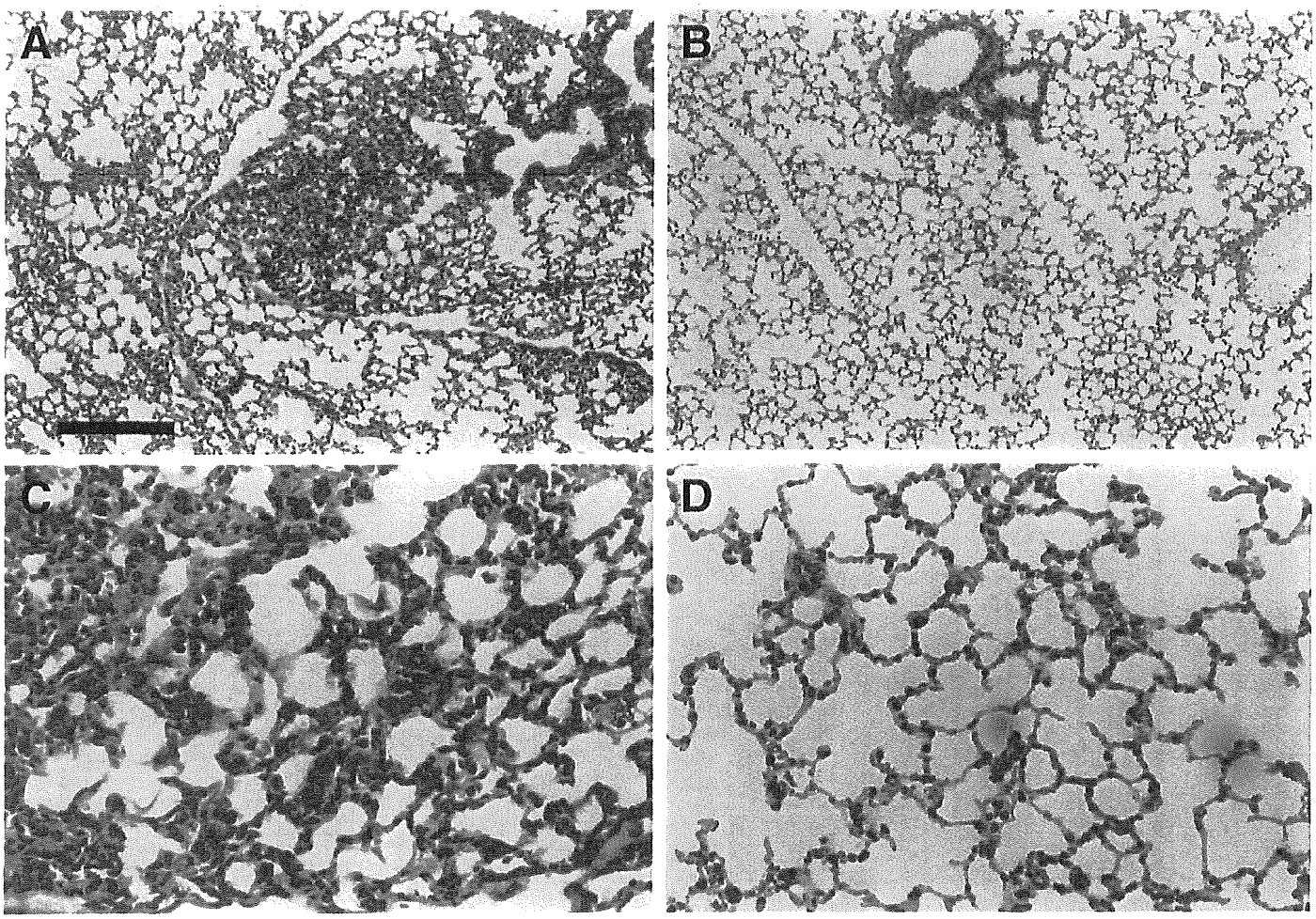

Fig. 9. Photomicrograph of lung tissues from LPS/zymosan-treated (*A*, *C*), and ATK/LPS/zymosan-treated (*B*, *D*) mice 4 h after LPS administration. Hematoxylin-eosin stain. Scale bar in *A* represents 200 μm in *A* and *B* and 50 μm in *C* and *D*.

ment of ARDS, the development of novel cPLA$_2$ inhibitors warrants future research.

In the present model of acute lung injury, we observed that the levels of Pa_{CO_2} and pH in the ATK/LPS/zymosan-treated group were the same as in saline-treated controls. However, LPS/zymosan-induced increases in E_L, severity of hypoxia, BALF protein, PMN, and eicosanoids were significantly attenuated but not eliminated by the treatment of ATK. These observations indicate that factors other than cPLA$_2$ may also play a role and contribute to physiological alteration. Recently, it has been demonstrated that secretory PLA$_2$ (sPLA$_2$), the other type of PLA$_2$, mediates LPS-induced lung injury and that the inhibition of sPLA$_2$ may also represent a therapeutic approach to acute lung injury (2). In addition, it has been suggested that oxygen radicals, adhesion molecules, and cytokines are also involved in this mechanism (8, 28). Recently, it was reported that cPLA$_2$ activation is essential for integrin-dependent adhesion of leukocytes (39). If one considers that there are as yet no pharmacological agents to reverse pulmonary edema and increase survival rates, these factors are potential targets to develop agents. The current study suggests that the intervention of cPLA$_2$ could be a promising clue to improve management of ARDS.

In summary, the inhibition of cPLA$_2$ significantly attenuated lung damage and respiratory failure induced by LPS/zymosan treatment. The current observations suggest that cPLA$_2$ products are involved in the pathogenesis of acute lung injury caused by septic syndrome. Inhibition of cPLA$_2$-initiated pathways might provide a novel and potential therapeutic approach to ARDS, to which no pharmaceutical agents are currently available.

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REFERENCES

1. **Aoki-Nagase T, Nagase T, Oh-hashi Y, Shindo T, Kurihara Y, Yamaguchi Y, Yamamoto H, Tomita T, Ohga E, Nagai R, Kurihara H, and Ouchi Y.** Attenuation of antigen-induced airway hyperresponsiveness in CGRP-deficient mice. *Am J Physiol Lung Cell Mol Physiol* 283: L963–L970, 2002.
2. **Arbibe L, Koumanov K, Vial D, Rougeot C, Faure G, Havet N, Longacre S, Vargaftig BB, Bereziat G, Voelker DR, Wolf C, and Touqui L.** Generation of lyso-phospholipids from surfactant in acute lung injury is mediated by type-II phospholipase A$_2$ and inhibited by a direct surfactant protein A-phospholipase A$_2$ protein interaction. *J Clin Invest* 102: 1152–1160, 1998.
3. **Blackwell TS, Lancaster LH, Blackwell TR, Venkatakrishnan A, and Christman JW.** Chemotactic gradients predict neutrophilic alveolitis in endotoxin-treated rats. *Am J Respir Crit Care Med* 159: 1644–1652, 1999.
4. **Chiang N, Gronert K, Clish CB, O'Brien JA, Freeman MW, and Serhan CN.** Leukotriene B$_4$ receptor transgenic mice reveal novel protective roles for lipoxins and aspirin-triggered lipoxins in reperfusion. *J Clin Invest* 104: 309–316, 1999.
5. **Clark JD, Lin LL, Kriz RW, Ramesha CS, Sultzman LA, Lin AY, Milona N, and Knopf JL.** A novel arachidonic acid-selective cytosolic PLA$_2$ contains a Ca^{2+}-dependent translocation domain with homology to PKC and GAP. *Cell* 65: 1043–1051, 1991.
6. **Donnelly SC and Haslett C.** Cellular mechanisms of acute lung injury: implications for future treatment in the adult respiratory distress syndrome. *Thorax* 47: 260–263, 1992.
7. **Figueroa DJ, Breyer RM, Defoe SK, Kargman S, Daugherty BL, Waldburger K, Liu Q, Clements M, Zeng Z, O'Neill GP, Jones TR, Lynch KR, Austen CP, and Evans JF.** Expression of the cysteinyl leukotriene 1 receptor in normal human lung and peripheral blood leukocytes. *Am J Respir Crit Care Med* 163: 226–233, 2001.
8. **Folkesson HG, Matthay MA, Hebert CA, and Broaddus VC.** Acid aspiration-induced lung injury in rabbits is mediated by interleukin-8-dependent mechanisms. *J Clin Invest* 96: 107–116, 1995.
9. **Fowler AA, Hamman RF, Good JT, Benson KN, Baird M, Eberle DJ, Petty TL, and Hyers TM.** Adult respiratory distress syndrome: risk with common predispositions. *Ann Intern Med* 98: 593–597, 1983.
10. **Hudson LD, Milberg JA, Anardi D, and Maunder RJ.** Clinical risks for development of the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 151: 293–301, 1995.
11. **Ichinose F, Ullrich R, Sapirstein A, Jones RC, Bonventre JV, Serhan CN, Bloch KD, and Zapol WM.** Cytosolic phospholipase A$_2$ in hypoxic pulmonary vasoconstriction. *J Clin Invest* 109: 1493–1500, 2002.
12. **Ishii S, Kuwaki T, Nagase T, Maki K, Tashiro F, Sunaga S, Cao WH, Kume K, Fukuchi Y, Ikuta K, Miyazaki J, Kumada M, and Shimizu T.** Impaired anaphylactic responses but intact sensitivity to endotoxin in mice lacking a platelet-activating factor receptor. *J Exp Med* 187: 1779–1788, 1998.
13. **Ishii S, Nagase T, Tashiro F, Ikuta K, Sato S, Waga I, Kume K, Miyazaki J, and Shimizu T.** Bronchial hyperreactivity, increased endotoxin lethality and melanocytic tumorigenesis in transgenic mice overexpressing platelet-activating factor receptor. *EMBO J* 16: 133–142, 1997.
14. **Ishii S and Shimizu T.** Platelet-activating factor (PAF) receptor and genetically engineered PAF receptor mutant mice. *Prog Lipid Res* 39: 41–82, 2000.
15. **Krawisz JE, Sharon P, and Stenson WF.** Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. *Gastroenterology* 87: 1344–1350, 1984.
16. **Leslie CC.** Properties and regulation of cytosolic phospholipase A$_2$. *J Biol Chem* 272: 16709–16712, 1997.
17. **Levy R, Dana R, Hazan I, Levy I, Weber G, Smoliakov R, Pesach I, Riesenberg K, and Schlaeffer F.** Elevated cytosolic phospholipase A$_2$ expression and activity in human neutrophils during sepsis. *Blood* 95: 660–665, 2000.
18. **Lin LL, Wartmann M, Lin AY, Knopf JL, Seth A, and Davis RJ.** cPLA$_2$ is phosphorylated and activated by MAP kinase. *Cell* 72: 269–278, 1993.
19. **Miotla JM, Williams TJ, Hellewell PG, and Jeffery PK.** A role for the beta2 integrin CD11b in mediating experimental lung injury in mice. *Am J Respir Cell Mol Biol* 14: 363–373, 1996.
20. **Myou S, Sano H, Fujimura M, Zhu X, Kurashima K, Kita T, Nakao S, Nonomura A, Shioya T, Kim KP, Munoz NM, Cho W, and Leff AR.** Blockade of eosinophil migration and airway hyperresponsiveness by cPLA$_2$-inhibition. *Nat Immunol* 2: 145–149, 2001.
21. **Nagase T, Fukuchi Y, Matsuse T, Sudo E, Matsui H, and Orimo H.** Antagonism of ICAM-1 attenuates airway and tissue responses to antigen in sensitized rats. *Am J Respir Crit Care Med* 151: 1244–1249, 1995.
22. **Nagase T, Ishii S, Katayama H, Fukuchi Y, Ouchi Y, and Shimizu T.** Airway responsiveness in transgenic mice overexpressing platelet-activating factor receptor: roles of thromboxanes and leukotrienes. *Am J Respir Crit Care Med* 156: 1621–1627, 1997.
23. **Nagase T, Ishii S, Kume K, Uozumi N, Izumi T, Ouchi Y, and Shimizu T.** Platelet-activating factor mediates acid-in-

duced lung injury in genetically engineered mice. *J Clin Invest* 104: 1071–1076, 1999.

24. **Nagase T, Ishii S, Shindou H, Ouchi Y, and Shimizu T.** Airway hyperresponsiveness in transgenic mice overexpressing platelet-activating factor receptor is mediated by an atropine-sensitive pathway. *Am J Respir Crit Care Med* 165: 200–205, 2002.
25. **Nagase T, Kurihara H, Kurihara Y, Aoki T, Fukuchi Y, Yazaki Y, and Ouchi Y.** Airway hyperresponsiveness to methacholine in mutant mice deficient in endothelin-1. *Am J Respir Crit Care Med* 157: 560–564, 1998.
26. **Nagase T, Matsui H, Aoki T, Ouchi Y, and Fukuchi Y.** Lung tissue behaviour in the mouse during constriction induced by methacholine and endothelin-1. *J Appl Physiol* 81: 2373–2378, 1996.
27. **Nagase T, Ohga E, Katayama H, Sudo E, Aoki T, Matsuse T, Ouchi Y, and Fukuchi Y.** Roles of calcitonin-gene related peptide (CGRP) in hyperpnea-induced constriction in guinea pigs. *Am J Respir Crit Care Med* 154: 1551–1556, 1996.
28. **Nagase T, Ohga E, Sudo E, Katayama H, Uejima Y, Matsuse T, and Fukuchi Y.** Intercellular adhesion molecule-1 mediates acid aspiration-induced lung injury. *Am J Respir Crit Care Med* 154: 504–510, 1996.
29. **Nagase T, Uozumi N, Ishii S, Kita Y, Yamamoto H, Ohga E, Ouchi Y, and Shimizu T.** A pivotal role of cytosolic phospholipase A_2 in bleomycin-induced pulmonary fibrosis. *Nat Med* 8: 480–484, 2002.
30. **Nagase T, Uozumi N, Ishii S, Kume K, Izumi T, Ouchi Y, and Shimizu T.** Acute lung injury by sepsis and acid aspiration: a key role for cytosolic phospholipase A_2. *Nat Immunol* 1: 42–46, 2000.
31. **Pittet JF, Mackersie RC, Martin TR, and Matthay MA.** Biological markers of acute lung injury: prognostic and pathogenetic significance. *Am J Respir Crit Care Med* 155: 1187–1205, 1997.
32. **Prescott SM, McIntyre TM, and Zimmerman G.** Two of the usual suspects, platelet-activating factor and its receptor, implicated in acute lung injury. *J Clin Invest* 104: 1019–1020, 1999.
33. **Riendeau D, Guay J, Weech PK, Laliberte F, Yergey J, Li C, Desmarais S, Perrier H, Liu S, Nicoll-Griffith D, and Street IP.** Arachidonyl trifluoromethyl ketone, a potent inhibitor of 85-kDa phospholipase A_2, blocks production of arachidonate and 12-hydroxyeicosatetraenoic acid by calcium ionophore-challenged platelets. *J Biol Chem* 269: 15619–15624, 1994.
34. **Shindou H, Ishii S, Uozumi N, and Shimizu T.** Roles of cytosolic phospholipase A_2 and platelet-activating factor receptor in the Ca-induced biosynthesis of PAF. *Biochem Biophys Res Commun* 271: 812–817, 2000.
35. **Street IP, Lin HK, Laliberte F, Ghomashchi F, Wang Z, Perrier H, Tremblay NM, Huang Z, Weech PK, and Gelb MH.** Slow- and tight-binding inhibitors of the 85-kDa human phospholipase A_2. *Biochemistry* 32: 5935–5940, 1993.
36. **Uozumi N, Kume K, Nagase T, Nakatani N, Ishii S, Tashiro F, Komagata Y, Maki K, Ikuta K, Ouchi Y, Miyazaki J, and Shimizu T.** Role of cytosolic phospholipase A_2 in allergic response and parturition. *Nature* 390: 618–622, 1997.
37. **Wong DA, Kita Y, Uozumi N, and Shimizu T.** Discrete role for cytosolic phospholipase A_2 alpha in platelets: studies using single and double mutant mice of cytosolic and group IIA secretory phospholipase A_2. *J Exp Med* 196: 349–357, 2002.
38. **Yokomizo T, Izumi T, Chang K, Takuwa Y, and Shimizu T.** A G-protein-coupled receptor for leukotriene B_4 that mediates chemotaxis. *Nature* 387: 620–624, 1997.
39. **Zhu X, Munoz NM, Kim KP, Sano H, Cho W, and Leff AR.** Cytosolic phospholipase A_2 activation is essential for beta 1 and 2 integrin-dependent adhesion of human eosinophils. *J Immunol* 163: 3423–3429, 1999.

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Effects of obstructive sleep apnea on circulating ICAM-1, IL-8, and MCP-1

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Ohga, Eijiro, Tetsuji Tomita, Hiroo Wada, Hiroshi Yamamoto, Takahide Nagase, and Yasuyoshi Ouchi. Effects of obstructive sleep apnea on circulating ICAM-1, IL-8, and MCP-1. *J Appl Physiol* 94: 179–184, 2003. First published September 27, 2002; 10.1152/japplphysiol.00177.2002.—Obstructive sleep apnea syndrome (OSAS) is one of the most important risk factors of cardiovascular disorders. In the treatment of OSAS, nasal continuous positive airway pressure (nCPAP) has been widely used and found to be effective. In the present study, we hypothesized that the hypoxic stress caused by obstructive sleep apnea would increase circulating intercellular adhesion molecule-1 (ICAM-1), interleukin-8 (IL-8), and monocyte chemoattractant protein-1 (MCP-1) in untreated OSAS patients compared with an age-matched control group. In addition, we hypothesized that nCPAP may decrease OSAS-induced hypoxic stress and mediators. To examine these hypotheses, we measured circulating ICAM-1 and IL-8 before and after nCPAP therapy in OSAS patients. We observed that nCPAP decreased apnea, desaturation, and the circulating ICAM-1 and IL-8 levels in OSAS patients. The circulating levels of ICAM-1, IL-8, and MCP-1 in untreated OSAS patients were significantly greater than those in the controls. These observations suggest that nCPAP therapy could reduce OSAS-induced hypoxia and generation of inflammatory mediators. Treatment of OSAS using nCPAP can be, therefore, a potential approach to decrease risk of the progression of OSAS-associated disorders.

cytokines; cardiovascular disorders; ischemic heart disease; desaturation magnitude; hypoxic stress; intracellular adhesion molecule-1; monocyte chemoattractant protein-1; interleukin-8

RECENTLY, IT HAS BEEN SHOWN that obstructive sleep apnea syndrome (OSAS) is related to obesity, insulin resistance, and diabetes mellitus (17, 28, 33). Moreover, OSAS could be one of the most important risk factors of cardiovascular disorders, including hypertension, ischemic heart disease, and cerebrovascular events (12, 15, 23, 25), whereas hypoxic stress elicited by OSAS may be involved in the development of cardiovascular disorders. However, the exact mechanism remains to be elucidated.

One of the potential mechanisms is that OSAS-induced hypoxic stress increases circulating inflammatory mediators, leading to cardiovascular lesions. It has been recently suggested that atherosclerosis is related to inflammatory process induced by activation of proinflammatory mediators, including adhesion molecules (11) and cytokines (10, 31). To induce leukocyte migration to inflamed tissue, it is essential for leukocytes to adhere to microvascular endothelium (32). Potential mediators responsible for leukocyte attachment to endothelium include intercellular adhesion molecule-1 (ICAM-1), interleukin-8 (IL-8), and monocyte chemoattractant protein-1 (MCP-1). It has been reported that ICAM-1, a member of the immunoglobulin superfamily, is required for leukocyte migration into inflamed area (3, 6, 35) and plays an important role in inflammatory disease, including bronchial asthma, lung injury, and ischemic heart disease (18–22). IL-8, a CXC chemokine that induces the migration and proliferation of endothelial cells and smooth muscle cells, is a potent angiogenic factor that may play a substantial role in atherosclerosis (4, 31). Increased expression of IL-8 has been reported in atherosclerotic lesions and circulating macrophages from patients with atherosclerosis (31). MCP-1 is upregulated in human atherosclerotic plaques, suggesting a role for MCP-1 in the development of early atherosclerotic lesions (5, 10).

Hypoxic stress increases the adherence of neutrophils to endothelial cells, and this increased adherence is mediated by proinflammatory mediators, including ICAM-1 (2) and IL-8 (13, 30). Furthermore, it has been reported that hypoxia induces the synthesis and expression of both ICAM-1 and IL-8 via the activation of nuclear transcription factor (NF)-κB (7, 36, 37).

In the treatment of OSAS, the efficacy of nasal continuous positive airway pressure (nCPAP) has been reported (8, 27). nCPAP improves sleepiness and quality of life in patients with OSAS, probably because nCPAP intervention removes sleeping upper airway collapse and decreases apnea episode (27). Although it is expected that nCPAP may ultimately improve the prognosis of various disorders associated with OSAS, its exact mechanism is not yet proven.

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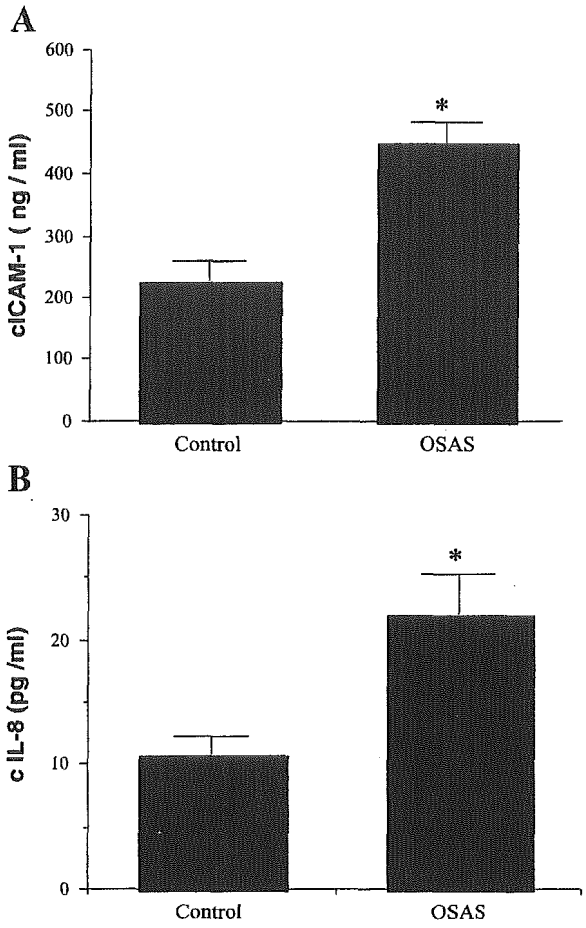

Fig. 1. Baseline levels of (*A*) circulating intercellular adhesion molecule-1 (cICAM-1) and (*B*) circulating interleukin-8 (cIL-8) in the control and obstructive sleep apnea syndrome (OSAS) groups. *$P <$ 0.05 vs. normal controls.

In the present study, we hypothesized that nCPAP may decrease OSAS-induced hypoxic stress and the generation of proinflammatory mediators. To examine this hypothesis, we measured circulating ICAM-1 and IL-8 before and after nCPAP therapy in OSAS patients.

METHODS

Subjects. Among patients diagnosed as OSAS in our department, 20 male subjects participated in the present study.

Table 1. *Characteristics of the subjects*

Group	*n*	Age, yr	Body Mass Index	Apnea Index
OSAS	20	47.8 ± 2.2	29.4 ± 1.4	38.9 ± 3.1*
Control	10	48.9 ± 2.9	28.4 ± 2.9	3.1 ± 0.4

Values are means ± SE; *n*, no. of subjects. OSAS; obstructive sleep apnea syndrome. *$P < 0.001$ vs. control group.

As age-matched controls, 10 male subjects were chosen and studied. No subjects had any history of cardiovascular, pulmonary, metabolic, or neuromuscular diseases. All subjects were in a stable condition for 1 mo before the study. The characteristics of the subjects in the OSAS and normal groups are shown in Table 1. There were no significant differences in age and body mass index (BMI) between the two groups, whereas apnea index (AI) in the OSAS group was markedly greater than that in the control.

After the polysomnography study, the patients with OSAS underwent therapeutic nCPAP treatment, and eight subjects continued to receive nCPAP successfully for 8–18 mo.

Polysomnography. The subjects underwent polysomnography for 2 consecutive nights. The polysomnography included an electroencephalogram, an electrooculogram, an electromyogram of the chin, and an electrocardiogram (DG Compact32, Medelec, Surrey, UK). We monitored ventilation and airflow using inductive plethysmography (Respitrace, Ambulatory Monitoring, Ardsley, NY) and thermistors (Fukuda-Sangyo, Chiba, Japan) placed at the nostril and mouth. Arterial oxygen saturation (Sa_{O_2}) was continuously measured via pulse oxymeter (Datex, Helsinki, Finland). Data acquisition was performed overnight from 9:00 PM to 6:00 AM the next morning.

Assessment of hypoxic episodes. To assess OSAS-induced hypoxia, we applied desaturation magnitude (DM) in this study. Desaturation episodes were defined as hypoxia of Sa_{O_2} <90%. We defined DM as

$$DM = \sum (90 - Sa_{O_2})t$$

where t is time of desaturation (in h). As shown in the equation, DM expresses the severity of hypoxic stress quantitatively.

Measurements of circulating ICAM-1, IL-8, and MCP-1. We obtained peripheral blood from the subjects at 9:00 AM before and after the nCPAP treatment. The blood samples were centrifuged at 250 *g* and 4°C for 10 min. The serum samples were then stored at −80°C until measurements. The concentrations of ICAM-1, IL-8 and MCP-1 in the serum were measured by ELISA method. The data are defined as

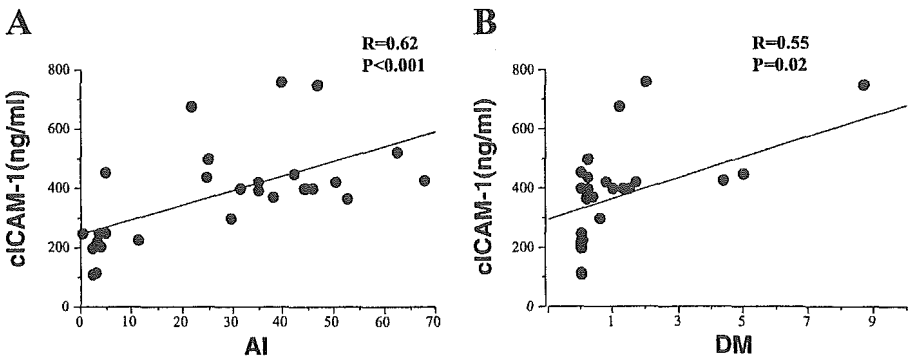

Fig. 2. Correlation between cICAM-1 and apnea-associated parameters, i.e., apnea index (AI; *A*) and desaturation magnitude (DM; *B*).

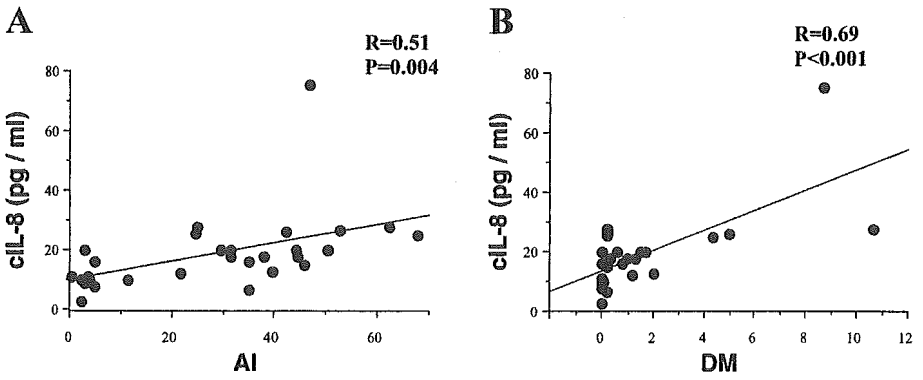

Fig. 3. Correlation between circulating IL-8 (cIL-8) and apnea-associated parameters, i.e., AI (*A*) and DM (*B*) desaturation magnitude (DM).

circulating ICAM-1 (cICAM-1), circulating IL-8 (cIL-8), and circulating MCP-1 (cMCP-1), respectively.

Data analysis. Comparisons of data between each experimental group were carried out with Student's *t*-test. Data are expressed as means ± SE. P values <0.05 were taken as significant.

RESULTS

Assessment of hypoxic episodes. There were significant differences in baseline DM between the OSAS and normal groups (2.01 ± 0.66 and 0.02 ± 0.01, respectively; $P < 0.001$), suggesting that the OSAS patients were exposed to significantly greater degree of hypoxia compared with the control subjects.

Baseline measurements of cICAM-1 and cIL-8. Figure 1 summarizes the cICAM-1 and cIL-8 levels in the baseline measurements. The levels of both cICAM-1 and cIL-8 in the OSAS group were significantly greater than those in the normal group.

Figure 2 demonstrates the relationships between cICAM-1 and AI and between cICAM-1 and DM. As shown, significant correlations are observed between cICAM-1 and apnea episodes. Similarly, significant correlation between cIL-8 and DM is detected, whereas the positive correlation is suggested between cIL-8 and AI (Fig. 3).

As indicated in Fig. 4, cICAM-1 is significantly correlated with cIL-8.

Effects of nCPAP on physiological parameters and circulating mediators. After nCPAP, the improvement in sleepiness was observed in all of the OSAS patients who successfully received therapeutic nCPAP. Consequently, nCPAP significantly decreased apnea and desaturation (Fig. 5).

Figure 6 summarizes the effects of long-term nCPAP on cICAM-1 and cIL-8 levels. As shown, nCPAP longer than 8 mo significantly decreased the levels of both cICAM-1 and cIL-8 in the treated OSAS group.

Figure 7 summarizes the cMCP-1 level in the OSAS group and normal group. The level of cMCP-1 in the

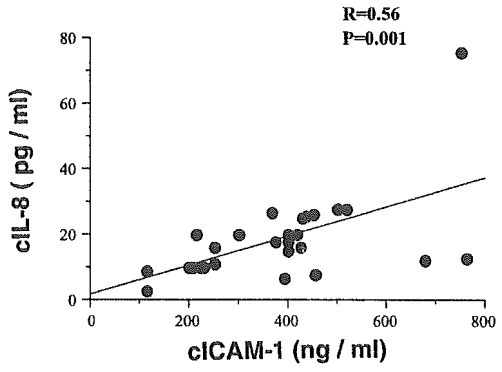

Fig. 4. Correlation between cICAM-1 and cIL-8.

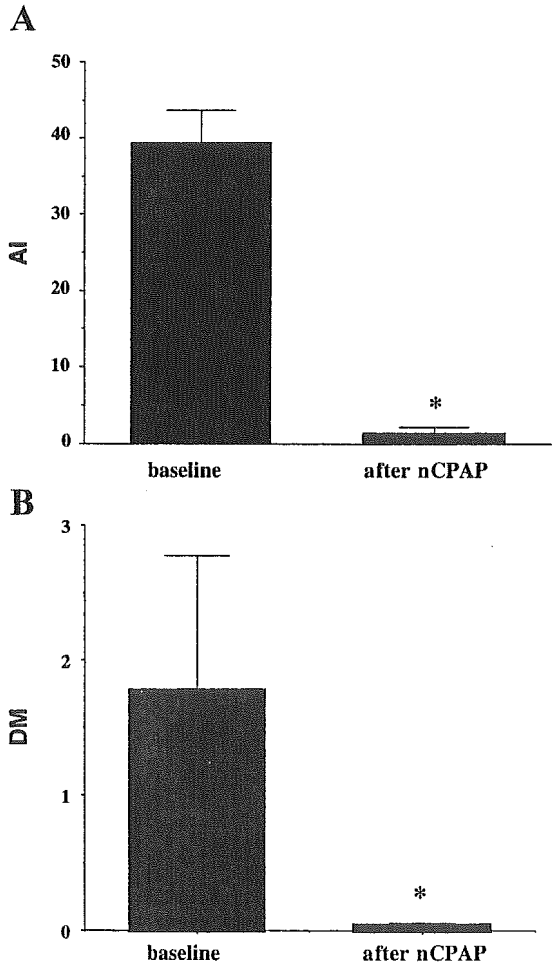

Fig. 5. Effects of nasal continuous positive airway pressure (nCPAP) on apnea-associated parameters, i.e., AI (*A*) apnea index and DM (*B*). *$P < 0.05$ vs. baseline.

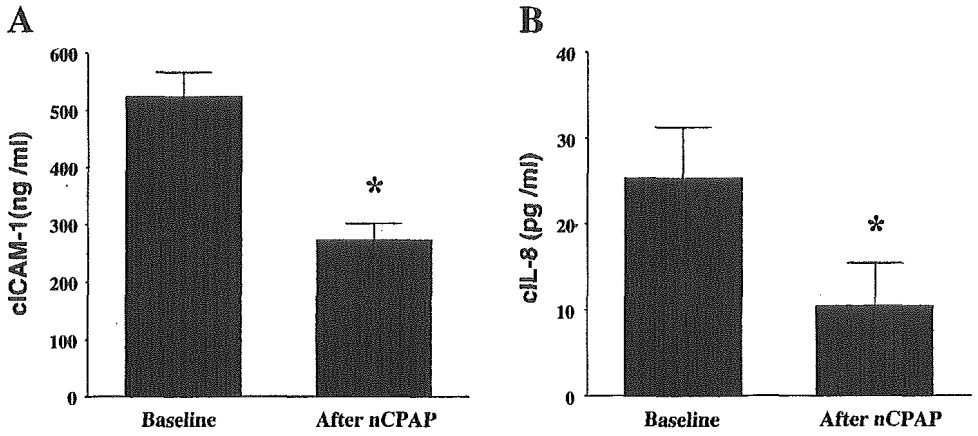

Fig. 6. Effects of nCPAP on cICAM-1 (*A*) and cIL-8 (*B*). *$P < 0.05$ vs. baseline.

OSAS group was significantly greater than that in the normal group.

DISCUSSION

The results of the present study demonstrate that nCPAP decreased apnea, desaturation, and the circulating ICAM-1 and IL-8 levels in the OSAS patients. In the baseline measurements, the levels of both ICAM-1 and IL-8 in the OSAS group were significantly greater than those in the control group. These observations suggest that nCPAP therapy could reduce OSAS-induced hypoxia and generation of inflammatory mediators, leading to the possible prevention of cardiovascular disorders.

Several issues warrant consideration before the results are discussed. First, we measured circulating ICAM-1 and IL-8 to assess the expression of cell-associated adhesion molecule and chemokine. Whereas this approach has been widely used (11, 20), it remains unclear whether the circulating levels of these mediators might precisely reflect the real expression of molecules attached to the endothelium or leukocytes. Second, the number of subjects in this study is relatively low, although the characteristics of the subjects were well matched. Increasing the number of subjects may be required to confirm the interpretation of the present results, and we should acknowledge this point.

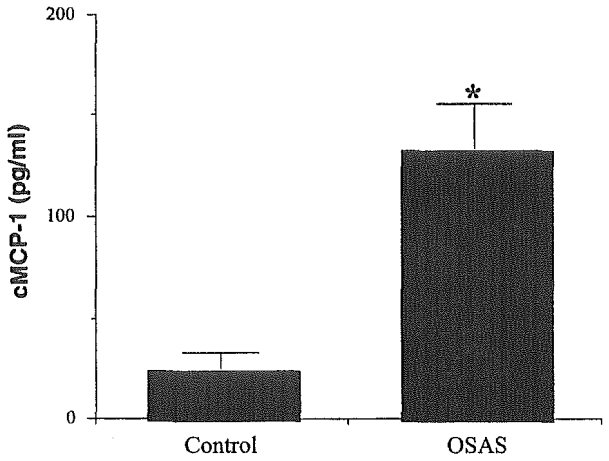

Fig. 7. Baseline levels of circulating monocyte chemoattractant protein-1 (cMCP-1) in the control and OSAS groups. *$P < 0.05$ vs. normal controls.

It has been recently postulated that inflammatory process has a crucial role in the pathogenesis of atherosclerosis, leading to the various cardiovascular disorders (1, 9). To promote migration of leukocytes from circulation to inflamed areas, it is essential for leukocytes to adhere to vascular endothelium via adhesion molecules (32). Especially, ICAM-1 has been reported to play important roles in leukocyte migration to inflamed area (2, 3, 29). ICAM-1 is an 80- to 110-kDa glycoprotein consisting of five immunoglobulin-like domains and a ligand for LFA-1α (18, 32). It has been demonstrated that the ICAM-1/LFA-1α pathway evolves to function in cell-cell adhesion (33) and mediates various inflammatory diseases (16, 19, 21, 22, 35). Recently, it has been reported that the circulating ICAM-1 levels are higher in patients with ischemic heart disease than those in controls (20). Moreover, the circulating ICAM-1 level may indicate a risk of future myocardial infarction, suggesting that antiadhesion therapies can be considered as a novel therapeutic means of cardiovascular disease (26). In the previous study, we have demonstrated that the circulating ICAM-1 level is significantly increased compared with the control group, suggesting that OSAS-induced hypoxia may induce the activation of ICAM-1 and the inflammation of endothelium in patients with OSAS (24). This observation may give rise to a hypothesis that the therapy for OSAS might be a potential approach to prevention of cardiovascular disorders via antiadhesion mechanism.

Recently, it has been demonstrated that IL-8 may play an important role in the development of atherosclerosis (4, 10, 31). Although monocytes contribute to the development of atherosclerotic lesions, IL-8 is a powerful trigger for firm adhesion of monocytes to vascular endothelium (10). It has been shown that hypoxia induces expression and/or generation of IL-8 (13, 30), indicating that OSAS-associated desaturation could lead to upregulatation of IL-8 expression. In addition, one could presume that the effective therapy

for OSAS may attenuate hypoxic stress, which may prevent the development of vascular lesions via the reduction of IL-8 production.

To treat patients with OSAS, nCPAP therapy is widely used, because nCPAP reduces excessive daytime sleepiness and improves quality of life (8, 27). Based on the recent studies, beneficial effects of nCPAP on the prognosis of OSAS-associated diseases are anticipated, but there exists little evidence to prove this notion. We therefore performed this study to address the question whether nCPAP could affect physiological phenomena and production of proinflammatory mediators. We observed that long-term nCPAP was effective to improve sleepiness, nocturnal apnea, and desaturation and that the levels of circulating mediators were reduced after nCPAP. One of the possible explanations is that nCPAP decreases hypoxic episodes, resulting in the reduction of hypoxia-induced inflammation and expression of ICAM-1 and IL-8. Considering the proinflammatory effects of ICAM-1 and IL-8, the attenuated production of these mediators elicited by nCPAP may suggest a novel approach to manage OSAS and prevent OSAS-associated inflammatory diseases.

To assess the severity of hypoxia induced by OSAS, we used DM. Possibly, this parameter may reflect OSAS-induced hypoxic stress more directly than AI. The usual way to assess the degree of OSAS includes the number of apnea episodes, but DM could reflect both decreases in Sa_{O_2} and time spent below 90%. However, to accurately analyze the hypoxic stress, exploring other indexes of hypoxic stress may be important and helpful.

We observed that there was a significant correlation between circulating ICAM-1 and IL-8 in the population studied. It has been demonstrated that nuclear transcription factor (NF)-κB regulates the synthesis and expression of both ICAM-1 and IL-8 (26, 27). In addition, NF-κB is upregulated by hypoxia, leading to the increased expression of both ICAM-1 and IL-8 (7, 37). These reports may explain the present findings that there were significant correlations between desaturation and mediators measured.

We further investigated the level of circulating MCP-1 in the normal and OSAS groups. Recently, it has been reported that the level of MCP-1 is increased in patients with coronary heart disease (14). In the present study, we observed that the level of MCP-1 in the OSAS group was increased compared with that of the normal group. Possibly, the increases in the circulating chemokines, including MCP-1, may play an important role in the pathogenesis in OSAS patients complicated with cardiovascular disease.

In summary, the circulating ICAM-1, IL-8, and MCP-1 levels increased in the OSAS patients compared with the normal subjects. After nCPAP therapy, significant decreases in the levels of ICAM-1 and IL-8 were observed in the OSAS group. Taken together, OSAS-induced hypoxia activates ICAM-1 and IL-8, resulting in the important risk factor of cardiovascular disorders. Treatment of OSAS with the use of nCPAP can be, therefore, a potential approach to decrease risk of the progression of OSAS-associated disorders.

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REFERENCES

1. **Alexander RW.** Inflammation and coronary artery disease. *N Engl J Med* 331: 468–469, 1994.
2. **Arnould T, Michiels C, and Remacle J.** Increased PMN adherence on endothelial cells after hypoxia: involvement of PAF, CD18/CD11b, and ICAM-1. *Am J Physiol Cell Physiol* 264: C1102–C1110, 1993.
3. **Barton RW, Rothlein R, Ksiazek J, and Kennedy C.** The effect of anti-intercellular adhesion molecule-1 on phorbol-ester-induced rabbit lung inflammation. *J Immunol* 143: 1278–1282, 1989.
4. **Boisvert WA, Santiago R, Curtiss LK, and Terkeltaub RA.** A leukocyte homoloue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice. *J Clin Invest* 101: 353–363, 1998.
5. **Boring L, Gosling J, Cleary M, and Charo IF.** Decreased lesion formation in CCR2−/− mice reveals a role for chemokines in the initiation of iniation of atherosclerosis. *Nature* 394: 894–897, 1998.
6. **Boyd AW, Wawryk SO, Burns GF, and Fecondo JV.** Intercellular adhesion molecule-1 (ICAM-1) has a central role in cell-cell contact-mediated immune mechanisms. *Proc Natl Acad Sci USA* 85: 3095–3099, 1988.
7. **Combe C, Burton CJ, Dufourco P, Weston S, Horsburgh T, Walls J, and Harris KP.** Hypoxia induces intercellular adhesion molecule-1 on cultured human tubular cells. *Kidney Int* 51: 1703–1709, 1997.
8. **Crispin J, Robert JOD, Rebecca M, and John RS.** Comparison of therapeutic and subtherapeutic nasal continuous positive airway pressure for obstructive sleep apnoea: a randomised prospective parallel trial. *Lancet* 353: 2100–2105, 1999.
9. **Entman ML and Ballantyne CM.** Inflammation in acute coronary syndromes. *Circulation* 22: 800–803, 1993.
10. **Gerszten RE, Gercia-Zepeda EA, Lim YC, Yoshida M, Ding HA, Gimbrone MA Jr, Luster AD, Luscinskas FW, and Rosenzweig A.** MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. *Nature* 398: 718–723, 1999.
11. **Haught WH, Mansour M, Rothlein R, Kishimoto TK, Mainolfi EA, Hendricks JB, Hendricks C, Mehta JL, Fla G, and Conn R.** Alterations in circulating intercellular adhesion molecule-1 and L-selectin: further evidence for chironic inflammation in ischemic heart disease. *Am Heart J* 132: 1–8, 1996.
12. **He J, Kryger MH, Zorick FJ, Conway W, and Roth T.** Mortality and apnea index in obstructive sleep apnea. *Chest* 94: 9–14, 1988.
13. **Hirani N, Antonicelli F, Strieter RM, Wiesener MS, Ratcliffe PJ, Haslett C, and Donnelly SC.** The regulation of interleukin-8 by hypoxia in human macrophages: a potential role in the pathogenesis of the acute respiratory distress syndrome (ARDS). *Mol Med* 7: 685–697, 2001.
14. **Hojo Y, Ikeda U, Takahashi M, and Shimada K.** Increased level of monocyte-related cytokines in patients with unstable angina. *Atherosclerosis* 161: 403–408, 2002.
15. **Hung J, Whitford EG, Parsons RW, and Hillman DR.** Association of sleep apnoea with myocardial infarction in men. *Lancet* 336: 261–264, 1990.
16. **Kang BH, Crapo JD, Wegner CD, Letts LG, and Chang LY.** Intercellular adhesion molecule-1 expression on the alveolar epithelium and its modification by hyperoxia. *Am J Respir Cell Mol Biol* 9: 350–355, 1993.

17. **Levinson PD, McGarvey ST, Carlisle CC, Eveloff SE, Herbert PN, and Millaman RP.** Adiposity and cardiovascular risk factors in men with obstructive sleep apnea. *Chest* 103: 1336–1342, 1993.
18. **Marlin SD and Springer TA.** Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell* 51: 813–819, 1987.
19. **Montefort S, Lai CKW, Kapahi P, Leung J, Lai KN, Chan HS, Haskard DO, Howarth PH, and Holgate ST.** Circulating adhesion molecules in asthma. *Am J Respir Crit Care Med* 149: 1149–1152, 1994.
20. **Morisaki N, Saito I, Tamura Tashiro J K, Masuda M, Kanzaki T, Watanabe S, Masuda Y, and Saito Y.** New indices heart disease and aging: studies on the serum levels of soluble intercellular adhesion molecule-1 (ICAM-1) and soluble vascular cell adhesion molecule-1 (VCAM-1) in patients with hypercholesterolemia and ischemic heart disease. *Atherosclerosis* 131: 43–48, 1997.
21. **Nagase T, Fukuchi Y, Matsuse T, Sudo E, Matsui H, and Orimo H.** Antagonism of ICAM-1 attenuates airway and tissue responses to antigen in sensitized rats. *Am J Respir Crit Care Med* 151: 1244–1249, 1995.
22. **Nagase T, Ohga E, Sudo E, Katayma H, Uejima Y, Matsuse T, and Fukuchi Y.** Intercellular adhesion molecule-1 mediates acid aspiration-induced lung injury. *Am J Respir Crit Care Med* 154: 504–510, 1996.
23. **Noda A, Okada T, Hayashi H, Yasuma F, and Yokota M.** 24-Hour ambulatory blood pressure variability in obstructive sleep apnea syndrome. *Chest* 103: 1343–1347, 1993.
24. **Ohga E, Nagase T, Tomita T, Teramoto S, Matsuse T, Katayama H, and Ouchi Y.** Increased levels of circulating ICAM-1, VCAM-1, and L-selectin in obstructive sleep apnea syndrome. *J Appl Physiol* 87: 10–14, 1999.
25. **Partinen M, Jamieson A, and Guilleminault C.** Long-term outcome for obstructive sleep apnea syndrome patients. *Chest* 94: 1200–1204, 1988.
26. **Ridker PM, Hennekens CH, Roitman-Johnson B, Stampfer MJ, and Allen J.** Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. *Lancet* 351: 88–92, 1998.
27. **Robert JOD and John RS.** The efficacy of nasal continuous positive airway pressure in the treatment of obstructive sleep apnea syndrome is proven. *Am J Respir Crit Care Med* 161: 1775–1778, 2000.
28. **Roep BO, Heidenthal E, de Vries RRP, Kolb H, and Martin S.** Soluble forms of intercellular adhesion molecule-1 in insulin-dependent diabetes mellitus. *Lancet* 343: 1590–1593, 1994.
29. **Rothlein R, Mainolfi EA, and Czajkowski M.** A form of circulating ICAM-1 in human serum. *J Immunol* 147: 3788–3793, 1991.
30. **Shi Q, Le X, Abbruzzese JL, Wang B, Mujaida N, Matsushima K, Huang S, Xiong Q, and Xie K.** Cooperation between transcription factor AP-1 and NF-kappaB in the induction of interleukin-8 in human pancreatic adenocarcinoma cells by hypoxia. *J Interferon Cytokine Res* 19: 1363–1371, 1999.
31. **Simonini A, Moscucci M, Muller DW, Bates ER, Pagani FD, Burdick MD, and Strieter RM.** IL-8 is an angiogenic factor in human coronary atherectomy tissue. *Circulation* 101: 1519–1526, 2000.
32. **Springer TA.** Adhesion receptors of the immune system. *Nature* 346: 425–434, 1990.
33. **Stoohs RA, Facchini F, and Guilleminault C.** Insulin resistance and sleep-disordered breathing in healthy humans. *Am J Respir Crit Care Med* 154: 170–174, 1996.
34. **Smith CW, Rothlein R, Hughes BJ, Mariscalco MM, Rudloff HE, Schmalstieg FC, and Anderson DC.** Recognition of an endothelial determinant for CD18-dependent human neutrophil adherence and transendothelial migration. *J Clin Invest* 82: 1746–1756, 1988.
35. **Wegner CG, Gundel RH, Reilly P, Haynes N, Letts LG, and Rothlein R.** Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 247: 456–459, 1990.
36. **Wilson SJ, Wallin A, Della-Cioppa G, Sandstorm T, and Holgate ST.** Effects of budesonide and formoterol on NF-kappaB, adhesion molecules, and cytokines in asthma. *Am J Respir Crit Care Med* 164: 1047–1052, 2001.
37. **Zund G, Uezono S, Stahl GL, Dzus AL, McGowan FX, Hickey PR, and Colgan SP.** Hypoxia enhances induction of endothelial ICAM-1: role for metabolic acidosis and proteasomes. *Am J Physiol Cell Physiol* 273: C1571–C1580, 1997.

Am J Physiol Lung Cell Mol Physiol 283: L963–L970, 2002.
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Attenuation of antigen-induced airway hyperresponsiveness in CGRP-deficient mice

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[1]*Departments of Geriatric Medicine and* [2]*Cardiovascular Medicine, Graduate School of Medicine, University of Tokyo, Tokyo 113-8655; and* [3]*Division of Integrative Cell Biology, Department of Embryogenesis, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto 862-0976, Japan*

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Aoki-Nagase, Tomoko, Takahide Nagase, Yoshio Ohhashi, Takayuki Shindo, Yukiko Kurihara, Yasuhiro Yamaguchi, Hiroshi Yamamoto, Tetsuji Tomita, Eijiro Ohga, Ryozo Nagai, Hiroki Kurihara, and Yasuyoshi Ouchi. Attenuation of antigen-induced airway hyperresponsiveness in CGRP-deficient mice. *Am J Physiol Lung Cell Mol Physiol* 283: L963–L970, 2002. First published June 21, 2002; 10.1152/ajplung.00130.2002.—Bronchial hyperresponsiveness and eosinophilia are major characteristics of asthma. Calcitonin gene-related peptide (CGRP) is a neuropeptide that has various biological actions. In the present study, we questioned whether CGRP might have pathophysiological roles in airway hyperresponsiveness and eosinophilia in asthma. To determine the exact roles of endogenous CGRP in vivo, we chose to study antigen-induced airway responses using CGRP gene-disrupted mice. After ovalbumin sensitization and antigen challenge, we assessed airway responsiveness and measured proinflammatory mediators. In the sensitized CGRP gene-disrupted mice, antigen-induced bronchial hyperresponsiveness was significantly attenuated compared with the sensitized wild-type mice. Antigen challenge induced eosinophil infiltration in bronchoalveolar lavage fluid, whereas no differences were observed between the wild-type and CGRP-mutant mice. Antigen-induced increases in cysteinyl leukotriene production in the lung were significantly reduced in the CGRP-disrupted mice. These findings suggest that CGRP could be involved in the antigen-induced airway hyperresponsiveness, but not eosinophil infiltration, in mice. The CGRP-mutant mice may provide appropriate models to study molecular mechanisms underlying CGRP-related diseases.

asthma; bronchial hyperreactivity; eosinophilia; leukotriene; knockout mouse

BRONCHIAL HYPERRESPONSIVENESS and inflammation, including eosinophilia, are major characteristics of asthma (11, 12, 23). Recent studies have shown that various mediators, including cytokines, eicosanoids, and adhesion molecules, are involved in the development of asthma. Genetic features are also potentially associated with the etiology of asthma. On the basis of the inheritance pattern, a number of genes could have substantial roles in the pathogenesis of bronchial asthma (43). However, the exact molecular mechanisms of bronchial asthma remain to be elucidated.

Calcitonin gene-related peptide (CGRP), a 37-amino acid neuropeptide, has various biological actions, including responses to sensory stimuli, cardiovascular regulation, and vasodilation (2, 3, 19, 20). CGRP belongs to the calcitonin family of peptides, which includes calcitonin, amylin, and adrenomedullin. The calcitonin receptor-like receptor functions as a CGRP receptor in the presence of receptor activity-modifying protein 1 (RAMP1) (24). There are two CGRP isoforms: α-CGRP, which is present in the central and peripheral nervous system (42), and β-CGRP, which is expressed in specific neuronal sites (1). It has been shown that CGRP, a potent vasodilator (22), modulates hypoxic pulmonary vasoconstriction (17). Recent studies using genetically engineered mice have shown that CGRP-knockout mice exhibit increased blood pressure and overactivation of the sympathetic nervous system (38).

In the respiratory system, CGRP is synthesized by sensory C-fibers throughout the respiratory tree (47). CGRP is also found in neuroepithelial cells of the lung and coexists with tachykinins in many airway sensory nerves (20), and CGRP receptors have been found to densely populate lung vessels (17). In terms of its physiological role, it has been reported that CGRP potently constricts airway smooth muscle in humans (39) and guinea pigs (41). In addition, it has been shown that CGRP has a significant role in eosinophilia in allergic inflammation (7, 37). On the basis of these observations, it is assumed that CGRP might be involved in the pathogenesis of bronchial asthma.

In the present study, we questioned whether α-CGRP might have pathophysiological roles in airway

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