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Induction of Cyclooxygenase-2 in Alveolar Macrophages after Acid Aspiration

Selective Cyclooxygenase-2 Blockade Reduces Interleukin-6 Production

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Background: **Gastric acid aspiration can result in acute lung injury. In this study, the authors determined whether alveolar macrophages express cyclooxygenase-2 as a source of inflammatory mediators after acid aspiration.**

Methods: **Seventy-five microliters of hydrochloric acid solution, *p*H 1.15, was instilled into one lung in mice. After exposure, alveolar macrophages were harvested, and competitive polymerase chain reaction and enzyme-linked immunosorbent assay were performed to measure expression of cyclooxygenase-1 and -2, interleukin-1β and -6, tumor necrosis factor-α, and inducible nitric oxide synthase (iNOS). The authors used immunocytochemistry to demonstrate expression of cyclooxygenase-2 in alveolar macrophages. Selective cyclooxygenase-2 blockade using *N*-2(-cyclohexyloxy-4-nitrophenyl) methane-sulphonamide was done to characterize prostaglandin–cytokine interaction.**

Results: **Acid aspiration induced upregulation of cyclooxygenase-2 and interleukin-6. Tumor necrosis factor-α and iNOS were not upregulated. Interleukin-1β was upregulated even with saline instillation but could not be detected in the supernatant of the cell culture. Alveolar macrophages harvested from mice instilled with acid showed a trend toward more production of prostaglandin E_2 and produced higher concentrations of interleukin-6 compared with alveolar macrophages from mice instilled with saline. Selective cyclooxygenase-2 blockade significantly decreased release of interleukin-6 from alveolar macrophages harvested from mice instilled with acid.**

Conclusions: **Acid aspiration induces strong expression of cyclooxygenase-2 and production of interleukin-6 in alveolar macrophages. Selective cyclooxygenase-2 blockade reduced production of interleukin-6 by acid-stimulated alveolar macrophages. These studies suggest that the induction of cyclooxygenase-2 plays an important role in the systemic inflammatory response induced by acid aspiration. (Key words: Competitive PCR; immunocytochemistry; murine model; NS-398; prostaglandin E_2; proinflammatory cytokine.)**

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GASTRIC acid aspiration can result in acute lung injury. The mechanism causing this injury has been shown to involve proinflammatory mediators, including cytokines and lipid mediators.[1] Numerous attempts have been made to reduce lung and systemic organ injury by blocking the chemical mediators released from stimulated cells.[2-4] The alveolar macrophage, situated at the air-tissue interface in the alveoli, is the first cell to encounter injurious stimuli and to release various chemical mediators in the early stages of the inflammatory response.[5]

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The importance of prostanoids produced by prostaglandin G/H synthase, also known as *cyclooxygenase,* in acid aspiration has been described previously.[6,7] Two isoforms of cyclooxygenase are now known: cyclooxygenase-1 and cyclooxygenase-2. Cyclooxygenase-1 is a 65-kDa protein, constitutively expressed in most tissues, notably the kidney, stomach, vascular smooth muscle,[8] and platelets.[9] Enzyme expression remains fairly constant, and cyclooxygenase-1 is thought to be responsible for "housekeeping" functions, such as gastric protection and renal blood flow regulation *via* tissue-specific production of prostaglandins.[10] In 1989, a second, inducible form of the cyclooxygenase enzyme, cyclooxygenase-2, was identified.[10] Cyclooxygenase-2 is a 70-kDa protein and is 65% homologous to cyclooxygenase-1.[11] Unlike cyclooxygenase-1, however, cyclooxygenase-2 is highly inducible in certain cell types after stimulation with various substances. Cyclooxygenase-2 is responsible for the production of large amounts of prostaglandins in the early stages of the inflammatory response. Macrophages are thought to be a major source of prostaglandins produced from upregulation of cyclooxygenase-2.[12–14]

The regulation of cytokines by prostaglandins in inflammatory states has been described.[15,16] Of the major proinflammatory cytokines, interleukin (IL)-6 appears to be most closely regulated by prostaglandins.[13,17] IL-6 is a multifunctional cytokine that has been implicated in a variety of inflammatory conditions. Prostaglandin E_2 (PGE_2) produced by cyclooxygenase-2 has been demonstrated to be responsible for increased concentrations of IL-6 in inflammatory conditions *in vivo.*[13,17]

We studied the role of cyclooxygenase-2 in alveolar macrophages after acid aspiration, and by using a specific cyclooxygenase-2 blocker we investigated the interaction between cyclooxygenase-2 and various proinflammatory cytokines early in the inflammatory response.

Methods

All animal experiments were performed in compliance with the guidelines given by the Committee on Animal Research of the University of California, San Francisco.

Acid Aspiration in Mice

Eight- to twelve-week-old male C57BL/6 mice (body weights, 25–30 g) were purchased from Simonsen Laboratories (Gilroy, CA). The mice were fed regular rodent chow and water *ad libitum.* Mice were anesthetized briefly with inhaled methoxyflurane (Metofane; Pitman-Moore, Inc., Mundelein, IL). The acid group was instilled with 75 μl hydrochloric acid solution (*p*H 1.15, 0.1 N, endotoxin-free; Sigma Chemical Co., St. Louis, MO) mixed with normal saline (normal saline:hydrochloric acid ratio was 1:2). The saline group was instilled with 75 μl of one third normal saline solution (normal saline:H_2O ratio was 1:2) into one lung *via* a blunt end feeding needle (24 G; Popper and Sons, Inc., New Hyde Park, NY) inserted orally.[18] Mice were allowed to recover from anesthesia and breathe room air spontaneously for the experimental period. The treatment group received a specific cyclooxygenase-2 blocker, *N*-2(-cyclohexyloxy-4-nitrophenyl) methane-sulphonamide (NS-398; Biomol, Plymouth Meeting, PA); each mouse was given 250 μg intraperitoneally, 2.5 mg/ml suspended in dimethyl sulfoxide, 1 h before acid instillation.[12,19] The nontreatment group received sham treatments of 100 μl dimethyl sulfoxide by intraperitoneal injection.

Harvest of Alveolar Macrophages by Bronchoalveolar Lavage

Mice were reanesthetized 2 and 4 h after acid instillation with 50 mg/kg of pentobarbital sodium. Their tracheas were exposed, and 20-gauge stub adapters were inserted and secured. Lungs were lavaged with cold 0.1% ethylenediaminetetraacetic acid/phosphate-buffered saline (PBS; 5 ml). Lavage fluid from two or three mice was pooled to obtain enough alveolar macrophages for analysis. Lavage fluid was centrifuged for 10 min at 4°C at 1,000 $\times$ *g* to separate the cells from supernatant. Cells were washed and resuspended in PBS (5 ml). Alveolar macrophage numbers and viability were quantified by hemacytometer with trypan blue staining ($>$ 90%). Macrophage concentration was adjusted to 1 $\times$ 10^6 cells/ml in RPMI1640 supplemented with 10% heat-inactivated fetal bovine serum and antibiotic agents. Macrophages were purified by allowing them to adhere to the bottom of the wells by incubation for 1 h in multiwell tissue culture plates. Nonadherent cells were removed by replacing the RPMI medium.

Semiquantitative Competitive Polymerase Chain Reaction for Determination of Levels of Cyclooxygenase-1, Cyclooxygenase-2, and Cytokine Genes in Messenger Ribonucleic Acid

Total ribonucleic acid (RNA) was extracted from 5 $\times$ 10^5 freshly harvested alveolar macrophages with TRIzol reagent (Gibco BRL, Gaithersburg, MD). RNA concen-

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trations were measured carefully by a microspectrophotometer (Spectramax; Molecular Devices, Menlo Park, CA), and 2.0 μg total RNA was reverse transcribed using reverse transcriptase, M-MLV (Promega, Madison, WI), and oligo-dT_{12-18} primers (Gibco BRL). The following specific primers for cyclooxygenase-1 and cyclooxygenase-2 were made for polymerase chain reaction (PCR):

cyclooxygenase-1
- 5′-primer: 5′-GGT TGA GGC ACT GGT GGA TGC CTT C-3′
- 3′-primer: 5′-AGA CAG ACC CGT CAT CTC CAG GGT A-3′

cyclooxygenase-2
- 5′-primer: 5′-ACT CAC TCA GTT TGT TGA GTC ATT C-3′
- 3′-primer: 5′-TTT GAT TAG TAC TGT AGG GTT AAT G-3′.

The size of the PCR products from cyclooxygenase-1 and cyclooxygenase-2 are 524 and 583 base pairs, respectively. The specificity of the PCR reaction for cyclooxygenase-1 and cyclooxygenase-2 complementary deoxyribonucleic acid (cDNA) was confirmed by digestion of PCR products by sequence-specific restriction enzymes (cyclooxygenase-1: Sac I and Sma I; cyclooxygenase-2: Pst I and Nco I). For quantitation of cyclooxygenase-1 and cyclooxygenase-2 mRNA levels, the *Escherichia coli* plasmid *pT2M* containing specific internal standards for cyclooxygenase-1 and cyclooxygenase-2 was constructed by internal deletion of sequences: The truncated fragments (320 base pairs) from cyclooxygenase-1 and cyclooxygenase-2 PCR products were incorporated into the *E. coli* cloning plasmid *pBSII SK(+)* (Stratagene, La Jolla, CA) at the EcoR I and Kpn I restriction sites and multiplied as plasmids in an *E. coli* strain *Db5* α. Plasmid solutions were adjusted to 1×10^4 molecules/μl after double digestion by restriction enzymes (EcoR I and Kpn I) and used as internal standards for competitive PCR. For IL-1β, IL-6, tumor necrosis factor (TNF)-α, inducible nitric oxide synthase (iNOS), and hypoxanthine-guanine phospho-ribosyltransferase (HPRT), a multiple cytokine competitor construct *pLOC* (provided by David B. Corry, University of California, San Francisco, San Francisco, CA) was used with specific cytokine primers as described.[20,21] We obtained confirmation that the cDNA samples contained the same amount of DNA by measuring the constitutively expressed HPRT gene first. The amounts of cDNA for cyclooxygenase-1, cyclooxygenase-2, and the various cytokine genes were measured based on this standardization, and the ratio of wild-type genes to competitor genes was amplified by PCR. For HPRT measurement, 1×10^5 competitor molecules were added. Polymerase chain reaction was performed for 35 cycles with the following conditions: 30 s at 94°C for denaturing DNA, 20 s at 55–60°C annealing temperature, and 40 s at 72°C for extension of the reaction. Products of PCR were separated by electrophoresis on 1.5% agarose gels and visualized by ethidium bromide staining.

Immunocytochemical Detection of Cyclooxygenase-2 in Alveolar Macrophages

Alveolar macrophages were harvested 4 h after instillation. Cells were counted with a hemacytometer, and cell viability was calculated. After washing the cells once with PBS, they were resuspended in PBS, and 1×10^5 cells were attached to glass slides by cytospin preparation. Slides were air-dried and stored at −80°C until staining. Immunocytochemistry was performed using the avidin-biotinylated alkaline phosphatase complex method (Vectastain ABC-AP complex kit; Vector, Burlingame, CA). Frozen slides were brought to room temperature and fixed in 2% paraformaldehyde. Cells were permeabilized with 0.1% triton-X/PBS. All washing procedures were performed with high-salt PBS (2.7% NaCl/PBS). Blocking of nonspecific reactions to the secondary antibody was performed with 5% normal goat serum plus 10% normal horse serum in PBS. Slides were incubated with the primary antibody (polyclonal anti-PGHS-2, polyclonal rabbit immunoglobulin G; or control, preimmune rabbit serum; Oxford Biomedical Res., Inc., Oxford, MI) at $^1/_{50}$ dilution in the blocking buffer (overnight at 4°C). After the secondary antibody (goat anti-rabbit immunoglobulin G, biotinylated anti-rabbit immunoglobulin G; Vector) was applied (1 μg/ml in blocking buffer for 30 min at room temperature), slides were incubated with the avidin-biotinylated alkaline phosphatase complex and developed with the substrate (Fast Red TR/Naphthol AS-MX; Sigma). After mounting, slides were viewed at high magnification and photographed. Cells were counted at low magnification (100 cells per visual area), and the ratio of positively stained cells was calculated.

Alveolar Macrophage Culture

Alveolar macrophages harvested from mice as described in the harvesting method were cultured in RPMI1640 at 37°C in a humidified atmosphere containing 5% CO_2 for 24 h at a concentration of 1×10^6 cells/ml, 200 μl/well, in 96-well tissue culture plates.

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When indicated, NS-398, a specific cyclooxygenase-2 blocker, was added to the culture medium at 100-μM concentration at the beginning of the incubation. Culture media was collected after 24 h of incubation and stored at −80°C until assayed for PGE_2, cytokines, or nitrite. Cell viability was 90–95% at the end of the 24-h incubation period, in media with or without NS-398.

Determination of Prostaglandin E_2 Concentrations by Enzyme Immunoassay

Prostaglandin E_2 was measured in the supernatant of the primary culture medium of alveolar macrophages by PGE_2 enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). Each sample was measured in triplicate. The specificity of this assay for PGE_2 is 100%. The lower detection limit was 5 pg/ml.

Measurement of Cytokines by Enzyme-linked Immunosorbent Assay

Enzyme-linked immunosorbent assays were performed for IL-1β, IL-6 and TNF-α as previously described.[18] The supernatant of the alveolar macrophage culture was used for measurements. For IL-6, microtiter plates (enzyme-linked immunosorbent assay plate, Easy wash; Corning, Cambridge, MA) were coated overnight at 4°C with capture antibody (rat anti-mouse IL-6 monoclonal antibody, MP5-20F3, rat immunoglobulin G_1; PharMingen, San Diego, CA) and blocked with 3% bovine serum albumin/PBS for 2 h at room temperature. Recombinant standards and samples were then added to plates and incubated for 4 h at room temperature. After incubation with biotinylated developing antibody (rat anti-mouse IL-6 monoclonal antibody, MP5-32C11, rat immunoglobulin G_{2a}; PharMingen) for 45 min at room temperature and alkaline phosphatase-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA) for 45 min at 37°C, plates were developed with *p*-nitrophenyl-phosphate (Sigma 104) dissolved in 0.1 M alkaline buffer solution (2-amino-2-methyl-1-propanol buffer, 1.5 M, *p*H 10.3, Sigma 221) and read at an OD of 405 nm using a microplate reader. Plates were washed two to six times with 0.05% Tween/PBS between each procedure. Sample concentrations were calculated by comparison with a standard curve of mouse recombinant IL-6 (PharMingen). Interleukin-1β and TNF-α were measured with the same methods using the following reagents: rabbit anti-mouse IL-1β polyclonal antibody, biotinylated anti-mouse IL-1β monoclonal antibody (1400.24.17, mouse immunoglobulin G_1), recombinant mouse IL-1β (all from Endogen, Cambridge, MA), rat anti-mouse TNF-α monoclonal antibody (MP6-XT22, rat immunoglobulin G_1), biotinylated rabbit anti-mouse TNF-α polyclonal antibody, and recombinant mouse TNF-α (all from PharMingen). Each sample was measured in triplicate. Lower detection limits for IL-1β, IL-6, and TNF-α were 50.0, 1.0, and 5.0 pg/ml, respectively.

Measurement of Metabolites of Nitric Oxide

Nitrite concentrations in the supernatant of the alveolar macrophage culture were measured by Griess reaction. The reagents were prepared immediately before assay. One hundred microliters of supernatant was mixed with 100 μl Griess reagent (final concentration, 1% sulfanilamide, 0.1% *n*-(1-naphthyl)-ethylene-diamine, 1.7% H_3PO_4) and incubated for 30 min in the dark. Absorbance was measured at 550 nm using a microplate reader. Concentrations were determined using a $NaNO_2$ standard (0.3–100.0 μM).

Data Analysis

Results are expressed as mean ± SD. Comparisons between groups were performed with a one-way analysis of variance, and a Bonferroni/Dunn test was used for multiple comparison. A probability value <0.05 was considered statistically significant and is identified with an asterisk in the figures.

Results

Expression of the Cyclooxygenase-2 Gene in Alveolar Macrophages after Acid Aspiration

In the control group, mRNA levels for the cyclooxygenase-1 and cyclooxygenase-2 genes in alveolar macrophages were lower than 10% of the HPRT mRNA (fig. 1B, rows 1 and 2). Saline instillation did not affect these levels. Two hours after acid instillation, however, a larger amount of cyclooxygenase-2 mRNA was detected; this large amount was the same at 4 h (fig. 1B, row 2). In contrast, the cyclooxygenase-1 mRNA was not increased after acid instillation (fig. 1B, row 1).

Levels of Proinflammatory Cytokine Genes in Messenger Ribonucleic Acid in Alveolar Macrophages after Acid Aspiration

We found more IL-1β gene expression in alveolar macrophages from mice instilled with either saline or acid compared with the control mice (fig. 1B, row 3). There was a slight increase in IL-6 gene expression in alveolar

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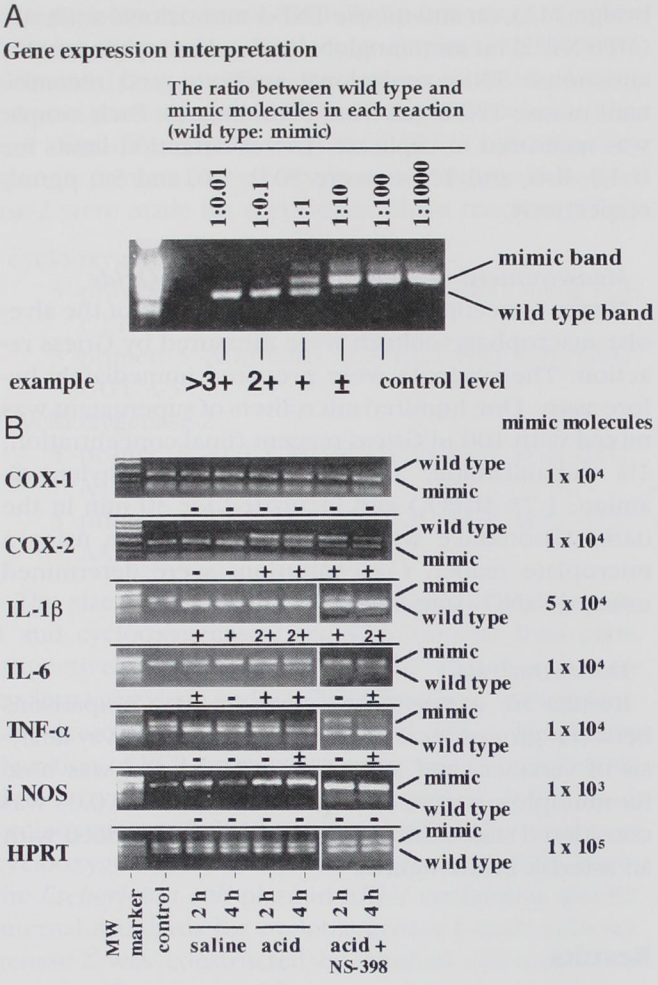

Fig. 1. (*A*, upper) Interpretation of competitive polymerase chain reaction (PCR). The PCR in this figure was run with a set amount of complementary deoxyribonucleic acid (cDNA), adding increasing amounts of competitor—increasing by 10-fold going from the left lane to the right. It demonstrates the ratio of mimic to wild-type genes in each gel picture. For semi-quantitation, we designated ±, +, 2+, and 3+ for each 10-fold increase from control level and estimated the amount of gene relative to control sample level. (*B*, lower) Cyclooxygenase-1, -2, and cytokine messenger ribonucleic acid (mRNA) levels estimated by competitive PCR performed with 2.0 μg total RNA extracted from alveolar macrophages from acid-instilled mice, 2 and 4 h after instillation, with and without pretreatment with NS-398. Upper bands are PCR fragments from wild-type mRNA for cyclooxygenase-1 and -2; competitors (mimic bands) are shown in the lower portion. For cytokines and hypoxanthine-guanine phospho-ribosyltransferase, competitors are upper bands. Note the cyclooxygenase-2 induction with acid instillation; the amount of mRNA was unaffected by pretreatment with NS-398 (row 2). Cyclooxygenase-1 levels are unchanged. Interleukin-1β was upregulated transiently 2 h after saline instillation but returned to control levels at 4 h. Interleukin-6 was upregulated at 2 and 4 h after acid instillation and decreased with cyclooxygenase-2 blockade (row 4). Tumor necrosis factor-α concentrations changed minimally, and iNOS mRNA levels did not change (rows 5 and 6). Each lane represents pooled macrophages from three mice. Each experiment was repeated three times. Representative results are shown in this figure. MW marker = molecular weight marker.

macrophages transiently 2 h after saline instillation, but the level was the same as that of controls at 4 h. In the acid group, however, there was more IL-6 gene expression at 2 h after acid instillation compared with the control group, and it remained at this level after 4 h (fig. 1B, row 4). Messenger RNA levels of TNF-α and iNOS genes were not different among control, saline, and acid groups (fig. 1B, rows 5 and 6).

Intense Cyclooxygenase-2 Expression in the Cytoplasm of Alveolar Macrophages after Acid Aspiration

Two different staining patterns for cyclooxygenase-2 in alveolar macrophages were detected from immunocytochemical analysis. One pattern was the localized staining of the perinuclear membrane, suggesting minimal expression of cyclooxygenase-2 (fig. 2A). The other pattern was strong staining not only in the perinuclear region but also in the cytoplasmic region, indicating high expression of cyclooxygenase-2 (fig. 2B). Almost all macrophages showed baseline regional perinuclear cyclooxygenase-2 expression. None of the alveolar macrophages from control mice showed cytoplasmic expression of cyclooxygenase-2. In the saline group, <1% of the cells (0.8 ± 1.5%) showed cytoplasmic expression. Significantly more alveolar macrophages (11.3 ± 4.4%) harvested from mice instilled with acid showed the strong cytoplasmic cyclooxygenase-2 expression ($P < 0.01$).

Alveolar Macrophages Produce High Concentrations of Prostaglandin E_2 and Interleukin-6 after Acid Aspiration

In 24-h culture media, we detected a trend of release of more PGE_2 from alveolar macrophages harvested 1 h after acid instillation compared with saline instillation (fig. 3). A significantly higher level of IL-6 production also was detected in the alveolar macrophages harvested after acid instillation compared with the saline group (fig. 4). The concentrations of IL-1β and TNF-α were lower than detectable levels in the culture media

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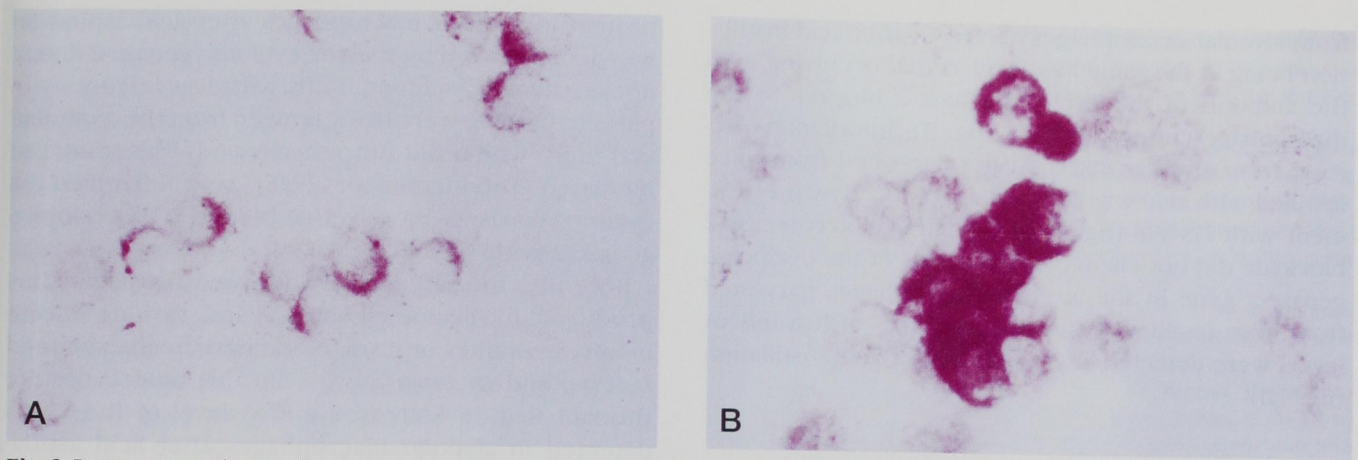

Fig. 2. Immunocytochemical staining of alveolar macrophages showing two patterns of staining. (*A*) Minimal localized cyclooxygenase-2 staining of the perinuclear membrane of alveolar macrophages. (*B*) Strong staining found not only in the perinuclear region but also in the cytoplasmic region, demonstrating high expression of cyclooxygenase-2.

from all groups of macrophages (data not shown). Low concentrations of nitric oxide metabolites ($NO_2^- < 3$ μM) were detected in all groups of the macrophage culture, and there was no significant difference between groups (data not shown).

Selective Cyclooxygenase-2 Blockade Reduces Interleukin-6 Release from Alveolar Macrophages after Acid Aspiration

Pretreatment of mice with the selective cyclooxygenase-2 blocker, NS-398, resulted in the PGE_2 production

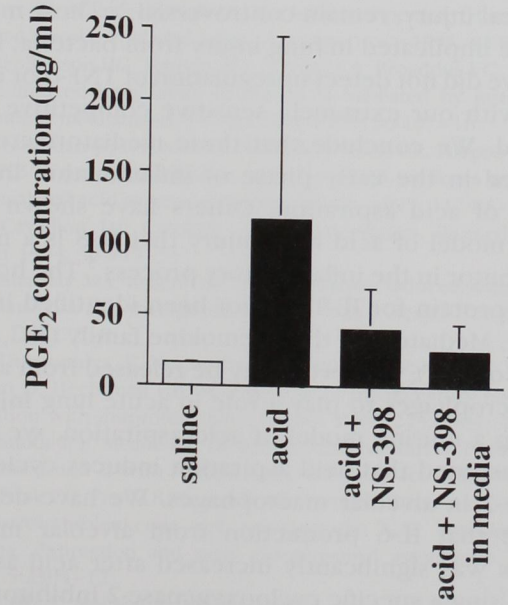

Fig. 3. Prostaglandin E_2 levels in 24-h alveolar macrophage culture supernatant. Macrophages were freshly harvested from mice instilled with acid or saline, and cell concentration was adjusted to 1×10^6 cells/ml. Cells were suspended in media, and 2×10^5 cells/200 μl media of each sample was aliquoted into 96-well microplates. Values are mean ± SD. The experiment was repeated three times. No statistical significance was found.

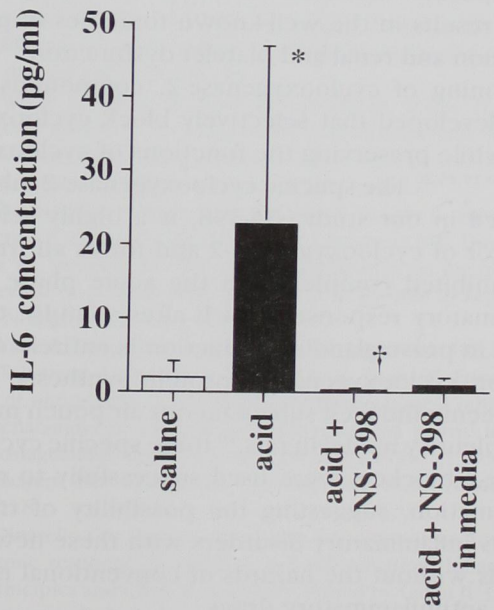

Fig. 4. Interleukin-6 concentrations in 24-h alveolar macrophage culture supernatant. Macrophages were freshly harvested from mice instilled with acid or saline, and cell concentration was adjusted to 1×10^6 cells/ml. Cells were suspended in media, and 2×10^5 cells/200 μl media of each sample was aliquoted into 96-well microplates. Values are mean ± SD. The experiment was repeated three times. *$P = 0.006$ *versus* saline group; †$P = 0.0054$ *versus* acid group.

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from alveolar macrophages harvested after acid instillation being at the same low level as that occurring with the addition of the cyclooxygenase-2 blocker to the macrophage culture medium (fig. 3). Interleukin-6 release from alveolar macrophages harvested from mice instilled with acid was significantly reduced by pretreatment with NS-398 (fig. 4). Although cyclooxygenase-2 blockade did not change mRNA levels of the cyclooxygenase-2 gene in the alveolar macrophages harvested from mice instilled with acid, decreases of IL-6 mRNA levels were detected at 2 and 4 h after acid instillation (fig. 1B).

Discussion

The role of prostaglandins in acid aspiration remains controversial.[22] In canine acid aspiration models, nonsteroidal antiinflammatory drugs have been used successfully to reduce lung edema and improve pulmonary function,[7,23] whereas other investigators have found that nonsteroidal antiinflammatory drugs improved the physiologic status of the lung but did not change mortality in sepsis.[24]

Nonspecific blockade of both cyclooxygenase-1 and -2 results in the well-known toxicities of gastric ulceration and renal and platelet dysfunction.[12] Since the cloning of cyclooxygenase-2, compounds have been developed that selectively block cyclooxygenase-2 while preserving the functions of cyclooxygenase-1.[12,19,25,26] The specific cyclooxygenase-2 inhibitor we used in our study, NS-398, is a highly selective inhibitor of cyclooxygenase-2 and forms an irreversibly inhibited complex.[19] In the acute phase of an inflammatory response (2–4 h after stimuli), the increase in prostaglandin production is entirely dependent on cyclooxygenase-2 protein synthesis.[16] In a carrageenin-induced subcutaneous air pouch model[20] and a pleurisy model in rats,[26] these specific cyclooxygenase-2 blockers were used successfully to reduce inflammation, suggesting the possibility of treating various inflammatory disorders with these new compounds without the hazards of conventional nonsteroidal antiinflammatory drugs.

In this study, significant induction of cyclooxygenase-2 was detected in alveolar macrophages after acid aspiration. First, the marked upregulation of cyclooxygenase-2 was confirmed by measurement of mRNA levels using a highly sensitive method, competitive PCR.[27] Second, intense expression of the cyclooxygenase-2 protein in alveolar macrophages after acid aspiration was demonstrated by specific cyclooxygenase-2 immunocytochemical staining. Cells with less intense cytoplasmic staining were likely lavaged from the contralateral lung, which did not receive acid. The source of increased production of PGE_2 was identified as cyclooxygenase-2 by selective blockade of cyclooxygenase-2 with NS-398.

Recently, the interactions between prostaglandins produced by cyclooxygenase-2 and various inflammatory cytokines or nitric oxide have been examined *in vivo* and *in vitro*.[16,17,28–30] In this model, despite the fact that an increased mRNA level of IL-1β was detected in alveolar macrophages after acid aspiration, the relation of IL-1β released from alveolar macrophages to cyclooxygenase-2 upregulation was unclear, as similar upregulation also was observed in alveolar macrophages from mice instilled with saline. In addition, no detectable concentrations of IL-1β were measured in the macrophage culture medium. We found a close relationship between cyclooxygenase-2 and IL-6, however; increased production of IL-6 in alveolar macrophages after acid aspiration was prevented by cyclooxygenase-2 blockade. The roles of TNF-α[1,31] and nitric oxide in acid aspiration, a chemical injury, remain controversial.[32] These mediators are implicated in lung injury from bacteria; however, we did not detect upregulation of TNF-α or iNOS even with our extremely sensitive competitive PCR method. We conclude that these mediators are not involved in the early phase of inflammation in our model of acid aspiration. Others have shown in a rabbit model of acid lung injury that IL-8 is a major contributor in the inflammatory process.[3] The homologous protein for IL-8 has not been identified in the mouse. Mediators of the chemokine family (KC, MIP-2, and others), however, may be released from alveolar macrophages to play a role in acute lung injury.

Using a murine model of acid aspiration, we have demonstrated that acid aspiration induces cyclooxygenase-2 in alveolar macrophages. We have demonstrated that IL-6 production from alveolar macrophages was significantly increased after acid aspiration. Using a specific cyclooxygenase-2 inhibitor, we also have shown that cyclooxygenase-2–derived prostanoids were responsible for induction of macrophage IL-6 production. It is well established that IL-6 plays a major role in the propagation of inflammatory responses, such as fever induction in the hypothalamus and induction of the acute phase response in the

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liver.[33,34] Interleukin-6-deficient mice were found to have impaired fever induction and acute phase response to inflammatory stimuli.[35] In addition, IL-6 production correlates with clinical outcome in the systemic inflammatory response syndrome.[36,37] In this model, the upregulation of IL-6 was reduced by selective cyclooxygenase-2 blockade. This result is consistent with previous reports using a rat arthritis model[17] and peritonitis model[13] in that induction of cyclooxygenase-2 is responsible for the increase in PGE_2 concentrations, which in turn induces IL-6.

We conclude that cyclooxygenase-2 is strongly induced in alveolar macrophages after acid aspiration, and specific blockade of cyclooxygenase-2 may be used in decreasing production of inflammatory mediators such as PGE_2 and IL-6, which may cause tissue injury.

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