Reduction of neutrophil influx diminishes lung injury and mortality following phosgene inhalation

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GHIO, ANDREW J., THOMAS P. KENNEDY, GARY E. HATCH, AND JEFFREY S. TEPPER. *Reduction of neutrophil influx diminishes lung injury and mortality following phosgene inhalation.* J. Appl. Physiol. 71(2): 657–665, 1991.—Phosgene inhalation causes a severe noncardiogenic pulmonary edema characterized by an influx of neutrophils into the lung. To study the role of neutrophils in lung injury and mortality after phosgene, we investigated the effects of leukocyte depletion with cyclophosphamide, inhibiting the generation of the chemotaxin leukotriene B_4 with the 5-lipoxygenase inhibitor AA861 and impairing neutrophil migration with the microtubular poison colchicine. Cyclophosphamide, AA861, and colchicine injected before exposure significantly reduced percent neutrophils, protein, and thiobarbituric acid-reactive products in bronchoalveolar lavage fluid of rats exposed to phosgene (0.5 ppm × 60 min). Cyclophosphamide, AA861, and colchicine also significantly decreased mortality from phosgene (2.0 ppm × 90 min) in mice. Colchicine significantly reduced neutrophil influx, lung injury, and mortality even when given 30 min after phosgene exposure. We conclude that lung injury and mortality after phosgene exposure are associated with an influx of neutrophils into the lung. Prevention of neutrophil migration with colchicine may hold therapeutic potential in phosgene poisoning.

cyclophosphamide; leukotrienes; colchicine; respiratory function tests; respiratory distress syndrome, adult; rat; mouse

PHOSGENE ($COCl_2$) is a toxic gas used commercially as a basic monomer in the synthesis of a wide variety of industrial agents. Chemically it reacts with a multitude of nitrogen, oxygen, sulfur, and carbon centers to acylate, chlorinate, decarboxylate, and dehydrate (1). The clinical presentation and lung morphology observed after inhalation of phosgene are similar to those after oxidant gas exposure and include a clinical latent period followed by noncardiogenic pulmonary edema and death at some time after a significant concentration × time product is attained (6). The mechanism of phosgene toxicity may be in part the consequence of oxidant injury (17, 18). Like oxidant gases, phosgene can generate compounds with unpaired electrons (1). After exposure of phosgene to organic compounds with unsaturated carbon-carbon bonds, carbon-centered free radicals are formed that can behave as oxidants to initiate damage to the pulmonary alveolar-capillary membrane (1).

In several forms of lung injury, the original causative agent or event produces chemotaxins that recruit polymorphonuclear leukocytes into the lung, resulting in magnification of injury (29). This sequence of events is illustrated by pulmonary oxygen toxicity, in which rapid worsening of lung injury and death are heralded by the influx of neutrophils into the lung (11), and morbidity and mortality are reduced by depleting neutrophils (28) or inhibiting production of leukotriene B_4 (LTB_4), the chemotaxin directing their influx (30). As a result of the association of lung neutrophils with phosgene inhalation (5), we tested the hypothesis that lung injury and mortality from phosgene were also associated with a LTB_4-directed influx of neutrophils.

METHODS

Reagents. Cyclophosphamide was purchased from Mead Johnson (Evansville, IN). Colchicine was from Eli Lilly (Indianapolis, IN), and halothane was from Halocarbon Laboratories (Hackensack, NJ). 2,3,5-trimethyl-6-(12-hydroxy)-5,10-dodecadiynyl)-1,4-benzoquinone (AA861) was generously donated by Takeda Chemical Industries (Osaka, Japan). All other reagents were purchased from Sigma Chemical (St. Louis, MO).

Animals. Sixty-day-old male Sprague-Dawley rats weighing 250–300 g (Charles River Breeding Laboratories, Wilmington, MA) were used within 1 wk of their arrival. Thirty-day-old female CD-1 mice weighing 25–30 g (Charles River) were used for mortality studies. Animals were housed in temperature- and humidity-controlled rooms and fed a standard diet (Ralston Purina, St. Louis, MO). Food and water were available ad libitum except while the animals were in the exposure chambers.

Phosgene exposures. Animals were placed in individual stainless steel wire-mesh cages and exposed in Rochester-type chambers. A mass-flow controller (Tylan, Torrance, CA) metered phosgene (300 ppm in nitrogen, Matheson Gas Products, Morrow, GA), with an airflow of 0.32 m^3/min. The gas mixture passed downward through the cages holding the test animals and was exhausted at the bottom into a water scrubber. Temperature and humidity in the chambers were adjusted to 23.0 ± 3.4°C and 60 ± 10%, respectively. Control animals were placed simultaneously in an identical chamber and allowed to breathe filtered air.

Phosgene concentrations in the chamber were deter-

mined every 10 min with a Hewlett Packard 11831 gas chromatograph containing a 6 ft × ⅛-in. Teflon column filled with Chromosil 310 packing (Supelco, Bellefonte, PA) operating at 70°C. Methane (5%) in argon was used as the mobile phase at a flow rate of 43 cm^3/min. Phosgene was detected by electron capture. A Wilks model 80 infrared analyzer was employed as a second check of the phosgene concentration. Uniform distribution of phosgene was ensured by sampling from various areas while animals were in the chambers. The chamber phosgene concentrations were maintained within 2–6% of the intended target concentrations.

Experimental protocols. Six separate protocols were conducted. In the first, we studied the effect of neutrophil depletion on phosgene lung injury. Rats were depleted of neutrophils by intraperitoneal injection of 100 mg/kg cyclophosphamide 4 days before and 75 mg/kg the day of phosgene exposure. Controls were treated with injection of a similar volume of saline. To verify depletion, blood leukocytes were counted using a hemocytometer and percentage of neutrophils was determined from blood smears stained with crystal violet 1% (wt/vol) in acetic acid 4% (vol/vol). Four groups were studied: rats exposed to phosgene 0.5 ppm × 60 min without treatment, rats exposed to phosgene 0.5 ppm × 60 min after neutrophil depletion, rats exposed to air without treatment, and rats exposed to air after neutrophil depletion. Lung injury was measured immediately within 2 h of the completion of the exposure (during the clinical latent period) and 24 h after exposure. The total number of rats studied was 48 (6 per group at the 2 times specified).

In the second protocol, we used the 5-lipoxygenase inhibitor AA861 (30) to study the effect on phosgene lung injury of inhibiting lung generation of the neutrophil chemotaxin LTB_4. Rats were treated intraperitoneally with 5 mg/kg AA861 at 24 h, 12 h, and immediately before exposure. Four groups (n = 6 per group) were studied: rats exposed to phosgene 0.5 ppm × 60 min without treatment, rats exposed to phosgene 0.5 ppm × 60 min after AA861, rats exposed to air without treatment, and rats exposed to air after AA861. Lung injury was studied 24 h after exposure. In identically treated groups of rats, LTB_4 was measured in bronchoalveolar lavage fluid immediately and 24 h after exposure to phosgene 0.5 ppm × 60 min and air.

In the third protocol, the microtubular inhibitor colchicine, which impairs neutrophil chemotaxis (21), was used to study the effect on phosgene lung injury of inhibiting neutrophil migration into the lung. Rats were treated with 1.0 mg/kg colchicine intraperitoneally 1 h before exposure. This treatment had no significant effect on neutrophil counts in the peripheral blood. Four groups (n = 6 per group) were studied: rats exposed to phosgene 0.5 ppm × 60 min without treatment; rats exposed to phosgene 0.5 ppm × 60 min after colchicine; rats exposed to air without treatment; and rats exposed to air after colchicine. Lung injury was studied 24 h after exposure. In separate but identically treated animals, bronchoalveolar lavage content of LTB_4 was measured immediately and 24 h after exposure. In a final set of experiments the effect of colchicine on phosgene-induced changes in lung function was evaluated. Lung volumes,

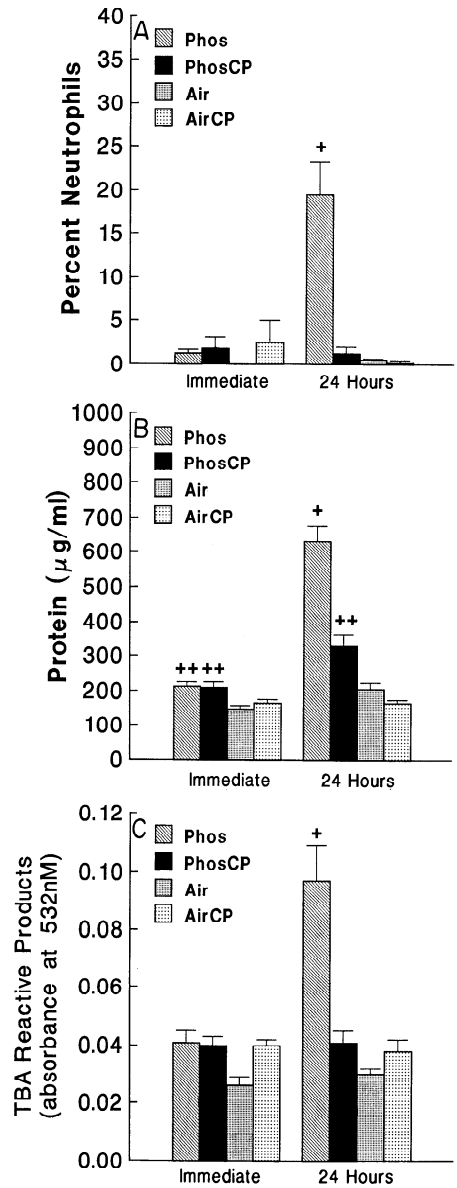

FIG. 1. Percent neutrophils, protein, and thiobarbituric acid-(TBA) reactive products in lavage fluid of rats exposed to phosgene after depletion of leukocytes by pretreatment with cyclophosphamide. Values are means ± SE. Phosgene has no immediate effect on percent neutrophils (P = 0.70) or TBA-reactive products (P = 0.58), but protein is increased (P = 0.005) and is unaffected by leukocyte depletion. Leukocyte depletion prevents influx of neutrophils (P = 0.0001) and decreases content of protein (P < 0.0001) and TBA-reactive products (P = 0.0003) 24 h after phosgene exposure. Phos, phosgene exposed and injected with saline; PhosCP, phosgene exposed after leukocyte depletion with cyclophosphamide; Air, air exposed and injected with saline; AirCP, air exposed after leukocyte depletion with cyclophosphamide. $^{+}P < 0.05$ compared with all other groups; $^{++}P < 0.05$ compared with air-exposed rats.

diffusing capacity, and peak expiratory flow were assessed in a group of 32 rats (n = 8 per group) 24 h after exposure to phosgene 1.0 ppm × 60 min and air. A higher dose of phosgene was necessary in these experiments to induce injury reflected by changes in lung function.

In the fourth protocol we studied the combined effects of depletion of neutrophils and inhibition of their migration on phosgene lung injury. Rats were treated with cyclophosphamide, colchicine 1.0 mg/kg ip, or the combina-

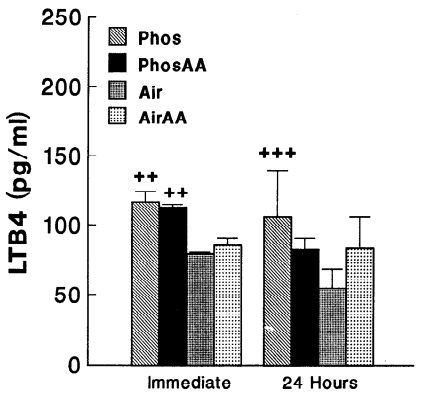

FIG. 2. Leukotriene B_4 (LTB_4) in lavage fluid of rats exposed to phosgene after pretreatment with 5-lipoxygenase inhibitor AA861. Values are means ± SE. LTB_4 is increased by phosgene ($P = 0.007$). AA861 does not attenuate increase in lavage LTB_4 after exposure. Phos, phosgene exposed and injected with saline; PhosAA, phosgene exposed after treatment with AA861; Air, air exposed and injected with saline; AirAA, air exposed after treatment with AA861. $^{++}P < 0.05$ compared with air-exposed and air-exposed treated with AA861 rats; $^{+++}P < 0.05$ compared with air-exposed rats.

tion of both treatments before phosgene 0.5 ppm × 60 min. Four groups (n = 5 per group) were studied: rats exposed to phosgene without treatment; rats exposed to phosgene after neutrophil depletion; rats exposed to phosgene after colchicine; and rats exposed to phosgene after both neutrophil depletion and colchicine. Lung injury was assessed 24 h after phosgene exposure.

The fifth protocol examined the potential therapeutic utility of colchicine. Colchicine (1.0 mg/kg) was injected 30 min after exposure. Four groups (n = 6 per group) were studied: rats exposed to phosgene 0.5 ppm × 60 min without treatment; rats exposed to phosgene 0.5 ppm × 60 min and then treated with colchicine; rats exposed to air without colchicine; and rats exposed to air and then treated with colchicine. Lung injury was assessed 24 h after exposure. To evaluate the dose-response relationship of lung injury to colchicine therapy, total lavage protein was determined at 24 h after both exposure to varying concentrations of phosgene (0–120 ppm × min) and postexposure treatment with varying doses of colchicine (0–1.0 mg/kg). There were six rats at each concentration of colchicine at the four different exposures (n = 96).

In the final protocol, the effect of interventions on mortality was studied. Mice rather than rats were employed as a result of expense. Eighty mice were exposed to phosgene 2.0 ppm × 90 min. One-half were sham-treated, and the other one-half treated with one of four interventions: neutrophil depletion with cyclophosphamide before exposure; pretreatment with AA861 at 24 h, 12 h, and immediately before exposure; 1.0 mg/kg colchicine 1 h before exposure; and 1.0 mg/kg colchicine 30 min after exposure. After phosgene exposure, survivors were counted every 6 h for 48 h and daily for an additional 5 days. All deaths were observed between 6 and 48 h after exposure. Results are reported as survivors beyond 48 h.

Measurement of lung injury. After anesthesia with halothane inhalation (2–5%), rats were exsanguinated through the abdominal aorta and the lungs were collapsed by puncturing the diaphragm. A cannula was inserted into the trachea and secured with a ligature. The volume of saline injected was determined from published allometric equations and equaled ~90% of the total lung capacity. Saline was withdrawn after a 3-s pause, reinjected an additional two times with similar delays, and then stored on ice. The volume of lavage fluid recovered from each animal was recorded. Mean return of fluid was 80% (7.7 ml), and there were no significant differences between groups.

Lavage fluid was centrifuged at 700 g for 10 min to remove cells. The pellet was resuspended in 5 ml of Geys balanced salt solution (Microbiological Associates, Bethesda, MD). An aliquot of this cell suspension was pelleted on a microscope slide using a cytocentrifuge (Shandon Southern Instruments, Sewickley, PA) at 300 g for 3 min, dried, and stained with modified Wright's stain (Diff-Quick stain; American Scientific Products, McGaw Park, IL). The percentage of neutrophils rather than an absolute cell number in lavage fluid was used to measure neutrophil influx, because we have previously documented this to be a more sensitive index of phosgene lung injury (5). Cell differential counts were enumerated by counting 500 cells per sample. Values were expressed as the percentage of total cells recovered.

Supernatant fractions of the lavage fluid were stored at −70°C until they could be assayed for protein and thiobarbituric acid (TBA) reactivity. Lavage protein was selected as a sensitive index of injury to the respiratory epithelial barrier. TBA-reactive products were chosen as a simple measure of oxidative damage. Lavage protein was determined using a modification of Lowry's method (20). TBA-reactive products were assayed in 1.0 ml of lavage fluid by adding 1.0 ml of 1% (wt/vol) TBA plus 1.0 ml of 2.8% (wt/vol) trichloracetic acid. This was heated at 100°C for 10 min, cooled, and centrifuged, and the concentration of the resulting chromophore was determined by its absorbance at 532 nm.

Measurement of LTB_4. LTB_4 was assayed using a commercially available radioimmunoassay kit (Advanced Magnetics, Cambridge, MA). Analysis employed [^{3}H]-LTB_4 tracer in phosphate-buffered saline and dextran-coated charcoal as the separation technique. Concentrations were measured in 100 μl of supernatant lavage fluid after elution through C_{18} Sep-Pak cartridges (Waters Associates, Milford, MA).

Measurement of pulmonary function. Rats were anesthetized with 2–5% halothane, intubated with an endotracheal tube, and placed supine in an acrylic constant-volume whole body plethysmograph. The endotracheal tube was connected to a bias flow of air through a low dead space (0.5-ml) valving system on the plethysmograph face plate. A computer-controlled solenoid valve was used to occlude the airway at the end of a tidal breath for measurement of end-expiratory volume. Airway pressure was measured at a side port of the face plate. Total lung capacity, residual volume, and multiple breath diffusing capacity (DL_{CO}) were determined using neon gas dilution. Pulmonary flow-volume properties were assessed in lungs slowly inflated to +30 cmH_2O and then rapidly deflated to −40 cmH_2O pressure to generate the maximum expiratory flow-volume curve for each animal. Analog output from conditioned transducer signals was digitized using a PDP 11/73+ microcomputer system

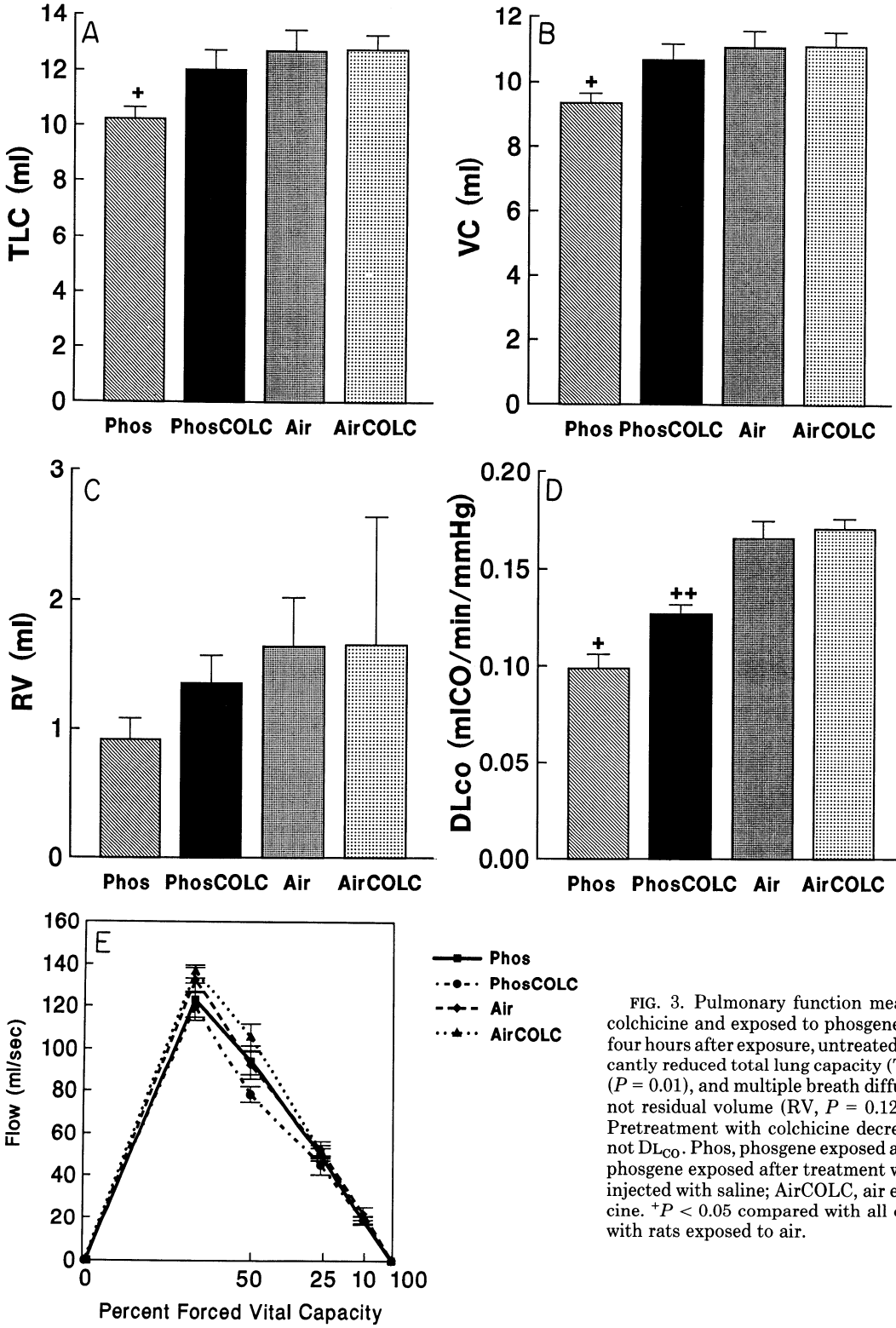

FIG. 3. Pulmonary function measurements of rats pretreated with colchicine and exposed to phosgene. Values are means ± SE. Twenty-four hours after exposure, untreated phosgene-exposed rats have significantly reduced total lung capacity (TLC) ($P = 0.01$), vital capacity (VC) ($P = 0.01$), and multiple breath diffusing capacity ($D_{L_{CO}}$) ($P = 0.02$) but not residual volume (RV, $P = 0.12$) or flow-volume curve ($P = 0.22$). Pretreatment with colchicine decreased fall in TLC, RV, and VC but not $D_{L_{CO}}$. Phos, phosgene exposed and injected with saline; PhosCOLC, phosgene exposed after treatment with colchicine; Air, air exposed and injected with saline; AirCOLC, air exposed after treatment with colchicine. $^{+}P < 0.05$ compared with all other groups; $^{++}P < 0.05$ compared with rats exposed to air.

(Digital Equipment, Maynard, MA). The data were computed using FORTRAN 77 programs.

Statistical analysis. Data are expressed as mean ± SE, and t tests of independent means and analysis of variance were used to determine differences between two and multiple groups, respectively (4). When F ratios were significant, means were compared using Duncan's multiple range testing. For mortality studies, χ^2 values were calculated and used to analyze differences in total survival (4). Significance was assumed at $P < 0.05$.

RESULTS

Phosgene caused marked lung injury. Phosgene had no significant effect on percent neutrophils (Fig. 1*A*) or TBA-reactive products (Fig. 1*C*) in lavage fluid immedi-

TABLE 1. *Effect of cyclophosphamide on numbers of white blood cells in rats and mice*

	Saline Treated	Cyclophosphamide Treated
	Rats	
Leukocyte count	10,250±182	188±10
Neutrophil count	1,979±142	10±4
	Mice	
Leukocyte count	4,800±650	370±204
Neutrophil count	769±75	50±18

Values are group means ± SE in mm^{-3} for 16 rats and mice. Cyclophosphamide significantly decreased both leukocyte and neutrophil counts in rats (t = 102.2, P = 0.005; t = 27.0, P = 0.01, respectively) and mice (t = 10.4, P = 0.03; t = 15.4, P = 0.02, respectively).

ately after exposure. However, both lavage fluid protein (Fig. 1*B*) and LTB_4 (Fig. 2) were increased immediately after phosgene. Twenty-four hours after exposure, percent neutrophils (Fig. 1*A*), protein (Fig. 1*B*), TBA-reactive products (Fig. 1*C*) and LTB_4 (Fig. 2) were all elevated in lavage fluid. Dissimilarities in percent neutrophils, lavage protein, and lavage TBA-reactive products in air-exposed groups were interpreted as normal variability of the measures. Phosgene also caused a significant decrease in lung volumes (Fig. 3, *A–C*) and DL_{CO} (Fig. 3*D*) at 24 h but had no effect on the flow volume curve (Fig. 3*E*).

Cyclophosphamide significantly decreased leukocyte and neutrophil counts in the blood of both rats and mice (Table 1). Leukocyte depletion before exposure significantly decreased neutrophil influx, protein, and TBA-reactive products in lavage fluid 24 h after phosgene (Fig. 1, *A–C*).

AA861 also dramatically reduced percent neutrophils, protein, and TBA-reactive products in lavage fluid 24 h after phosgene exposure (Fig. 4, *A–C*). LTB_4 elevation after phosgene was unaffected by AA861 (Fig. 2).

Pretreatment with colchicine significantly reduced the increase in neutrophils, protein, and TBA-reactive products found in lavage fluid 24 h after phosgene exposure (Fig. 5, *A–C*). Colchicine also protected against the fall in lung volumes and DL_{CO} after phosgene (Fig. 3, *A–D*). However, colchicine had no significant effect on phosgene-induced increases in lavage fluid LTB_4 (immediately after exposure, LTB_4 concentrations were 149 ± 5 pg/ml for phosgene and 184 ± 12 pg/ml for phosgene treated with colchicine; 24 h after exposure, concentrations were 128 ± 17 pg/ml for phosgene and 175 ± 37 pg/ml for phosgene treated with colchicine).

The combination of leukocyte depletion with cyclophosphamide and inhibition of neutrophil migration with colchicine was not superior to either intervention alone. The phosgene-induced increase of percent neutrophils, protein, and TBA-reactive products in lavage fluid were all significantly decreased by leukocyte depletion, colchicine, or both (Fig. 6, *A–C*). However, there were no differences in percent neutrophils or indexes of lung injury among the three interventions.

Colchicine reduced phosgene lung injury even when given after exposure. Postexposure treatment with colchicine significantly decreased percent neutrophils, protein, and TBA-reactive products in lavage fluid 24 h after exposure (Fig. 7, *A–C*). A comparison of the effect of varying doses of colchicine given after different doses of phosgene showed that the effect of colchicine was most pronounced at the highest dose of both drug (1.0 mg/kg) and phosgene (120 ppm × min), but there was significant protection of injury employing colchicine 0.1 mg/kg (Fig. 8).

The effect of each treatment intervention on mortality 48 h after phosgene exposure is shown in Table 2. All interventions significantly reduced mortality after phos-

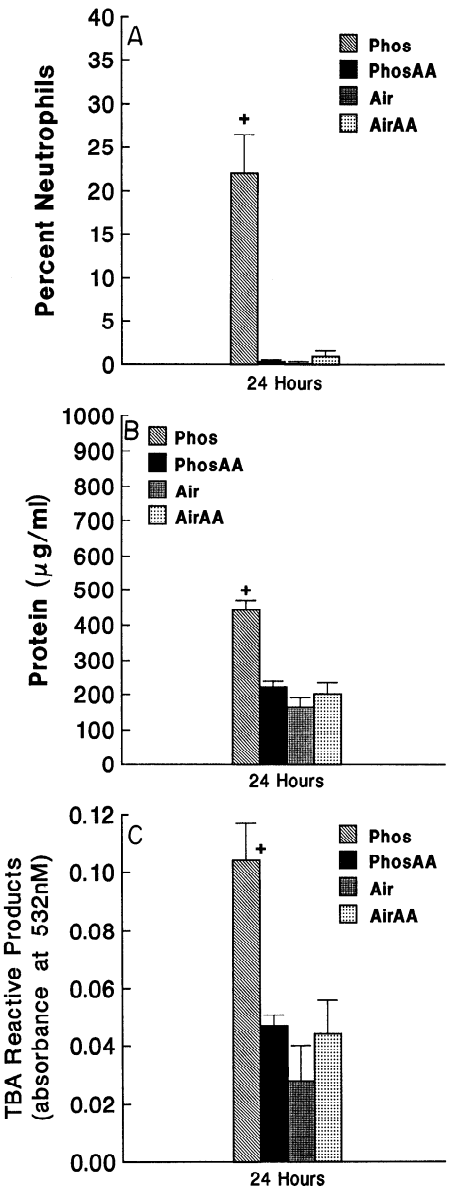

FIG. 4. Percent neutrophils, protein, and TBA-reactive products in lavage fluid of rats exposed to phosgene after pretreatment with 5-lipoxygenase inhibitor AA861. Values are means ± SE. AA861 prevents influx of neutrophils (P = 0.02) and decreases content of protein (P = 0.004) and TBA-reactive products (P = 0.007) 24 h after phosgene exposure. Phos, phosgene exposed and injected with saline; PhosAA, phosgene exposed after treatment with AA861; Air, air exposed and injected with saline; AirAA, air exposed after treatment with AA861. $^+P < 0.05$ compared with all other groups.

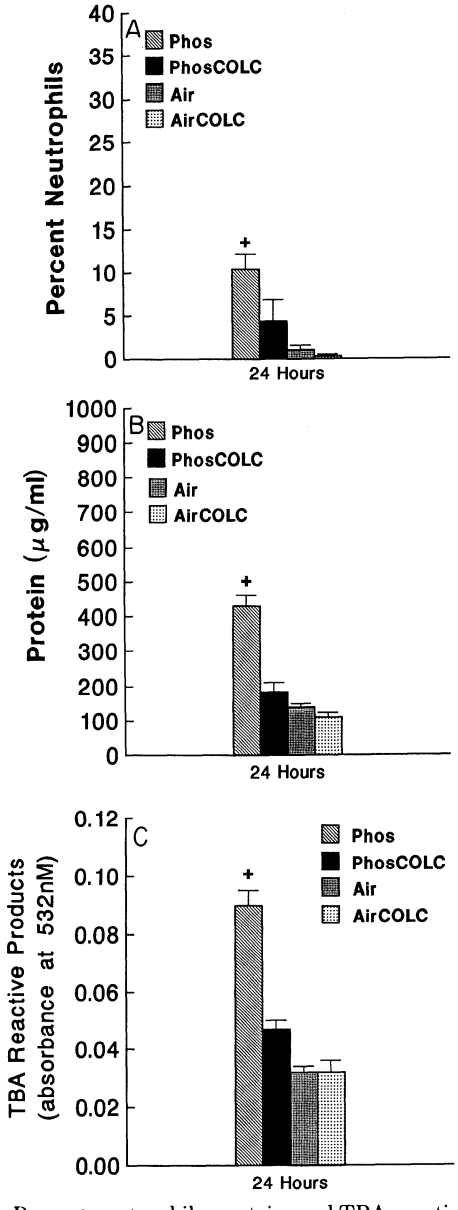

FIG. 5. Percent neutrophils, protein, and TBA-reactive products in lavage fluid of rats pretreated with microtubular inhibitor colchicine and exposed to phosgene. Values are means ± SE. Colchicine prevents increase in percentage neutrophils ($P = 0.0001$) and decreases protein ($P < 0.0001$) and TBA-reactive products ($P = 0.0005$) in lavage fluid after phosgene. Phos, phosgene exposed and injected with saline; PhosCOLC, phosgene exposed after treatment with colchicine; Air, air exposed and injected with saline; AirCOLC, air exposed after treatment with colchicine. $^{+}P < 0.05$ compared with all other groups.

gene. Colchicine protected against death even when the treatment was given after phosgene exposure.

DISCUSSION

Polymorphonuclear leukocytes have been implicated in the pathogenesis of several forms of lung injury, including adult respiratory distress syndrome (31), emphysema (14), interstitial fibrosis (15), and pulmonary oxygen toxicity (11, 28). In this study we have shown that neutrophil migration into the lung plays an important role in magnifying the intensity of lung injury after inhalation of phosgene. Phosgene exposure of rats caused an immediate increase in lavage fluid protein, but the increase in protein was substantially greater by 24 h (Fig. 1*B*), corresponding to the time when increased neutrophils were present in bronchoalveolar lavage fluid (Fig. 1*A*). Surprisingly, there was no increase in lavage fluid TBA-reactive products immediately after phosgene exposure, but a marked elevation was present by 24 h (Fig. 1*C*). The phosgene-induced increases in lavage fluid neutrophils, protein, and TBA-reactive products were prevented by depleting neutrophils with cyclophosphamide (Fig. 1, *A–C*), inhibiting generation of chemotactic 5-lipoxygenase products with AA861 (Fig. 4, *A–C*), and impairing neutrophil chemotaxis with colchicine (Fig. 4, *A–*

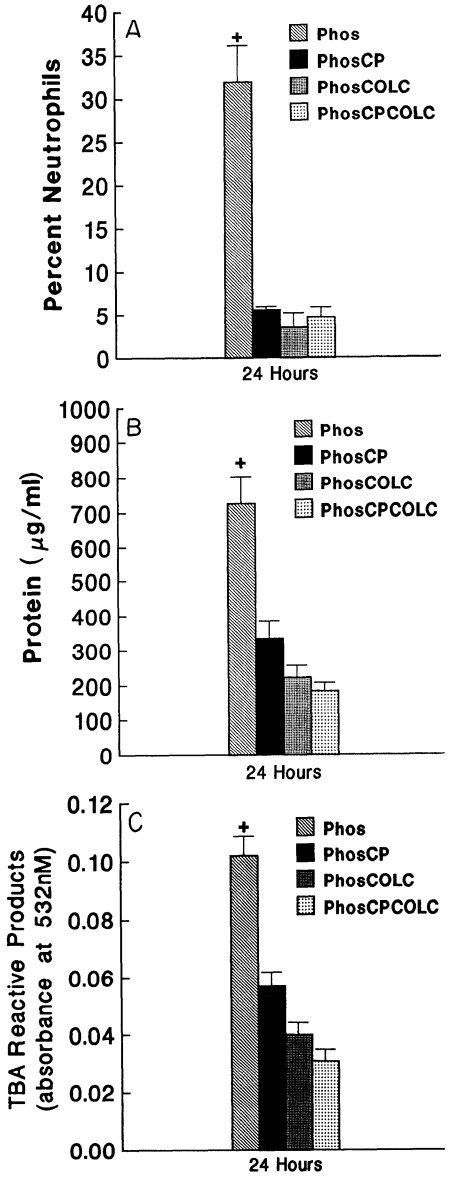

FIG. 6. Percent neutrophils, protein, and TBA-reactive products in lavage fluid of rats pretreated with cyclophosphamide, colchicine, or both and exposed to phosgene. Values are means ± SE. Depleting leukocytes with cyclophosphamide, impairing neutrophil migration with colchicine, or combination of both therapies provided equivalent protection from phosgene lung injury. Phos, phosgene exposed and injected with saline; PhosCP, phosgene exposed after leukocyte depletion with cyclophosphamide; PhosCOCL, phosgene exposed after treatment with colchicine; PhosCPCOCL, phosgene exposed after treatment with both cyclophosphamide and colchicine. $^{+}P < 0.05$ compared with all other groups.

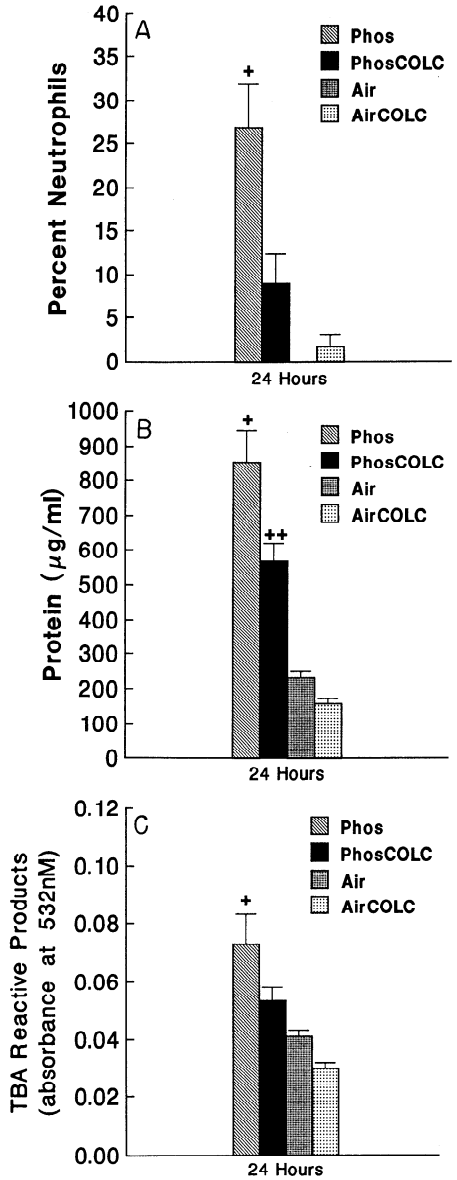

FIG. 7. Percent neutrophils, protein, and TBA-reactive products in lavage fluid of rats exposed to phosgene and treated with colchicine 30 min after exposure. Values are means ± SE. Colchicine significantly reduced percent neutrophils (P = 0.006), protein (P = 0.0002), and TBA-reactive products (P = 0.03) even when given after phosgene exposure. Phos, phosgene exposed and then injected with saline; PhosCOCL, phosgene exposed and then treated with colchicine; Air, air exposed and then treated with colchicine. $^{+}P < 0.05$ compared with all other groups; $^{++}P < 0.05$ compared with air-exposed rats.

C). These interventions also significantly decreased mortality from phosgene exposure (Table 2). Finally, colchicine reduced the fall in lung volumes (Fig. 3, *A–C*) and DL_{CO} (Fig. 3*D*) observed 24 h after phosgene. Examined as a whole, these data suggest that a substantial degree of lung injury after the inhalation of phosgene is mediated by influx of neutrophils into the lung.

We have previously shown that phosgene causes an increase in pulmonary alveolar-capillary membrane permeability (17, 18). In this study lavage protein was selected as a sensitive index of injury to the respiratory epithelial barrier. Similarities of banding patterns from polyacrylamide gel electrophoresis of protein from serum and lavage fluid after ozone exposure suggest that

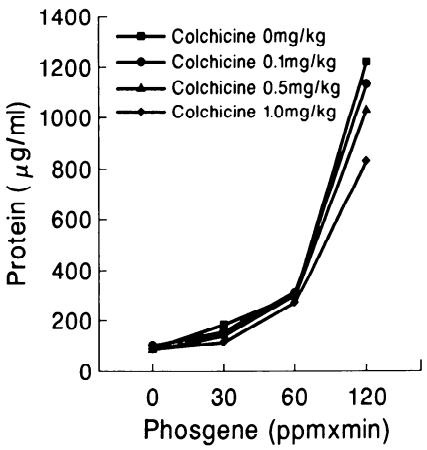

FIG. 8. Lavage fluid protein in rats exposed to varying doses of phosgene (0–120 ppm × min) and treated 30 min after exposure with varying doses of colchicine (0–1.0 mg/kg). Values are means ± SE. Colchicine exerts a beneficial effect even at lowest dose of 0.1 mg/kg.

lavage protein after oxidant gas inhalation is derived from serum (27). However, we cannot be certain whether all of the protein in lavage fluid after phosgene inhalation was from increased alveolar-capillary membrane permeability or might have been due in part to increases in lavage fluid surfactant (13). An increase in the apoprotein of pulmonary surfactant might also contribute to increased lavage protein after phosgene.

As a consequence of phosgene inhalation, lung volumes (TLC and vital capacity) and DL_{CO} declined by 24 hours after exposure, while airway function remained unaffected. Injury to the pulmonary alveolar-capillary epithelial barrier results in an increased transport of fluid into the alveolar compartment. The lung's ability to clear this excess fluid through the lymphatics is exceeded, and accumulation of edema fluid in both the interstitial and alveolar compartments of the lung begins. This alveolar and interstitial edema is presumed responsible for both decreased pulmonary function and, at larger phosgene doses, mortality. Among the pulmonary function parameters measured, diffusion capacity was the most sensitive indicator of lung damage after phosgene inhalation. DL_{CO} decreases early after phosgene exposure in both

TABLE 2. *Effect of treatment intervention on mortality*

Intervention	Alive	Dead
Leukocyte depletion		
Untreated	17	23
Treated	29	11
Preexposure AA861		
Untreated	8	32
Treated	21	19
Preexposure colchicine		
Untreated	6	34
Treated	30	10
Postexposure colchicine		
Untreated	8	32
Treated	17	23

Values are no. of mice (40/group). Leukocyte depletion, AA861, and colchicine before phosgene 2.0 ppm × 90 min increased surviving mice (χ^2 = 6.19, P = 0.02; χ^2 = 5.59, P = 0.03; and χ^2 = 26.72, $P < 0.001$ respectively). Finally, colchicine treatment after phosgene 2.0 ppm × 90 min also increased numbers of mice surviving (χ^2 = 3.72; P = 0.05).

animals and humans (19). Decreases in lung volumes are also consistent with fluid accumulation. Finally, the lack of significant changes in airways function is predictable. Only at very large concentration × time values has phosgene been observed to induce bronchoconstriction (6).

Cyclophosphamide diminished both percent neutrophils in lavage fluid and lung injury 24 h after phosgene inhalation. In addition, cyclophosphamide reduced mortality after phosgene. Although it is likely that these effects are the result of neutrophil depletion, cyclophosphamide does decrease the number of platelets, which might also prevent lung injury (9). However, other strategies of preventing lung neutrophil influx, such as inhibiting production of chemotactic leukotrienes with AA861 or impairing neutrophil migration with colchicine, also protected from phosgene lung injury. In addition, neutrophil depletion attenuates oxidant lung injury, including that from hyperoxia (28) and ozone (23). Thus it is likely that the protection afforded by cyclophosphamide in our model was a consequence of depleting neutrophils.

Because previous investigations have shown that the 5-lipoxygenase product LTB_4 is a major chemotactic agent recruiting neutrophils into the lung after oxidant exposure (30), in this study we sought to determine the role of LTB_4 in mediating neutrophil migration into the lung after phosgene exposure. LTB_4 was increased in lung lavage fluid both immediately and 24 h after phosgene (Fig. 2), and neutrophil migration into the phosgene-exposed lung was prevented by the 5-lipoxygenase inhibitor AA861 (Fig. 4*A*). AA861 has been previously reported to decrease LTB_4 generation and subsequent neutrophil influx into the lung after exposure to 100% O_2 at 1 atm (30). We attribute the efficacy of AA861 in preventing damage and mortality after phosgene to its reduction in leukotriene products. However, because it failed to decrease the elevation in lavage LTB_4 after phosgene, the possibility remains that AA861 reduced phosgene lung injury by a mechanism other than inhibiting generation of chemotactic factors for neutrophils. Alternately, 5-lipoxygenase products other than LTB_4 may be important in directing neutrophils into the lung after phosgene exposure.

Colchicine was extremely effective in decreasing neutrophil influx, lung injury, and mortality from phosgene. Colchicine was even effective when given after phosgene exposure. Colchicine acts primarily as a microtubule poison by binding to tubulin and disrupting microtubule formation and function (24). After parenteral administration, colchicine is widely distributed in the body, but levels found in leukocytes are 10–12 times greater than those in plasma after a single dose (8). In other diseases such as gout, colchicine is thought to act primarily by decreasing neutrophil migration into the inflamed joint space (21). Decreased neutrophil influx, lung injury, and mortality after phosgene can be explained by an effect of colchicine to impair neutrophil migration into the injured lung. However, recently, Martin and associates (22) have suggested that LTB_4 is the predominant neutrophil chemotactic factor secreted by alveolar macrophages stimulated by either calcium ionophores or phagocytosis. The initial event in LTB_4 generation is activation of 5-lipoxygenase when it is translocated from cytosolic to membrane compartments by microtubular transport (26). Translocation of 5-lipoxygenase and subsequent LTB_4 generation can be blocked by pretreatment of cells with colchicine (24). Thus colchicine might also be effective in phosgene lung injury by preventing generation of chemotactic factors by lung macrophages or neutrophils. Against this possibility is the failure of colchicine to decrease LTB_4 in lung lavage fluid immediately or 24 h after phosgene exposure.

Oxidant gases produce lung injury both by direct production of free radicals and recruitment of activated neutrophils (7, 12). Reactive oxygen intermediates and other free radicals participate in lung injury after oxidant exposures through the direct oxidation of cellular lipids and proteins, activation of phospholipase A_2, and the liberation of arachidonic acid (3). Recruited neutrophils magnify lung injury through release of a variety of neutral proteases such as elastase, cathepsin G, and proteinase 3, as well as oxidants such as O_2-, •OH, H_2O_2, and HOCl. These proteases and oxidants are capable of degrading lung matrix elements and causing direct cytotoxicity to lung endothelium and epithelium (29). The contribution of neutrophils to various forms of lung injury is a spectrum that depends on the initial injury and is assumed to be greater when the inciting agent is a powerful neutrophil activator. Conversely, insults that act more directly on the pulmonary alveolar-capillary membrane cause an injury that may be less dependent on neutrophils. Interactions between the generation of reactive oxygen intermediates and recruitment of neutrophils in mediating damage can also occur. Oxidant exposures activate 5-lipoxygenase, which can generate chemotactic leukotrienes such as LTB_4 from liberated arachidonic acid (10). Oxygen intermediates also inactivate antiproteases, thereby enhancing toxicity of neutrophil-derived proteases (16). Finally, oxidant-induced membrane damage enhances neutrophil adherence (2). All these interactions predispose cells to further injury by neutrophils.

There is presently no effective treatment for phosgene inhalation. Evidence that colchicine is effective in preventing injury and death even when given after phosgene exposure suggests that this drug may have a potential role in the therapy of phosgene inhalation. Doses approaching 1 mg/kg cannot be given to humans without either death or severe side effects, including bone marrow suppression, disseminated intravascular coagulation, and cell injury (32). However, the dose-response study of phosgene and colchicine indicated a beneficial effect of colchicine at doses as low as 0.1 mg/kg. Colchicine is well recognized as effective in gout, another intensely inflammatory neutrophil-mediated process. Also, colchicine has recently been proposed as a treatment for fibrosing lung disorders (25). Thus colchicine might offer a strategy for decreasing neutrophil-mediated injury of the lung after phosgene inhalation and other forms of adult respiratory distress syndrome.

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