Role of the Intercellular Adhesion Molecule-1 (ICAM-1) in Endotoxin-induced Pneumonia Evaluated Using ICAM-1 Antisense Oligonucleotides, anti–ICAM-1 Monoclonal Antibodies, and ICAM-1 Mutant Mice

Toshio Kumasaka,* William M. Quinlan,* Nicholas A. Doyle,* Thomas P. Condon,‡ James Sligh,§ Fumio Takei,‖¶ Arthur L. Beaudet,§ C. Frank Bennett,‡ and Claire M. Doerschuk*

**Herman B Wells Center for Pediatric Research and the Section of Pulmonology, Department of Pediatrics, Indiana University, Indianapolis, Indiana 46202; ‡ISIS Pharmaceuticals, Carlsbad, California 92008; ‖Terry Fox Laboratory, British Columbia, Canada V5Z 1L3; ¶Department of Pathology, University of British Columbia, Vancouver, British Columbia, Canada V6T 2B5; and the §Department of Molecular and Human Genetics, Baylor College of Medicine and Howard Hughes Medical Institute, Houston, Texas 77030*

Abstract

This study examined the effectiveness of antisense oligonucleotides targeted to intercellular adhesion molecule-1 (ICAM-1) to inhibit endotoxin-induced upregulation of ICAM-1 and neutrophil emigration and compared the apparent role of ICAM-1 when examined using antisense oligonucleotides, anti–ICAM-1 antibodies, and ICAM-1 mutant mice. Antisense oligonucleotides inhibited upregulation of ICAM-1 mRNA at 4 and 24 h after instillation of endotoxin in a dose-dependent manner. Neutrophil emigration into the alveolar spaces at 24 h was inhibited by 59%, similar to inhibition using the anti–ICAM-1 antibodies 3E2 (58%) and YN1/1 (75%). No inhibition was observed in the ICAM-1 mutant compared to wild-type mice. These data show that antisense oligonucleotides targeted to ICAM-1 inhibit the endotoxin-induced upregulation of ICAM-1 in the lung and are as effective as anti–ICAM-1 antibodies in preventing neutrophil emigration. The incomplete inhibition by either antisense oligonucleotides or antibodies suggests that alternative adhesion pathways that do not require ICAM-1 are important in neutrophil emigration in the lungs. The disparity in the role of ICAM-1 when evaluated using antisense or antibodies compared to mutant mice suggests that either these inhibitors are exerting additional effects on endothelial cells other than blockade of ICAM-1 or mutant mice have upregulated the ICAM-1–independent pathways to compensate for the long-term loss of ICAM-1. (*J. Clin. Invest.* 1996. 97: 2362–2369.) Key words: adhesion molecules • lung injury • pneumonia • neutrophils • endothelial cells

Introduction

The presence of endotoxin within the distal airspaces of the lung induces an inflammatory response that results in the accumulation of neutrophils and the formation of edema within 24 h (1–5). This response is attributed to the effect of endotoxin on epithelial cells, alveolar macrophages, and endothelial cells to induce the production of cytokines. These cytokines subsequently induce upregulation of adhesion molecules and emigration of neutrophils acutely and mononuclear cells at later times.

Previous studies have shown that intraairway instillation of endotoxin-induced neutrophil emigration into the alveolar space through adhesion pathways that required CD11/CD18 and was associated with upregulation of intercellular adhesion molecule-1 (ICAM-1)[1] mRNA and protein expression on both capillary endothelial cells and type II alveolar epithelial cells (4). However, the function of ICAM-1 in mediating CD11/CD18–dependent emigration has not been clearly defined.

Antisense oligonucleotides that hybridize to a specific mRNA or pre-mRNA have the potential to inhibit the expression of the targeted molecule in vivo (6–8). Many studies have shown that antisense oligonucleotides that hybridized to message specific for cytokines, adhesion molecules, or viruses prevented upregulation of expression (9–21). In particular, antisense oligonucleotides targeted to human ICAM-1 mRNA inhibited its upregulation in human umbilical vein endothelial cells and a lung epithelial cell line, A549, in response to TNF-α (14, 15). While unmodified phosphodiester oligonucleotides are effective in vitro, the phosphorothioate oligodeoxynucleotides, in which sulfur is substituted for one of the nonbridging oxygen atoms in the phosphate backbone, are less susceptible to hydrolysis by nucleases and are potentially more effective in vivo (22, 23). In particular, the antisense oligonucleotide ISIS 3082 has been shown to be effective in delaying acute cardiac allograft rejection (17, 18), inhibiting colitis (Bennett, C.F., D. Kornbrust, S. Henry, S. Dutson, W. Hall, and H.I. Jacoby, manuscript submitted for publication), and preventing carrageenin-induced neutrophil emigration into the subcutaneous space (Bennett, C.F., unpublished data).

The purpose of these studies was to determine if the antisense oligonucleotide ISIS 3082 inhibited the upregulation of ICAM-1 expression induced by intratracheal instillation of *Escherichia coli* endotoxin into the distal airways and if inhibiting the upregulation of ICAM-1 using this technology prevented the acute inflammatory response. These studies also compared

This work was presented in part in abstract form at the American Thoracic Society meetings in Seattle, WA on 20–24 May 1995.

Address correspondence to Claire M. Doerschuk, Harvard School of Public Health, Rm I-305, 665 Huntington Avenue, Boston, MA 02115. Phone: 617-432-1706; FAX: 617-432-3468; E-mail: cdoersch@hsph.harvard.edu

Received for publication 6 November 1995 and accepted in revised form 23 February 1996.

J. Clin. Invest.

0021-9738/96/05/2362/08 $2.00
Volume 97, Number 10, May 1996, 2362–2369

1. *Abbreviations used in this paper:* ^{125}I-Albumin, ^{125}I-labeled HSA; EVA, extravascular albumin; G3PDH, glyceraldehyde 3-phosphate dehydrogenase; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.

the function of ICAM-1 when inhibited by antisense oligonucleotides, by antibodies that are specific for murine ICAM-1 and block its function, and by genetic elimination of ICAM-1 using ICAM-1 mutant mice (24).

Methods

Animals. BALB/c female mice weighing 20–25 g were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). ICAM-1 mutant mice (129/Sv × C57BL/6J) were produced using homologous recombination in embryonic stem cells as previously described (24). Wild-type mice of the same genetic background were used as controls.

Oligonucleotides. ICAM-1 antisense oligonucleotide (3082; ISIS Pharmaceuticals, Carlsbad, CA) was made against the 3′-untranslated region of the murine ICAM-1 gene (17). The unrelated oligonucleotide (1082; ISIS Pharmaceuticals) was used as a sequence control, as the A+T/C+G ratio was similar to ISIS 3082. The sequences for ISIS 3082 and ISIS 1082 are 5′-TGCATCCCCCAGGCCACCAT-3′ and 5′-GCCGAGGTCCATGTCGTACGC-3′, respectively. ISIS 1082 targets the herpes virus UL-13 gene sequence.

These phosphorothioate oligonucleotides were synthesized on a 0.5 mmol scale on a DNA synthesizer (Milligen 8800; Millipore Corp., Bedford, MA) using modified phosphoramidite chemistries with β-cyanoethoxyphosphoramidites as previously described (25). A crude product of approximately 70% purity was further purified by column chromatography using a column (HC18-HA; Millipore Corp.). The purified material was ethanol precipitated, redissolved, and further desalted by ultrafiltration. The samples were depyrogenated by ultrafiltration to yield endotoxin levels reduced to below detectable levels. Purity of the material was assessed by capillary electrophoresis, anion exchange HPLC, and nuclear magnetic resonance. The oligonucleotides were found to be greater than 92% full-length material and to contain less than 0.3 mol% phosphodiester linkages.

Monoclonal antibodies. Two blocking antimurine ICAM-1 (CD54) antibodies were used in these studies. Rat antimurine ICAM-1 monoclonal antibody YN1/1 was purified as previously described (26, 27). Rat IgG was used as a control antibody (Sigma Chemical Co., St. Louis, MO). Azide-free hamster antimurine ICAM-1 monoclonal antibody 3E2 was purchased from PharMingen (San Diego, CA). This antibody has also been shown to inhibit the function of murine ICAM-1 (28). Nonimmune hamster IgG was purchased from Pierce (Rockford, IL).

Radioisotopes. ^{125}I-labeled human serum albumin (^{125}I-albumin) was purchased from Mallinckrodt Inc. (St. Louis, MO) (#352; specific activity 8.5 μCi/mg albumin, concentration 6.67 μCi/ml and 0.8 mg albumin/ml). Thin layer chromatography using silica gel–impregnated glass fiber sheets (Gelman Sciences Inc., Ann Arbor, MI) and 80% methanol as the solvent showed that 97–99% of the I was bound to protein.

Sodium chromate-51 (^{51}Cr) was purchased from DuPont-NEN (Boston, MA) (concentration 1 mCi/ml) and used to label murine RBC (29).

The effect of ICAM-1 antisense oligonucleotide on E. coli endotoxin-induced expression of ICAM-1 mRNA

4-h pneumonia. BALB/c mice were anesthetized with ketamine hydrochloride (80–100 mg/kg i.m.) and acepromazine maleate (8–10 mg/kg i.m.). Anesthetized BALB/c mice received an intravenous injection of ISIS 3082 at doses of 0, 1, 10, 30, or 100 mg/kg (n = 4 at 0 dose, n = 3 in all other groups). 2 h after administering the oligonucleotide, endotoxin (100 μl of 2 mg/ml) was instilled into the airways through the trachea. Control mice received no endotoxin. 4 h after endotoxin was instilled, the lungs were frozen in liquid nitrogen. Lung tissue was pulverized and total RNA was extracted using guanidinium isothiocynate as described by Chirgwin et al. (30). Total RNA was separated on agarose/formaldehyde gels and transferred to nylon membranes. Northern blots were probed for ICAM-1, vascular cell adhesion molecule-1 (VCAM-1), and glyceraldehyde 3-phosphate dehydrogenase (G3PDH) mRNA using random primed labeled cDNA probes. RNA was quantitated using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The increase in ICAM-1 or VCAM-1 mRNA was normalized for differences in loading using the G3PDH mRNA and was expressed as a fold increase compared to animals that received no endotoxin.

24-h pneumonia. Anesthetized BALB/c mice received intravenous injections of ISIS 3082 (100 mg/kg) or saline either 2 h before or 2 h before and 4 h after intratracheal instillation of endotoxin (100 μl of 2 mg/ml, n = 3 in each group). After 24 h, the lungs were frozen in liquid nitrogen. Both ICAM-1 and VCAM-1 mRNA were quantitated as described above.

The effect of ICAM-1 antisense oligonucleotide on neutrophil emigration and edema formation induced by E. coli endotoxin

Anesthetized mice were pretreated with either ISIS 3082 (100 mg/kg, n = 10), ISIS 1082 (100 mg/kg, n = 4), or saline (n = 10) through the tail vein. After 1 h and 45 min, ^{125}I-albumin was injected through the tail vein to measure the extravascular accumulation of albumin in the lungs (29). The trachea was exposed through a midline incision in the neck and a 24-gauge catheter was inserted. After 15 min, endotoxin (100 μl of 2 mg/ml, n = 5 of either ISIS 3082– or saline-pretreated mice) or saline (100 μl, n = 5 of either ISIS 3082– or saline-pretreated mice) was instilled through the intratracheal catheter into the lungs. 4 h after the intratracheal instillation, a second injection of the same pretreatment (either ISIS 3082 (100 mg/kg), ISIS 1082 (100 mg/kg), or saline) was given through the tail vein. At 24 h after instillation of endotoxin, ^{51}Cr-labeled RBC were injected intravenously and allowed to circulate for 2 min to measure the pulmonary blood volume, and the mice received an overdose of halothane. The chest was rapidly opened, the base of the heart was tied, and a blood sample was taken from the inferior vena cava. The heart and lungs were removed en bloc and the lungs were fixed by intratracheal instillation of 4% glutaraldehyde in phosphate buffer at a constant pressure of 25 cm H_2O over a scintillation vial to retrieve any spilled fixative. After the heart and mediastinal structures were removed, radioisotope levels were measured in the lungs, blood sample, and plasma sample using a gamma counter interfaced with a PC and software to correct for overlap of one isotope into other channels, background, and radiodecay. Paraffin-embedded histologic 5–6-μm sections of lungs were cut and stained with hematoxylin and eosin to measure neutrophil emigration as described below.

The effect of antimurine ICAM-1 monoclonal antibodies on neutrophil emigration and edema formation induced by E. coli endotoxin

Anesthetized BALB/c mice were pretreated with hamster antimurine ICAM-1 monoclonal antibody 3E2 (5 mg/kg, n = 4) or hamster IgG (5 mg/kg, n = 5) mixed with ^{125}I-albumin. After 15 min, *E. coli* endotoxin (2 mg/ml, 100 μl) was instilled intratracheally. After 24 h, ^{51}Cr-labeled RBC were given intravenously and neutrophil emigration and extravascular albumin (EVA) were evaluated as described below.

Anesthetized wild-type mice were pretreated with rat antimurine ICAM-1 monoclonal antibody YN1/1 (5 mg/kg, n = 5) or rat IgG (5 mg/kg, n = 4) mixed with ^{125}I-albumin. The mice received intratracheal instillations of *E. coli* for 24 h as described above. Neutrophil emigration and EVA were evaluated as described below.

Neutrophil emigration and edema formation induced by E. coli endotoxin in ICAM-1 mutant and wild-type mice

Anesthetized ICAM-1 mutant and wild-type mice were given an intravenous injection of ^{125}I-albumin to the extravascular accumulation of albumin in the lungs. After 15 min, 100 μl of 2 mg/ml of endotoxin (n = 5 in each group) was instilled intratracheally as described above. After 24 h, the pulmonary blood volume was measured using ^{51}Cr-labeled RBC, and the lungs, blood sample, and plasma sample were evaluated as described above.

Quantitation of neutrophil emigration and edema formation in the lungs. Neutrophil emigration into the alveolar spaces was quantitated on paraffin-embedded histologic sections using point-counting techniques to determine the percentage of the distal airspace that was occupied by neutrophils (31, 32). 500 points were counted in each of five fields of alveolar space and alveolo-capillary walls on histologic sections at 200 magnification. The percentage of the alveolar tissue (alveolar space + alveolo-capillary walls) occupied by intraalveolar neutrophils was calculated.

Edema formation was quantitated by measuring the EVA. EVA was expressed as microliter plasma equivalents/murine lung and calculated as (29):

$$\text{EVA} = \text{total albumin volume} - \text{intravascular albumin volume}$$

$$\text{where total albumin volume} = \frac{^{125}\text{I-albumin in lungs}}{^{125}\text{I-albumin/gram plasma}}$$

and intravascular albumin volume =

$$\frac{^{51}\text{Cr-RBC in lungs}}{^{51}\text{Cr-RBC/gram blood}} \times (1 - \text{hematocrit})$$

Statistics. One way analyses of variance were used to compare the percentages of alveolar tissue occupied by neutrophils and edema formation. After a significant ANOVA, multiple contrasts were used with a Bonferroni adjustment to determine which groups were significantly different (33, 34). $P < 0.05$ was considered significant. The data are expressed as mean±SEM.

Results

The effect of ICAM-1 antisense oligonucleotide on E. coli endotoxin-induced expression of ICAM-1 mRNA. Endotoxin induced a fivefold increase in ICAM-1 mRNA in the lungs within 4 h of instillation, but no change in VCAM-1 mRNA (Figs. 1 and 2). The increase in ICAM-1 mRNA was inhibited by ISIS 3082 when given at 30 or 100 mg/kg, but not at 1 or 10 mg/kg (Figs. 1 and 2). When animals were studied at 24 h after instillation of endotoxin, there was still a significant increase in the ICAM-1 mRNA and no change in VCAM-1 mRNA expression (Fig. 3). This increase was inhibited when ISIS 3082 was given at a dose of 100 mg/kg both 2 h before and 4 h after endotoxin, but there was no statistically significant effect when the compound was given only 2 h before (Fig. 3). ISIS 3082 had no effect on the expression of VCAM-1.

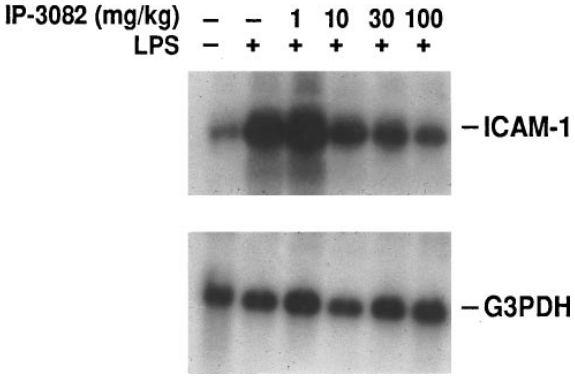

Figure 1. Northern blot demonstrating inhibition of endotoxin-induced upregulation of ICAM-1 mRNA at 4 h after instillation by ISIS 3082 in a dose-dependent manner.

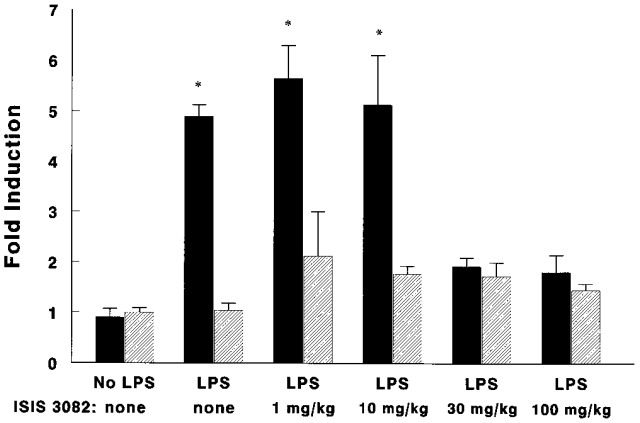

Figure 2. Inhibition of endotoxin-induced upregulation of ICAM-1 mRNA at 4 h by antisense oligonucleotide ISIS 3082. Each bar represents the mean data describing the fold increase in ICAM-1 mRNA (*filled bars*) or VCAM-1 mRNA (*slashed bars*) normalized for differences in loading using expression of G3PDH mRNA at 4 h after instillation of the stimulus. ISIS 3082 at doses of 30 or 100 mg/kg inhibited ICAM-1 mRNA expression. *Significantly greater fold induction of ICAM-1 mRNA than in mice that received no endotoxin (*LPS*) or from mice given LPS and 30 or 100 mg/kg ISIS 3082 ($P < 0.05$).

The effect of ICAM-1 antisense oligonucleotide on neutrophil emigration and edema formation induced by E. coli endotoxin. Histologic sections of lung tissue from mice with endotoxin-induced pneumonia given intravenous injections of either the control antisense oligonucleotide ISIS 1082 or the blocking oligonucleotide ISIS 3082 are shown in Fig. 4. In control BALB/c mice given injection of saline instead of antisense oligonucleotide at 2 h before and 4 h after endotoxin, 2.60±0.20% of the distal lung was occupied by emigrated neutrophils 24 h after instillation of E. coli endotoxin (Fig. 5). In the mice pre-

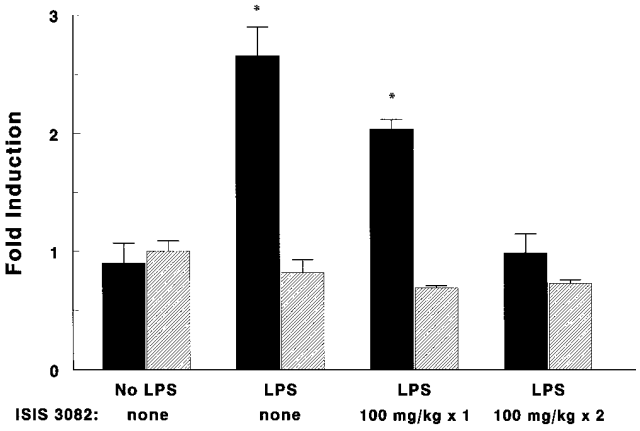

Figure 3. Inhibition of endotoxin-induced upregulation of ICAM-1 mRNA at 24 h by antisense oligonucleotide ISIS 3082. Each bar represents the mean data describing the fold increase in ICAM-1 mRNA (*filled bars*) or VCAM-1 mRNA (*slashed bars*) normalized for differences in loading using expression of G3PDH mRNA at 24 h after instillation of the stimulus. ISIS 3082 at a dose of 100 mg/kg given 2 h before and 2 h after instillation of endotoxin inhibited expression of ICAM-1 mRNA, but not when given only 2 h before instillation. *Significantly greater fold induction of ICAM-1 mRNA than in mice that received no endotoxin (*LPS*) or from mice given LPS and 100 mg/kg ISIS 3082 twice ($P < 0.05$).

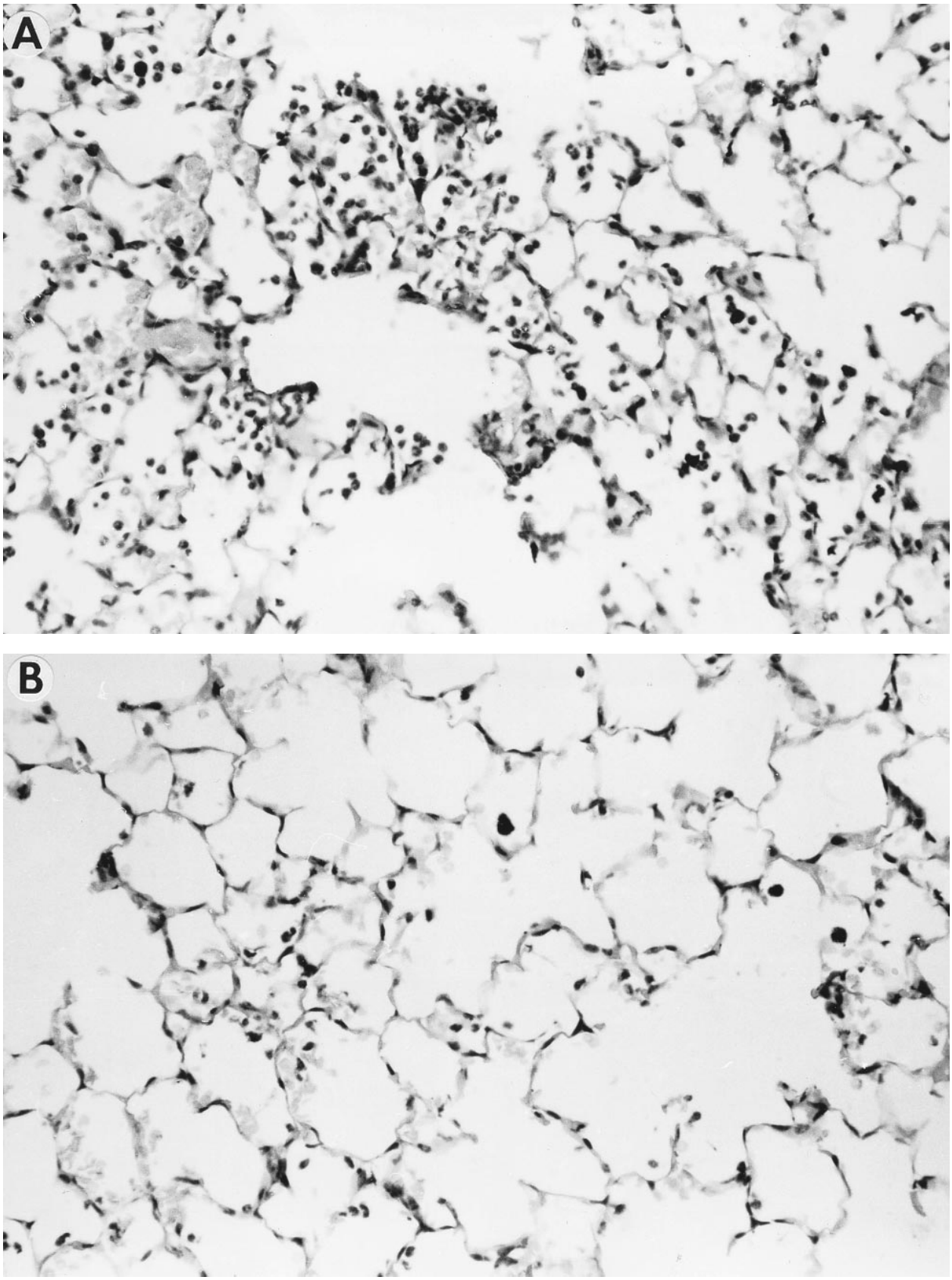

Figure 4. Micrographs of histologic sections of lung tissue from animals given the control antisense oligonucleotide ISIS 1082 (*A*) and the murine ICAM-1–targeted oligonucleotide ISIS 3082 (*B*) and instillation of *E. coli* endotoxin. Treatment of mice with ISIS 3082 inhibited neutrophil emigration into the alveolar spaces, but lung injury, as evaluated by the extravasation of RBC, is still present. ×200.

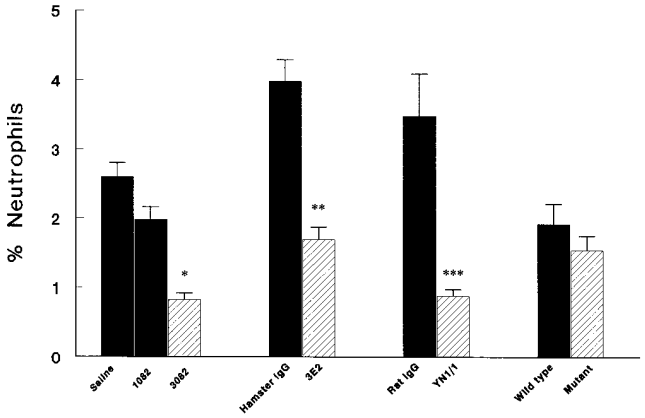

Figure 5. Neutrophil emigration induced by instillation of *E. coli* endotoxin for 24 h. The increase in neutrophil emigration induced by this stimulus, quantitated morphometrically as the fraction of the alveolar tissue occupied by emigrated neutrophils, was inhibited when ICAM-1 expression was prevented by intravenous injection of the antisense oligonucleotide ISIS 3082. Neutrophil emigration was also inhibited in either BALB/c or wild-type mice when the function of ICAM-1 was blocked using anti–ICAM-1 antibodies. However, no inhibition of neutrophil emigration was observed when ICAM-1 expression was eliminated using ICAM-1 mutant mice compared to wild-type mice. Significantly less ($P < 0.05$) than neutrophil emigration in mice given: *ISIS 1082; **hamster IgG; ***rat IgG.

treated with ISIS 1082, this value was similar (1.98±0.18%, $P > 0.05$). However, in the mice pretreated with ISIS 3082, only 0.82±0.10% of the distal lung was occupied by emigrated neutrophils ($P < 0.05$), indicating that the ICAM-1 antisense oligonucleotide inhibited neutrophil emigration into the lung parenchyma by 59%.

The accumulation of edema, as measured by EVA, is shown in Fig. 6. The lungs of mice that were given ISIS 3082 but did not receive instillation of endotoxin showed no increase in EVA (Fig. 6). Intratracheal instillation of endotoxin induced a significant increase in edema formation that was not inhibited by either ISIS 1082 or ISIS 3082 (Fig. 6).

The effect of antimurine ICAM-1 monoclonal antibodies on neutrophil emigration and edema formation induced by E. coli endotoxin. In the lungs of BALB/c mice pretreated with hamster IgG, 3.98±0.31% of the alveolar tissue was occupied by emigrated neutrophils 24 h after instillation of endotoxin (Fig. 5). When mice were pretreated with the hamster anti–ICAM-1 antibody 3E2, neutrophil emigration was prevented by 58%, as only 1.69±0.18% of the alveolar tissue was occupied by neutrophils (Fig. 5). This anti–ICAM-1 antibody had no significant effect on EVA (124±7 μl) compared to that in mice pretreated with hamster IgG (126±21 μl, Fig. 7).

In the lungs of rat IgG–pretreated wild-type mice (129/Sv × C57BL/6J), 3.48±0.61% of the alveolar tissue was occupied by emigrated neutrophils (Fig. 5). Pretreatment with the rat antimurine ICAM-1 antibody YN1/1 prevented neutrophil emigration by 75%, as emigrated neutrophils occupied only 0.88±0.07% of the alveolar tissue. The accumulation of EVA was inhibited by 64% (122±19 μl in rat IgG-treated mice, compared to 58±9 μl in wild-type mice, $P < 0.05$, Fig. 7).

Neutrophil emigration and edema formation induced by E. coli endotoxin in ICAM-1 mutant and wild-type mice. Emigrated neutrophils occupied 1.92±0.29% of the alveolar tissue in the lungs of wild-type mice and 1.54±0.21% in ICAM-1 mutant mice 24 h after instillation of endotoxin ($P > 0.05$, Fig. 5). The accumulation of EVA was also similar in the lungs of mutant mice (106±9 μl, Fig. 7) compared to wild-type mice (86±11 μl).

Discussion

E. coli endotoxin, when instilled into the distal airways of mice, induced an upregulation of ICAM-1 mRNA by 4 h that persisted through 24 h, by which time neutrophil emigration and edema formation were occurring. No change in VCAM-1 mRNA was observed. The antisense oligonucleotide ISIS 3082 prevented this upregulation of ICAM-1 mRNA in a dose-dependent manner at both 4 and 24 h after instillation and had no effect on the constitutive expression of VCAM-1. The high doses

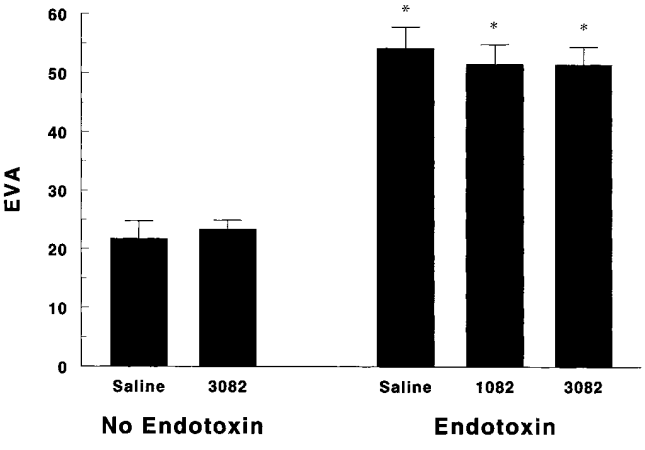

Figure 6. Extravascular albumin in mice pretreated with ICAM-1 antisense oligonucleotide ISIS 3082. Intratracheal instillation of endotoxin induced an increase in EVA compared to mice given instillations of saline. This increase in EVA was not prevented by treatment with either ISIS 1082 or ISIS 3082. ISIS 3082 in mice given instillation of saline did not increase EVA. *Significantly less than the EVA in mice given either saline or ISIS 3082 but no endotoxin ($P < 0.05$).

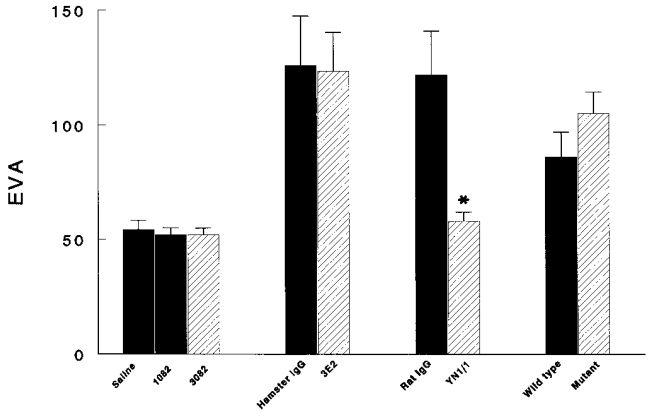

Figure 7. Extravascular albumin in mice given intratracheal instillation of endotoxin. Only the antibody YN1/1 in wild-type mice partially prevented the formation of edema. No inhibition was observed when evaluated using the antisense oligonucleotide, the 3E2 antibody, or mutant mice. *Significantly less than the EVA in mice given rat IgG ($P < 0.05$).

required for complete inhibition of message likely reflect the low uptake of the oligonucleotide by the lungs observed after intravenous injection, which was less than 2% of the total amount injected (35–38). The requirement for a second dose of ISIS 3082 likely reflects the rapid initial clearance of ISIS 3082 from the plasma (half time of 0.17 h) and its metabolism within tissues over 24 h (38). However, these doses in the absence of endotoxin did not injure the lung, as no increase in permeability was observed when instillation of saline was substituted for endotoxin. In addition, although the cardiovascular effects of ISIS 3082 in mice have not been evaluated, similar phosphorothioate oligonucleotides have been associated only with sporadic hypotension in monkeys (39–41). These data suggest that antisense oligonucleotides targeted to ICAM-1 may have important therapeutic uses in the treatment of patients with ICAM-1–dependent inflammatory responses that result in lung injury.

Inhibition of ICAM-1 expression by ISIS 3082 significantly reduced the accumulation of neutrophils within the alveolar spaces by 59% without an effect on edema formation. This partial inhibition of neutrophil emigration occurred despite complete inhibition of the upregulation of ICAM-1 mRNA. This inhibition was specific to the antisense oligonucleotide targeted to ICAM-1, as ISIS 1082, a sequence control, had no effect. However, normal murine lung tissue constitutively expresses detectable levels of ICAM-1 mRNA and protein (4, 42). The antisense oligonucleotide failed to reduce the level of ICAM-1 mRNA expression below the constitutive level. These data may suggest that the ICAM-1 expressed normally on the pulmonary capillaries may be capable of mediating some neutrophil emigration. Alternatively, endothelial cell adhesion molecules other than ICAM-1 may be mediating the firm adhesive step during neutrophil emigration, suggesting that ICAM-1–independent adhesion pathways are operant.

The antisense oligonucleotide was as effective as anti–ICAM-1 antibodies, in that neutrophil emigration was inhibited by 58% in BALB/c mice given 3E2 and by 75% in wild-type mice given YN1/1, similar to the 59% inhibition observed using the antisense oligonucleotide. The slightly greater effect of YN1/1 may reflect more efficient inhibition that resulted in partial inhibition of edema formation as well as neutrophil emigration. However, the lack of complete inhibition by either antibody or the antisense oligonucleotide support the hypothesis that alternative adhesion pathways exist.

The rat antimurine ICAM-1 antibody YN1/1 inhibited both neutrophil emigration and edema formation while the antisense oligonucleotide and the hamster antimurine ICAM-1 antibody 3E2 inhibited only neutrophil emigration. The YN1/1 provided the greatest degree of inhibition in neutrophil emigration, reducing emigration by 75%, compared to 58% by 3E2 and 59% by ISIS 3082. As the formation of edema occurs through both neutrophil-mediated and neutrophil-independent pathways, a large reduction in neutrophil emigration is likely to be required before a decrease in the total accumulation of edema is observed.

In striking contrast to either the antisense oligonucleotide or the antibodies, neither neutrophil emigration nor edema formation were inhibited when the function of ICAM-1 was evaluated using ICAM-1 mutant mice. These studies suggest that the manner in which the ICAM-1 is inhibited results in different apparent functions of this molecule in mediating the acute inflammatory response. Other studies have also suggested that the apparent function of an adhesion molecule may vary when evaluated using antibodies compared to mutations. In particular, ICAM-1 mutant mice showed no defect in neutrophil emigration during *Pseudomonas aeruginosa*–induced pneumonia while anti–ICAM-1 antibodies inhibited emigration by 65% (43) (Qin, L., W.M. Quinlan, N.A. Doyle, L. Graham, J.E. Sligh, F. Takei, A.L. Beaudet, and C.M. Doerschuk, manuscript submitted for publication). In addition, cobra venom factor–induced acute lung injury was prevented by anti–ICAM-1 or anti–P-selectin antibodies in wild-type mice, but was not altered in either ICAM-1 mutant, P-selectin mutant, or ICAM-1/P-selectin double mutant mice (44). In these two studies, antibodies against an adhesion molecule, either P-selectin or ICAM-1, had no effect when given to mutant mice deficient in the same adhesion molecule. One possibility is that antibodies and antisense oligonucleotides are inducing more changes in endothelial cells than simply inhibiting the function or the expression of the targeted adhesion molecule, although their effects are likely to be through interaction with the targeted molecule. These additional effects may include alterations in signal transduction or changes in the surface of endothelial cells. Alternatively, the apparent differences may be due to the time over which the mice are deficient in a molecule. Antibodies and antisense oligonucleotides inhibit the function of their targets for only a short time before evaluating the role of the adhesion molecule while mutations prevent the function of the adhesion molecule after conception. A defect in adhesion pathways throughout the development and adult life of mice may allow expression of alternative pathways. The observation in the present study that complete inhibition of neutrophil emigration was never observed using either of two antibodies or the antisense oligonucleotides that induced complete inhibition of ICAM-1 mRNA upregulation, as well as similar results by other investigators showing partial inhibition of the inflammatory response to other stimuli in several species (reviewed in reference 45), suggests that ICAM-1–independent pathways exist in the lungs and are important in neutrophil emigration. The mutant mice may have upregulated this pathway to compensate for a deficiency in ICAM-1.

The molecules involved in this alternative pathway have not been identified. Previous studies examining neutrophil emigration in response to *E. coli* have suggested that the compensating molecule in mutant mice is not P-selectin or E-selectin (46). Other studies have suggested that alternative adhesion molecules may be important in acute neutrophil emigration in the lung and in chronic inflammatory processes in the peritoneum. For example, *Streptococcus pneumoniae* induces CD11/CD18–independent, ICAM-1–independent emigration in the lung (4, 47). C5a and hydrochloric acid also induce CD11/CD18–independent adhesion pathways, although the function of ICAM-1 has not been evaluated (47, 48). Winn and Harlan have demonstrated that CD11/CD18–independent pathways of neutrophil emigration occur at later time points in peritonitis induced by stimuli that induced CD11/CD18–dependent emigration in the initial stages (49). Finally, *Pseudomonas aeruginosa*, a stimulus that elicits CD11/CD18–dependent emigration into the alveolar spaces in the initial pneumonia, induces CD11/CD18–independent emigration at the same time point in recurrent pneumonias at the previously inflamed site (50). These studies suggest that alternative adhesion pathways that, to date, are poorly defined, exist in the lungs and other organs. The mutant mice may provide an opportunity to iden-

tify either new functions for known molecules or novel adhesion molecules.

In summary, antisense oligonucleotides targeted to ICAM-1, but not sequence control oligonucleotides, completely prevented endotoxin-induced upregulation of ICAM-1 in the lung, inhibited neutrophil emigration into the alveolar spaces to a similar degree as anti–ICAM-1 antibodies, and did not induce lung injury in the absence of endotoxin. These data suggest that antisense oligonucleotides have a potentially important therapeutic role in the treatment of ICAM-1–mediated lung diseases. Despite very effective inhibition of ICAM-1 mRNA expression by ISIS 3082, neutrophil emigration was only partially prevented by this antisense oligonucleotide and by anti–ICAM-1 antibodies. No apparent function for ICAM-1 was observed when evaluated using ICAM-1 mutant mice. These studies suggest that alternative ligands exist and mediate interactions between neutrophils and pulmonary endothelial cells.

Acknowledgments

The authors thank Taffy Hooser in the Department of Anatomy, Indiana University (Indianapolis, IN) for the preparation of histologic sections and Bonnie Meek for the preparation of figures.

This study was supported by research grants from the Cystic Fibrosis Foundation (PHS HL 48160, PHS HL 52466, and AI 32177). T. Kumasaka is the recipient of a Fellowship from the Sasakawa Health Science Foundation. C.M. Doerschuk is the recipient of a Career Investigator Award from the American Lung Association.

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