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Halogenated Anesthetics Reduce Interleukin-1β–induced Cytokine Secretion by Rat Alveolar Type II Cells in Primary Culture

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Background: **Alveolar epithelial type II (AT_{II}) cells participate in the intraalveolar cytokine network by secreting cytokines and are widely exposed to volatile anesthetics during general anesthesia. The aim of the current study was to evaluate the effects of halothane, enflurane, and isoflurane on rat AT_{II} cell cytokine secretions in AT_{II} primary cell cultures.**

Methods: **Alveolar epithelial type II primary cell cultures were obtained from adult rat lungs. AT_{II} cells were stimulated by recombinant murine interleukin-1β (rmIL-1β) to mimic an inflammatory response, and immediately exposed for various duration to different concentration of halothane, enflurane, or isoflurane. Interleukin-6, macrophage inflammatory protein-2 (MIP-2), and monocyte chemoattractant protein-1 (MCP-1) protein concentrations were then measured in cell culture supernatants. Recombinant mIL-1β–stimulated AT_{II} cells exposed to air served as control.**

Results: **Halothane, isoflurane, and enflurane (1 minimum alveolar concentration [MAC], 4 h) decreased rmIL-1β–stimulated AT_{II} cell secretions of interleukin-6, MIP-2, and MCP-1, but did not modify total protein secretion. Halothane exposure decreased rmIL-1β–stimulated AT_{II} cell secretions of interleukin-6, MIP-2, and MCP-1 in a dose- and time-dependent manner. Total protein concentrations remained unchanged except at 1.5 MAC of halothane, and no cytotoxic effect could be evidenced by lactate dehydrogenase release. These effects were transient as rmIL-1β–stimulated AT_{II} cell secretions of interleukin-6 and MIP-2 progressively reached control values between 4 and 24 h after the end of halothane exposure. However, MCP-1 inhibition persisted until 24 h. rmIL-1β–induced MIP-2 and tumor necrosis factor-α mRNA expression were decreased by 36 and 24%, respectively, after halothane exposure.**

Conclusions: **The current study shows that exposure of rmIL-1β–stimulated AT_{II} cells to volatile anesthetics reversibly alters their cytokine secretion. Therefore, volatile anesthesia, by modulating pulmonary epithelial cell secretion of inflammatory cytokines, might affect the lung inflammatory response.**

This article is accompanied by an Editorial View. Please see: Gropper MA, Wiener-Kronish J: The alveolar epithelium: Suspect or innocent bystander? ANESTHESIOLOGY 2003; 98:3–4.

ALVEOLAR epithelial type II cells (AT_{II}) play an important role in the alveolar space. AT_{II} cells synthesize and secrete surfactant, regulate the epithelial fluid clearance, and proliferate and differentiate into alveolar type I cells, particularly in case of epithelial injury.[1] Moreover, AT_{II} cells participate with alveolar inflammatory cells, to the intraalveolar cytokine network, by secreting interleukin-6,[2] macrophage inflammatory protein-2 (MIP-2), a murine analog of interleukin-8,[3] cytokine-induced neutrophil chemoattractant,[4] monocyte chemoattractant protein-1 (MCP-1),[5] and tumor necrosis factor-α (TNF-α).[6,7] *In vitro*, unstimulated AT_{II} cells secrete low amounts of cytokines. However, several studies have documented a potent cytokine secretion by AT_{II} cells either *in vivo*, in response to lung injury, or *in vitro*, in response to interleukin-1β stimulation.[3–5,8–10] It has been also shown that AT_{II} cells secrete low amounts of TNF-α after lipopolysaccharide stimulation[6] and that TNF-α induces MIP-2 production by alveolar epithelial cells.[7]

Alveolar epithelial type II cells are exposed to volatile anesthetic agents throughout the duration of anesthesia, as these agents are delivered to and principally eliminated by the lungs. However, little is known regarding the effects of volatile anesthetics on pulmonary cell functions. Halothane and other volatile anesthetics are known to modify cellular metabolism.[11,12] Indeed, it has been demonstrated that elevated concentrations of halothane have an inhibitory effect on protein synthesis in perfused rat lung.[13–15] Volatile anesthetics can also modulate the immune response by decreasing neutrophil functions *in vitro*[16–18] and cytokine release by human peripheral blood mononuclear cells.[19] *In vitro* studies from our laboratory also showed that halothane inhibits

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surfactant phospholipids and apoprotein biosynthesis by rat AT_{II} cells[20,21] and decreases Na-K-ATPase and sodium channel activities.[22]

Little is known about the effects of volatile anesthetics on lung inflammatory response. In a previous *in vivo* study, we have shown that halothane reduces the lung inflammatory response induced by intratracheal lipopolysaccharide instillation in mechanically ventilated rats.[23] Indeed, 1% halothane delivered during 4 h through mechanical ventilation decreased the lipopolysaccharide-induced recruitment of neutrophils and secretion of TNF-α, interleukin-6, and MIP-2 in the bronchoalveolar fluid. MIP-2 and TNF-α mRNA expression in lung homogenates was also decreased. Several cellular sources of cytokines within the lung, including inflammatory cells and AT_{II} cells, might have been affected by halothane exposure. As yet the effects of halothane on cytokine secretion by AT_{II} cells remain unknown.

As AT_{II} cells, under various conditions, are a potent source of cytokines within the lungs as well as a potential target for volatile anesthetics, the aim of the current work was to evaluate the effects of halothane, enflurane, and isoflurane on cytokine secretions by rat AT_{II} primary cell culture. As in our previous *in vivo* study, the effects of halothane were only observed during a lung inflammatory process; recombinant murine interleukin-1β (rmIL-1β) was used to stimulate AT_{II} cells and to mimic an inflammatory setting.

Materials and Methods

This study, including care of the animals involved, was conducted according to the official edict presented by the French Ministry of Agriculture (Paris, France) and the recommendations of the Helsinski Declaration. Thus, these experiments were conducted in an authorized laboratory and under the supervision of authorized researchers.

Isolation of Rat Alveolar Type II Cells

Alveolar type II cells were isolated from adult rat lungs by enzymatic dissociation and purified by differential adherence to plastic as previously describe.[24] Cells (3×10^6) were plated in a 60-mm-diameter cell culture dish (Costar Corp., Cambridge, MA) with 2 ml of Dulbecco's modified Eagle medium (DMEM; Gibco Life Technologies, Cergy-Pontoise, France) containing 15% fetal bovine serum (Gibco Life Technologies), 10^5 IU/ml penicillin, 100 μg/ml streptomycin, and 250 ng/ml amphotericin B (complete DMEM). The dishes were incubated 24 h at 37°C with a 95% air–5% CO_2 mixture. The incubator atmosphere was continuously kept saturated with water.

After a 24-h period, nonadherent cells were removed by gently washing twice with phosphate-buffered saline. Subsequently, 2 ml fresh complete DMEM was added and the cells were used for the experiments. AT_{II} cells were identified by the modified Papanicolaou stain and by phosphine fluorescence staining.[25] A total of 93 ± 2% (mean ± SEM, n = 12) of adherent cells were AT_{II} cells 24 h after rat lung isolation. Adherent cell viability was higher than 98% as assessed by the trypan blue exclusion test.

Volatile Anesthetic Exposure and Experimental Design

Alveolar type II cells were stimulated with 1 ng/ml rmIL-1β (R&D Systems, Abingdon, UK) to induce their cytokine secretion and to mimic an inflammatory response.They were then exposed to either air or volatile anesthetics as previously described.[20,21] AT_{II} cells were incubated in a 12-l air-tight Lwolff chamber at 37°C and 5% CO_2. The chamber atmosphere was continuously kept saturated with water. Volatile anesthetic vapors were provided into the chamber by directing a 95% air–5% CO_2 mixture through volatile anesthetic vaporizers placed on the entrance of the chamber. A volatile anesthetic monitor (Capnomac; Datex, Helsinki, Finland) determined the concentration of the anesthetic agents exiting the chamber. The chambers were sealed when the desired concentration was obtained.

To analyze the effects of different volatile anesthetics on cytokine secretion, rmIL-1β–stimulated AT_{II} cells were incubated during 4 h at 37°C with 1 minimum alveolar concentration (MAC) of halothane (1%), isoflurane (1.5%), or enflurane (2.2%), *i.e.*, clinically relevant concentration.[26] Control cells were rmIL-1β–stimulated AT_{II} cells only exposed to a water-saturated 95% air–5% CO_2 mixture during 4 h. Each experimental condition was performed in duplicate.

To study a dose–response effect, rmIL-1β–stimulated AT_{II} cells were exposed to volatile anesthetic vapors provided into the chamber by directing a 95% air–5% CO_2 mixture through volatile anesthetic vaporizers placed on the entrance of the chamber for 4 h to 0, 0.5, 1, and 1.5% of halothane.

To determine a relation between duration of exposure to halothane and AT_{II} cell cytokine secretion, rmIL-1β–stimulated AT_{II} cells were exposed to volatile anesthetic vapors provided into the chamber by directing a 95% air–5% CO_2 mixture through volatile anesthetic vaporizers placed on the entrance of the chamber in the presence or absence of 1% halothane for 1, 2, 3, and 4 h as described above.

Finally, to determine the reversibility of halothane exposure (4 h, 1%), rmIL-1β–stimulated AT_{II} cells were maintained incubated in the same culture medium at 37°C with a 95% air–5% CO_2 mixture (without halothane) for 1, 2, 4, and 24 h following halothane or air exposure. The incubator atmosphere was kept continuously saturated with water.

At the end of the culture period or volatile anesthetic exposure, supernatants from duplicate cell cultures were collected and immediately frozen at −20°C to measure the concentration of interleukin-6, MIP-2, MCP-1, total proteins, and lactate dehydrogenase (LDH) activities. Total cellular mRNA from AT_{II} adherent cells (3×10^6 AT_{II}) were extracted using 2 ml of Trizol (Gibco) following the manufacturer's instructions.

Protein Assay

Total protein content in cell supernatants was measured with a pyrogallol-red-molybdate complex[27] on an automatic Hitachi-911 analyzer (Boehringer-Mannheim, Paris, France).

Interleukin-6 Assay

Bioactive interleukin-6 was measured in cell supernatants using the interleukin-6–dependent B9 hybridoma cell line.[28] B9 cells were cultured for 72 h in presence of duplicate serial dilutions of the specimens in 96-well plates. B9 cell proliferation was estimated after 72 h of incubation at 37°C in 5% CO_2 using MTT colorimetric assay.[29] Human recombinant interleukin-6 was used as an internal standard in all assays. One unit of interleukin-6 was defined by the half-maximal proliferation of B9 cells.

Macrophage Inflammatory Protein-2 and Monocyte Chemoattractant Protein-1 Assays

The amount of MIP-2 and MCP-1 in cell supernatants was determined with commercially available enzyme-linked immunosorbent assay kits (Cytoscreen MIP-2/MCP-1; Biosource, Montrouge, France), following the manufacturer's instruction. The detection limit was 1 pg/ml and 8 pg/ml for MIP-2 and MCP-1, respectively.

Reverse-transcriptase Polymerase Chain Reaction

After extraction, total cell RNA was submitted to a reverse transcription. Four microliters of total RNA (0.5 μg/μl) in presence of 3 μl of oligo-dT (0.8 μg/μl; Promega, Charbonnieres, France) was heated to 70°C for 5 min in a thermocycler (Gene Amp; Perkin Helmer, Norwalk, CT). Then, at 4°C, 2.5 μl of deoxyribonucleoside triphosphate (10 mM; Boerhinger-Mannheim, Mannheim, Germany) and 2 μl of avian myeloblastosis virus reverse transcriptase (32 IU/μl; Boerhinger-Mannheim) were added to a final volume of 25 μl in RNAse-free water. The reverse transcription was performed in a thermocycler that heated to 42°C for 45 min and to 99°C for 5 min. The final cDNA products were stored at −20°C.

Polymerase Chain Reaction for Macrophage Inflammatory Protein-2 and Tumor Necrosis Factor-α

Polymerase chain reaction for MIP-2 and TNF-α was performed by mixing 2 μl cDNA, 12.5 μl deoxyribonucleoside triphosphate (1 mM; Boerhinger-Mannheim), 5 μl $MgCl_2$ (50 mM; Gibco), 2 μl sense primer (12 μM, MIP-2 = 5'-GGC ACA ATC GGT ACG ATC CAG-3' or TNF-α = 5'-GCC ACC ACG CTC TTC TGT CT-3'), 2 μl antisense primer (12 μM, MIP-2 = 5'-ACC CTG CCA AGG GTT GAC TTC-3' or TNF-α = 5'-GGG CTA CGG GCT TGT CAC T-3'), 2 μl sense primer S14 (12 μM, 5'-ATC AAA CTC CGG GCC ACA GGA-3'), 2 μl antisense primer of S14 (5'-GTG CTG TCA GAG GGG ATG GGG-3') to a final volume of 48 μl in Rnase-free water. This mixture was heated at 80°C for 5 min, then 2 μl of Taq polymerase (1.25 μg/μl; Gibco) was added. Twenty-five repeated cycles of heat denaturation (94°C for 30 s), annealing of the primers (59°C for 30 s), and extension of the annealed primers (72°C for 30 s) were performed.

Macrophage inflammatory protein-2, TNF-α, and S14 cDNA were size fractionated by electrophoresis through a 3% agarose gel containing 2% of Tris-buffer saline (1 mM; Sigma, Saint-Quentin Falavier, France) and 0.01% of Vista Green nucleic acid gel stain (Amersham Life Science, Orsay, France). The intensity of each band was measured under ultraviolet light with a charge-coupled device camera using image analyzer (Gel Analyst; Iconix, Santa Monica, CA). The size of MIP-2, TNF-α, and S14 amplification products were, respectively, 287, 151, and 134 base pairs. MIP-2 mRNA was expressed as the percentage of the housekeeping gene S14 mRNA. This technique permitted semiquantitative analysis of the reverse-transcription polymerase chain reaction.

Lactate Dehydrogenase Assay

To determine whether halothane exposure had cytotoxic effect on AT_{II} cells, LDH activity was measured in the AT_{II} cell supernatants[30] with an automatic Hitachi-911 analyzer (Boehringer-Mannheim, Paris, France) and expressed as international units per milliliter.

Statistical Analysis

All results are expressed as the percentage of values obtained for air-exposed cells (mean ± SD) derived from at least n = 3 independent experiments, each conducted in duplicate. The statistical analysis was performed with the program Sigma Stat (Jandel Scientific, San Jose, CA). Between-group differences were first assessed with the Kruskall-Wallis test and then, in the case of global significant difference, individual group means were compared with a nonparametric Mann–Whitney U test. A P value < 0.05 was considered significant.

Results

Effects of Halothane, Isoflurane, and Enflurane on Cytokine and Protein Productions by Recombinant Murine Interleukin-1β–stimulated Alveolar Epithelial Type II Cells

As expected, rmIL-1β increased the secretion of interleukin-6, MIP-2, MCP-1 (50-, 13-, and 10-fold, respec-

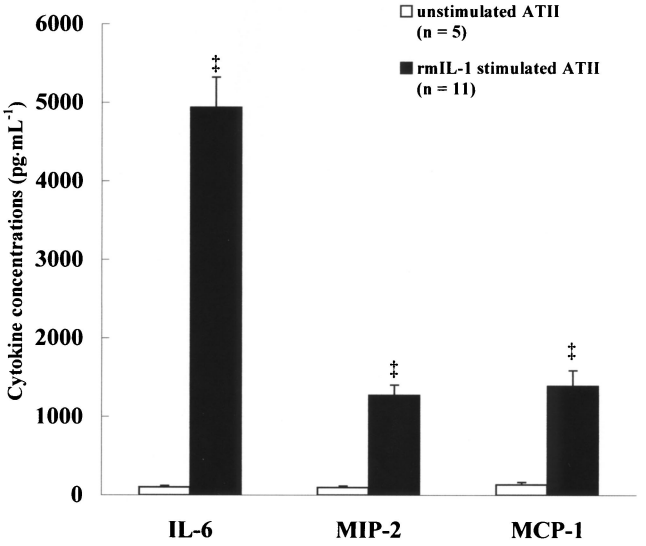

Fig. 1. Cytokine concentrations in alveolar type II (AT_{II}) cell supernatants. Effects of recombinant murine interleukin-1β (rmIL-1β) stimulation. The cytokine secretions by rat AT_{II} cells were induced by rmIL-1β (1 ng/ml). Results are expressed in picograms per milliliter, mean ± SD; ‡$P < 0.001$ *versus* unstimulated AT_{II} cells. MCP-1 = monocyte chemoattractant protein-1; MIP-2 = macrophage inflammatory protein-2.

tively; $P < 0.001$) and total protein concentrations as compared with unstimulated AT_{II} cells (fig. 1).

Exposure of rmIL-1β–stimulated AT_{II} cells to 1 MAC of halothane, isoflurane, and enflurane during 4 h decreased interleukin-6 concentration by 51 ± 18.5, 53 ± 17.3, and 49 ± 18.9%, respectively ($P < 0.001$), MIP-2 concentration by 53 ± 18.2, 57 ± 19.5, and 51 ± 17.7%, respectively ($P < 0.001$), and the MCP-1 concentration by 31 ± 15.2, 33 ± 14.3, and 30 ± 12.2%, respectively ($P < 0.05$), while total protein secretion was not changed (fig. 2). No difference was observed between the effects of halothane, isoflurane, and enflurane. Thus, all further experiments were only performed with halothane.

Dose-dependent Effect of Halothane on Cytokine Production

The exposure of rmIL-1β–stimulated AT_{II} cells during 4 h at increasing concentrations of halothane (*i.e.*, 0.5, 1, and 1.5 MAC) decreased interleukin-6 concentration by 22 ± 12.2, 61 ± 15, and 69 ± 16.6%, respectively ($P < 0.05$), MIP-2 concentration by 24 ± 16.4, 52 ± 17.4, and 64 ± 16.9%, respectively ($P < 0.05$), and MCP-1 concentration by 21 ± 12.8, 22 ± 14.1, and 55 ± 15.9%, respectively ($P < 0.05$). Total protein concentrations were not modified when using 0.5 and 1 MAC of halothane but were significantly decreased (19%; $P < 0.05$) at 1.5 MAC of halothane (fig. 3).

Lactate dehydrogenase activities were measured in cell culture supernatants to rule out a direct cytotoxic effect of halothane. LDH activities were not modified after exposure to 0.5 and 1 MAC of halothane (+1.7 ± 1.4 and +1.2 ± 0.9%, respectively, *vs.* control; not significant). By contrast, exposure to 1.5 MAC of halothane led to an increased LDH activity (+11.5 ± 3.2% *vs.* control; $P < 0.05$; fig. 4).

Time-dependent Effect of Halothane on Cytokine Productions

Exposure of rmIL-1β–stimulated AT_{II} cells to halothane at 1 MAC during 1, 2, 3, and 4 h induced a progressive inhibition of cytokine secretion 2 h after the onset of halothane exposure (fig. 5). Indeed, after a 2-h period of halothane exposure, both interleukin-6 and MIP-2 were significantly decreased ($P < 0.05$) as compared with air-exposed cells. The decrease in MCP-1 concentrations reached significance at the third hour as compared with air-exposed cells. Total protein concentration remained unchanged during all intervals of exposure to halothane.

Reversibility of Halothane Effects

After either air (control) or halothane exposure (1 MAC, 4 h), the AT_{II} cell chamber atmosphere was washed with air, and the AT_{II} cell supernatants were collected 1, 2, 4, and 24 h after the end of halothane exposure.

As shown in figure 6, both interleukin-6 and MIP-2 secretions increased progressively after the end of halothane exposure to reach control values between the 4th and 24th hour. As opposed, MCP-1 secretion remained inhibited until the 24th hour after the end of halothane exposure.

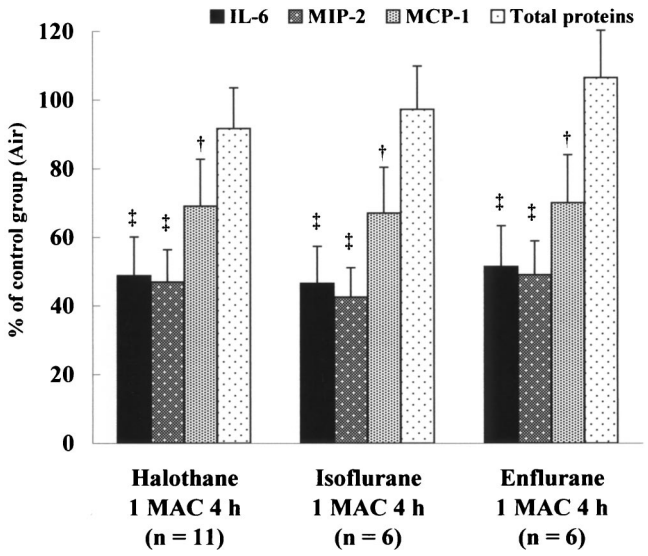

Fig. 2. Effects of volatile anesthetics on interleukin-6 (IL-6), macrophage inflammatory protein-2 (MIP-2), monocyte chemoattractant protein-1 (MCP-1), and total protein secretions by recombinant murine interleukin-1β (rmIL-1β)-stimulated alveolar type II cells. rmIL-1β–stimulated rat alveolar type II cells were exposed for 4 h either to air or volatile anesthetics (halothane, enflurane, isoflurane; 1 minimum alveolar concentration [MAC]). Cytokine concentrations were then measured in cell supernatants. Results are expressed as the percentage of values obtained for air-exposed cells, mean ± SD; †$P < 0.05$ and ‡$P < 0.001$ *versus* air.

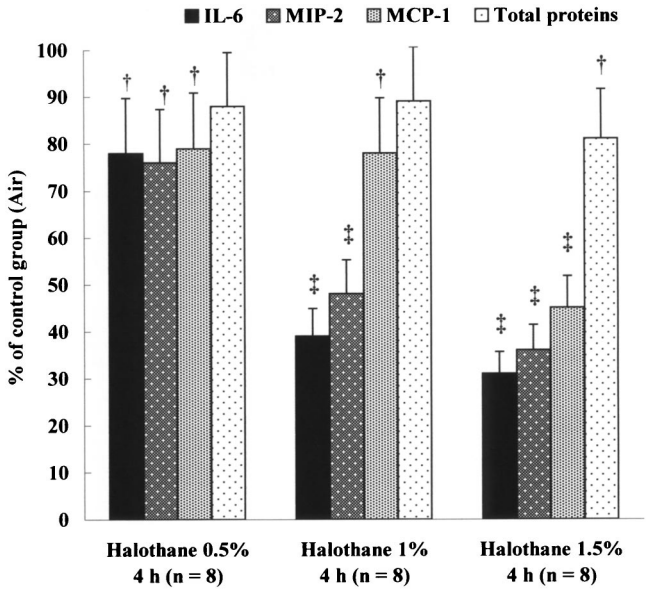

Fig. 3. Dose-dependent effect of halothane on interleukin-6 (IL-6), macrophage inflammatory protein-2 (MIP-2), monocyte chemoattractant protein-1 (MCP-1), and total protein secretions by recombinant murine interleukin-1β (rmIL-1β)–stimulated alveolar type II cells. Recombinant mIL-1β–stimulated rat alveolar type II cells were exposed during 4 h to 0, 0.5, 1, and 1.5 minimum alveolar concentration of halothane. Cytokine concentrations were then measured in cell supernatants. Results are expressed as the percentage of values obtained for air-exposed cells, mean ± SD; †P < 0.05 and ‡P < 0.001 *versus* air.

Messenger RNA Expression of Macrophage Inflammatory Protein-2 and Tumor Necrosis Factor-α

Messenger RNA expression of MIP-2 in unstimulated AT_{II} cells was under the detection limit (fig. 7). rmIL-1β stimulation induced an increase in MIP-2 mRNA in AT_{II} cells (air group). In agreement with MIP-2 protein concentration, MIP-2 mRNA expression was decreased by

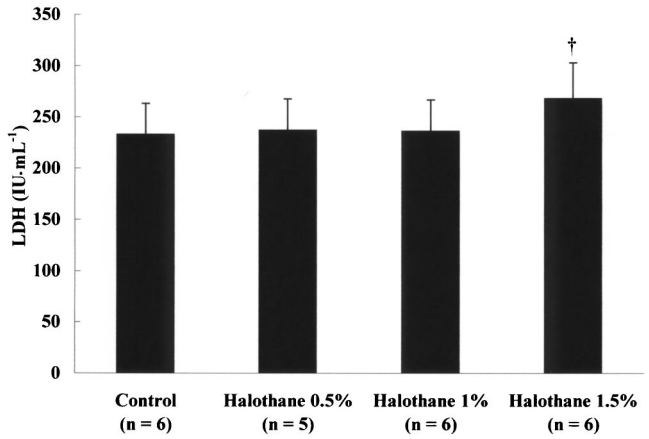

Fig. 4. Effect of halothane on lactate dehydrogenase (LDH) release by alveolar type II cells. Recombinant murine interleukin-1β–stimulated rat alveolar type II cells were exposed during 4 h to 0, 0.5, 1, and 1.5 minimum alveolar concentration of halothane. LDH activities were then measured in cell supernatants. Results are expressed as the percentage of values obtained for air-exposed cells, mean ± SD; †P < 0.05 *versus* air.

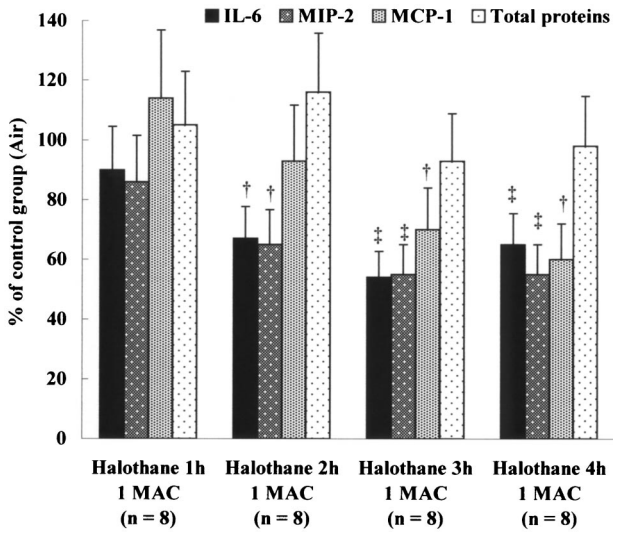

Fig. 5. Time-dependent effect of halothane on interleukin-6 (IL-6), macrophage inflammatory protein-2 (MIP-2), monocyte chemoattractant protein-1 (MCP-1), and protein secretions by recombinant murine interleukin-1β (rmIL-1β)–stimulated alveolar type II cells. rmIL-1β–stimulated rat alveolar type II cells were exposed either to air or halothane (1 minimum alveolar concentration [MAC]) during 1, 2, 3, and 4 h. Cytokine concentrations were then measured in cell supernatants. Results are expressed as the percentage of values obtained for air-exposed cells, mean ± SD; †P < 0.05 and ‡P < 0.001 *versus* air.

36 ± 13.9% (P < 0.001) in AT_{II} cells exposed to 1% halothane for 4 h.

Alveolar type II cells are well known to only produce low concentrations of TNF-α, even in the presence of

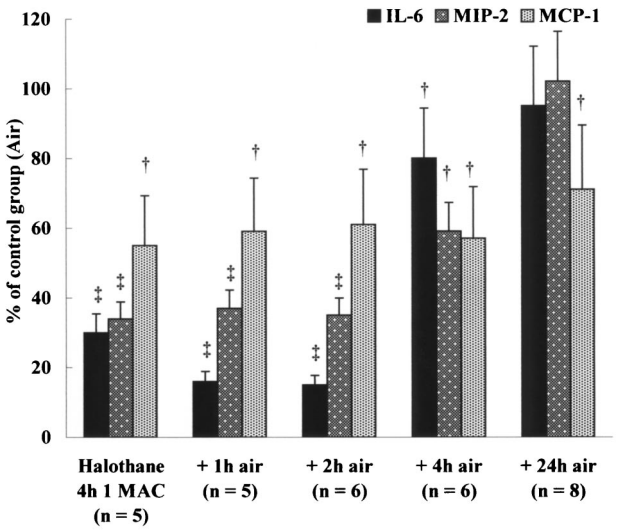

Fig. 6. Reversibility of halothane effects on interleukin-6 (IL-6), macrophage inflammatory protein-2 (MIP-2), and monocyte chemoattractant protein-1 (MCP-1) secretions by alveolar type II cells. Recombinant murine interleukin-1β–stimulated rat alveolar type II cells were exposed either to air or halothane during 4 h (1 minimum alveolar concentration [MAC]). Cytokine secretions were then measured in cell supernatants immediately after halothane exposure and 1, 2, 4, and 24 h after the end of halothane exposure. Results are expressed as the percentage of values obtained for air-exposed cells, mean ± SD; †P < 0.05 and ‡P < 0.001 *versus* air.

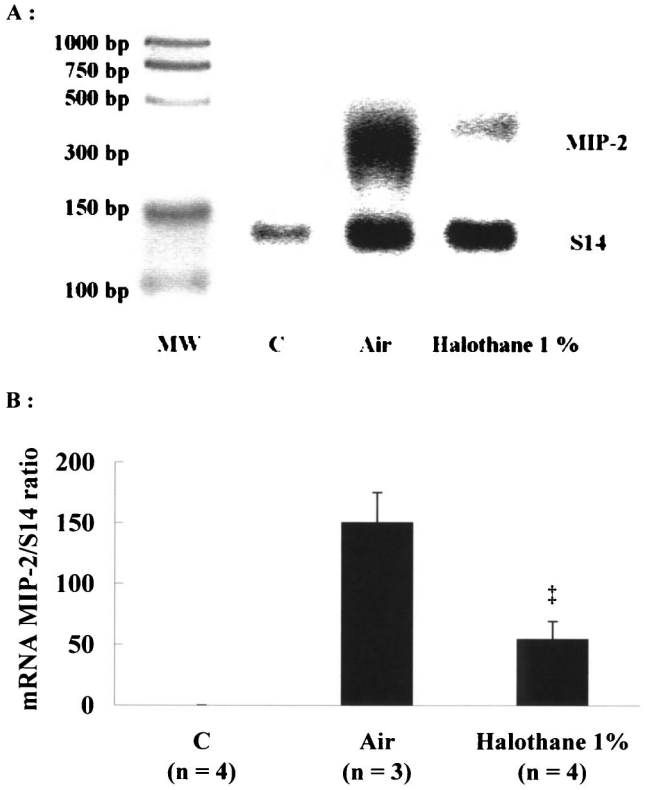

Fig. 7. Effect of halothane on macrophage inflammatory protein-2 (MIP-2) mRNA expression in alveolar type II cells. (*A*) Southern-blot analysis of MIP-2 cDNA after reverse-transcription polymerase chain reaction. Results of one of four representative experiments. (*B*) MIP-2 expression in alveolar type II cells expressed as MIP-2/S14 ratio. Results are expressed as mean ± SD; ‡$P < 0.001$ *versus* air. bp = base pair; C = control group; MW = molecular weight.

proinflammatory cytokine such as interleukin-1β. Therefore, we did not measure TNF-α protein secretion by AT_{II} cells exposed to volatile anesthetics. However, as TNF-α secreted by AT_{II} cells has been involved in the regulation of MIP-2 synthesis in an autocrine loop,[7] we also investigated the effect of halothane on TNF-α mRNA expression. TNF-α mRNA expression in unstimulated AT_{II} cells was under the detection limit (fig. 8), and rmIL-1β stimulation induced an increase of TNF-α mRNA expression in air-exposed cells. Halothane exposure (1 MAC, 4 h) decreased TNF-α mRNA expression by 24 ± 11.3% as compared with air-exposed rmIL-1β–stimulated AT_{II} cells ($P < 0.001$).

Discussion

The main result of our study is that halothane, isoflurane, and enflurane reduced the rmIL-1β–stimulated AT_{II} cell cytokine secretion. Halothane reduced the secretion of interleukin-6, MIP-2, and MCP-1 proteins in a time- and dose-dependent manner, in association with a decrease in MIP-2 and TNF-α mRNA expression. This reduced cytokine secretion was transient and reversible between the 4th and 24th hour after the end of volatile anesthetic exposure.

Most of our findings are in agreement with our previous *in vivo* study.[23] In the latter, we demonstrated, in mechanically ventilated rats, that 4 h of 1% (1 MAC) halothane exposure decreased the lung inflammatory response induced by intratracheal instillation of lipopolysaccharide. Neutrophil recruitment was reduced and was tightly correlated with MIP-2 secretion in the bronchoalveolar lavage fluid. This was accompanied by a decrease in interleukin-6, TNF-α protein concentrations, and MIP-2 and TNF-α mRNA expression in the bronchoalveolar lavage fluid and lung homogenates. These results observed *in vivo* might be explained, at least in part, by an inhibitory effect of volatile anesthetics on cytokine secretion by AT_{II} cells. Only a few investigations have documented the effects of volatile anesthetics on cell cytokine secretion, and contradictory effects have been reported. In agreement with our results, Mitsuhata *et al.*[19] have shown that *in vitro* production of TNF-α and IL-1 by peripheral blood mononuclear cells was inhibited by enflurane, isoflurane, and sevoflurane, the latter compound being the most effective to inhibit cytokine release. In the current study, we did not ob-

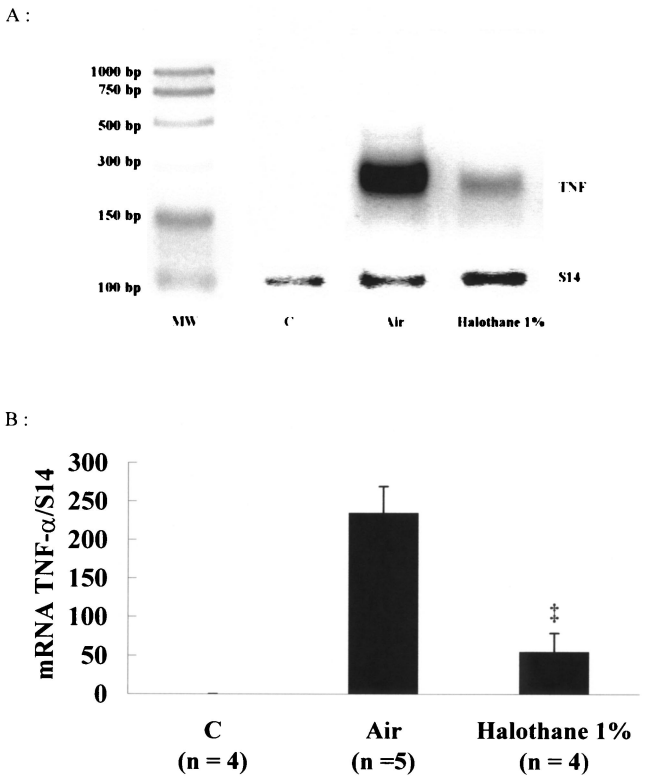

Fig. 8. Effect of halothane on tumor necrosis factor-α (TNF-α) mRNA expression in alveolar type II cells. (*A*) Southern-blot analysis of TNF-α cDNA after reverse-transcription polymerase chain reaction. Results of one of four representative experiments. (*B*) TNF-α expression in alveolar type II cells expressed as TNF-α/S14 ratio. Results are expressed as mean ± SD; ‡$P < 0.001$ *versus* air. bp = base pair; C = control group; MW = molecular weight.

serve any difference between halothane, enflurane, and isoflurane. By contrast, Kotani *et al.*[31] have found *ex vivo*, that exposure of rat alveolar macrophages to volatile anesthetics and mechanical ventilation augmented mRNA expression of interleukin-1β, MIP-2, interferon-γ, and TNF-α as compared with mechanical ventilation alone. These increases were associated with a significant increase in MIP-2 and TNF-α protein in the bronchoalveolar lavage fluid from ventilated rats.[31] However, in the latter work, rat alveolar macrophage cytokine expression was studied in basal conditions, without any further stimulation. In our study, the basal concentrations of interleukin-6, MIP-2, and MCP-1 in unstimulated AT_{II} cell supernatants were not modified by the volatile anesthetics.

In contrast to the results found in this *in vitro* investigation, our previous data obtained in an *in vivo* model, in which the animals received intratracheal lipopolysaccharide, did not show a significant change in MCP-1 concentration after exposure to halothane.[23] A possible explanation for these discordant results is that bronchoalveolar lavage fluid represents the cytokine secretion of the overall cells, including at least AT_{II} cells, alveolar macrophages, and neutrophils. These rat lung cells might have their cytokine secretions differentially altered by halothane, and the decrease of *in vitro* AT_{II} cell MCP-1 secretion by halothane might be blinded *in vivo* by the secretion of MCP-1 originating from other cells such as alveolar macrophages. Indeed, alveolar macrophages represented the most important producer of inflammatory cytokines,[32] and their cytokine secretions might be differently affected by halothane exposure.

The mechanisms by which AT_{II} cell cytokine secretion was decreased remain to be determined. As total protein concentration in cell supernatants was not modified until 1.5% of halothane, the decreased inflammatory cytokine secretion could not be caused by an inhibition of global protein secretion. These results are in agreement with two previous studies that showed that halothane decreased the protein synthesis for halothane concentrations higher than 2% and for time exposure longer than 6 h.[14,15] A cytotoxic effect can also be excluded as LDH activity remained unchanged even after 4 h of 1% halothane exposure. These findings are in agreement with the study of Molliex *et al.*,[20] who demonstrated a cytotoxic effect of halothane only for long exposure periods, *i.e.*, 8 and 12 h, and for elevated halothane concentrations, *i.e.*, 8%.

We showed that MIP-2 and TNF-α steady state mRNA concentrations were decreased in AT_{II} cells. Since halothane was able to decrease mRNA concentration,[33] halothane might have directly decreased MIP-2 and TNF-α secretion by acting, at least in part, at the transcriptional level. The decrease of MIP-2 protein in AT_{II} cell supernatants might have been indirectly induced by the decreased in TNF-α, which is able to induce MIP-2 secretion.[7]

Recently, Tschaikowsky *et al.*[34] have shown that the expression of inducible nitric oxide synthase by immunostimulated murine macrophage-like cell line was affected by volatile anesthetics through intracellular calcium changes. In this connection, volatile anesthetics have been shown to reversibly inhibit voltage-dependent calcium channels and affect intracellular calcium mobilization, resulting in a decreased concentration of intracellular Ca^{2+}.[17,35,36]

Whatever the mechanism involved, it is noteworthy that the effect of volatile anesthetic on AT_{II} cytokine secretion was transient. Indeed, we showed that the inhibitory effect of volatile anesthetic was rapidly reversible after the end of exposure. These findings are in agreement with our *in vivo* study and suggest that, in clinical practice, the inhibitory effect of volatile anesthetic might be limited to the operative or early postoperative period. It remains, however, to determine the effects of volatile anesthetics in clinical practice, particularly in the genesis of lung infection in the postsurgical period.

In conclusion, our study demonstrates that rmIL-1β–stimulated AT_{II} exposure to volatile anesthetics reversibly alters AT_{II} cell cytokine secretion. It is therefore possible that volatile anesthesia, by modulating pulmonary epithelial cell secretion of inflammatory cytokines, might affect lung inflammatory response.

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