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Effect of Intravenous Anesthetics on Spontaneous and Endotoxin-stimulated Cytokine Response in Cultured Human Whole Blood

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Background: **Various anesthetics have been suggested to interfere with the immune system. The ability of leukocytes to express surface receptors and mediators is fundamental to a successful host defense. Therefore, the effects of intravenous anesthetics on cytokine release by leukocytes and expression of surface molecules known to modulate this response were determined.**

Methods: **Concentration-dependent effects of thiopentone, etomidate, propofol, ketamine, midazolam, and fentanyl on spontaneous and endotoxin (lipopolysaccharide; 1 μg/ml)-stimulated cytokine release were studied in whole blood from volunteers (n = 6) cultured for 25 h. In addition, expression of the lipopolysaccharide-recognition molecule CD14 and the major histocompatibility complex class II molecule human leukocyte locus A system-DR (HLA-DR) on monocytes were assessed using flow cytometry.**

Results: **All anesthetics studied elicited only minor effects on spontaneous cytokine release even at pharmacologic concentrations. However, expression density of CD14 was reduced in the presence of thiopentone, etomidate, and propofol, whereas HLA-DR was unaffected. lipopolysaccharide-stimulated tumor necrosis factor response was inhibited by thiopentone (12.8% [median]; 7.6–18.8 [25–75 percentile]) of control, and ketamine (46.4% [median]; 44.4–56.4 [25–75 percentile]), at pharmacologic concentrations, whereas it was augmented even in the presence of low concentrations of propofol (172.3% [median]; 120.5–200.7 [25–75 percentile]). Ketamine additionally decreased the concentration of interleukin (IL)-1β (14.8% [median]; 12.0–18.0 [25–75 percentile]). Release of IL-1 receptor antagonist (IL-1ra) was inhibited by thiopentone, etomidate, and propofol, whereas the same anesthetics increased IL-10 concentration simultaneously. Midazolam and fentanyl did not alter the concentrations of any cytokine.**

Conclusions: **These results suggest a complex modulation of the cytokine response by the studied anesthetics in cultured whole blood. Although effects on spontaneous cytokine release by leukocytes were negligible, some anesthetics affected their ability to respond to lipopolysaccharide. (Key words: CD14; HLA-DR; lipopolysaccharide; leukocytes; monocytes.)**

AMPLE evidence suggests that stress and trauma associated with major surgery may affect the host defense system.[1–3] Among the different mediators involved in the regulation of the host defense, the orchestrated response of the cytokine network seems to be of particular importance.[4] Cytokines comprise a heterogenous group of peptide mediators that modulate cell growth, maturation, and intercellular communication of immune-competent cells, particularly during inflammation.[5] They are produced from activated leukocytes and, to a lesser extent, from fibroblasts and endothelial cells. Cytokines released by these cells during injury are important costimulatory molecules in the process of antigen presentation,[4] a critical link between innate and adaptive immunity and regulators of the acute phase response, a key component of the systemic adaptive changes during stress conditions.[6]

General anesthesia may influence the host defense system; however, the cellular mechanisms involved are far from clear. Anesthetics may either modulate the host defense indirectly by affecting the afferent neuronal input from the operative site, thus affecting the neurohumoral response to injury, or directly act on immune-competent cells.[7,8] For instance, an inhibitory effect of thiopentone on respiratory burst activity seems to be

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responsible for the inhibition of bacterial killing by polymorphonuclear neutrophils.[9] Similarly, inhibition of the release of cytokines by cultured mononuclear cells has been reported after exposure to opioids and various anesthetics, including volatile anesthetics and intravenous anesthetics, *e.g.*, ketamine.[10,11] In the current study, we attempted to gain further insight into the mechanisms underlying the perioperative modulation of immune function by intravenous anesthetics in a whole-blood culture system in which many of the cellular and humoral interactions remain intact. In particular, an effort was made to elucidate the influence of the intravenous anesthetics on the ability of leukocytes to express surface receptors and cytokines critical for the response to bacterial stimuli.

Materials and Methods

Bacterial endotoxin (*Escherichia coli* O111:B4) and all chemicals were obtained from Sigma Chemicals (St. Louis, MO) if not specified otherwise.

Processing of Blood Samples and Experimental Protocol

Venous blood was collected from six healthy volunteers aseptically in a pyrogen-free citrate-containing system (Sarsted Monovette, Muembrecht, Germany) and processed immediately as described by Wilson *et al.*[12] with minor modifications, as described previously.[13] Briefly, blood samples from each donor were diluted 1:5 with cell culture medium RPMI 1640 (Gibco, Paisly, Scotland) and recalcified by addition of 1 ml of a 250-mM calcium chloride ($CaCl_2$) stock solution per 100 ml diluted blood after addition of heparin (2 units/ml; tested for endotoxin < 5 pg/ml). Aliquots of diluted blood (25 ml) were placed in sterile polypropylene tubes (Falcon; Beckton Dickinson, Lincoln Park, NY) and incubated in a humidified atmosphere with 5% carbon dioxide (CO_2) at 37°C. One aliquot of blood was processed immediately, as described herein, and served as the baseline value. Two aliquots were cultured in the absence of any anesthetic but in the presence or absence of 1 μg/ml lipopolysaccharide (LPS) added 1 h after onset of the whole-blood culture for a total period of 25 h (time-matched controls). Anesthetics were added to aliquots of blood of each donor for a 1-h preincubation period and subsequently were cultured for another 24 h in the presence or absence of 1 μg/ml LPS, a dose that induces a maximum release of all cytokines studied. Anesthetics were added to the whole-blood cultures at concentrations achievable during clinical conditions (*i.e.*, thiopentone: $2.65*10^{-4}$ M; etomidate: $1.72*10^{-5}$ M; propofol: $1.57*10^{-4}$ M; ketamine: $5.89*10^{-5}$ M; midazolam: $4.30*10^{-6}$ M; fentanyl: $4.16*10^{-8}$ M) or at pharmacologic concentrations 10-fold more than these. The concentrations of anesthetics added to the culture system were calculated assuming a body weight (b.w.) of 70 kg, a blood volume of 5 l, and injection of doses of 5 mg/kg b.w. thiopentone (Trapanal, Byk Gulden, Konstanz, Germany); 0.3 mg/kg b.w. etomidate (Etomidat-Lipuro, B. Braun, Melsungen, Germany); 2 mg/kg b.w. propofol (Disoprivan, Zeneca, Plankstadt, Germany); 1 mg/kg b.w. ketamine (Ketanest, Parke Davis, Berlin, Germany); 0.1 mg/kg b.w. midazolam (Dormicum, Hoffmann-La Roche, Grenzach-Wyhlen, Germany); or 2 μg/kg b.w. fentanyl (Fentanyl Curamed, Schwabe-Curamed, Karlsruhe, Germany) to induce anesthesia, respectively. Osmolality and *p*H were measured in separate aliquots of diluted blood after addition of the different concentrations of the anesthetics and equilibration in the humidified atmosphere with 5% CO_2 at 37°C using a standard ion-selective electrode (*p*H Meter 523, WTW, Weilheim, Germany) and freezing-point osmometry (Microosmometer 3MO, Advanced Instruments, Needham Heights, MA), respectively.

The effects of the additives benzethonium chloride (20 μg/ml) and Intralipid (15 μl/ml; a soybean oil preparation; Pharmacia & Upjohn, Erlangen, Germany), which are contained in ketamine, etomidate, and propofol solutions, on spontaneous and LPS-stimulated cytokine response at concentrations corresponding to the supraclinical concentrations of the anesthetics added were assessed in control experiments (n = 6 each). Because *p*H remained well in the normal range even after addition of the high dose of thiopentone to cultured whole blood, no such experiments were conducted to rule out the effect of sodium carbonate, the additive used to dissolve thiopentone.

At 25 h after onset of culture, the samples were centrifuged over a Ficoll-Hypaque density gradient (d = 1.077) at 680 g for 20 min. The plasma was then removed and stored immediately at −70°C until it was assayed for cytokines. The interphase containing peripheral blood mononuclear cells (PBMCs) was separated for fluorescence-activated cell sorting analysis. The viability of PBMCs (> 95%) isolated by density-gradient centrifugation after whole-blood culture was evaluated using

trypan blue exclusion and was not found to change significantly during the incubation period.

Cytokine Assays

Cytokine concentrations were measured using specific enzyme-linked immunosorbent assays (EASIA; Medgenix/Biosource, Ratingen, Germany). Aliquots of supernatants, which were stored at −70°C for less than 2 months before the assay, were thawed, and LPS-stimulated samples were diluted up to 1:20 with the provided diluent to stay within the linear range of the assay. Controls provided with the kit were measured routinely with each assay. Calculated inter- and intraassay coefficients of variance were 5.2% (3.5%) for tumor necrosis factor (TNF)-α; 7.3% (6.1%) for interleukin (IL)-1β; 8.9% (11.5%) for IL-1ra; 5.2% (4.7%) for IL-6; and 9.9% (4.3%) for IL-10. The minimal detectable concentrations, as estimated from the average optical density (OD) reading of the zero standard plus two standard deviations, were approximately 3 pg/ml for TNF-α; 2 pg/ml for IL-1β; 4 pg/ml for IL-1ra; 2 pg/ml for IL-6; and 1 pg/ml for IL-10.

Flow Cytometry

The expression pattern of the surface receptors CD14 and HLA-DR on PBMCs was characterized by fluorescence-activated cell sorting analysis, as described previously.[14] The PBMCs were obtained by density gradient centrifugation, as already specified. The interphase containing PBMCs was washed in phosphate-buffered saline, pelleted at 150 g (4°C, 10 min) and resuspended in 500 μl phosphate-buffered saline. The PBMCs (approximately 3–5 $\times$ 10^9 cells/l) were incubated subsequently with monoclonal antibodies directed against CD14 (anti-CD14 (Leu-M3) fluorescent tagged with fluorescein isothiocyanate, clone MOP9; Becton Dickinson, San Jose, CA) and HLA-DR (anti-HLA-DR fluorescent tagged with phycoerythrin, clone L243.9; Becton Dickinson), 20 μl each at 4°C for 10 min, as specified by the manufacturer. After incubation with antibodies, cells were washed and resuspended in phosphate-buffered saline subjected to flow cytometric analysis in a FACSscan (Becton Dickinson) and evaluated with the LYSYS II software package (Becton Dickinson). Cells expressing HLA-DR are reported as percentage of all cells found to express CD14. Expression density of the LPS-recognition molecule CD14 is reported semiquantitatively according to published data[15] with $CD14^{++}$ reflecting a population of strongly positive cells in the monocyte gate (mean fluorescence intensity exceeding $468*10^{-2}$V) as opposed to a subpopulation expressing low levels of CD14, *i.e.*, $CD14^{+}$ cells.

Statistical Analysis

Data are presented as box plots indicating median 25 and 75 percentile and range. Because criteria for parametric testing were violated for many of the cytokine data, data were transformed logarithmically. Differences were analyzed subsequently by two-way analysis of variance after confirming that the log-transform succeeded in normalizing the distribution (Kolmogorov-Smirnow and Levene-Mediane tests). Two way analysis of variance was performed with factors "donor" and "drug" followed if significant by *post hoc* Dunnett's test to compare each drug with the time-matched aliquots of each donor cultured in the absence of anesthetics. All P values reported are two-sided and are considered statistically significant if $P = 0.05$ or less.

Results

Effect of Addition of the Anesthetics on pH and Osmolality

Concentrations of the anesthetics achievable during induction of anesthesia did not affect osmolality or *p*H of the cultured blood. Addition of thiopentone at pharmacologic doses increased the *p*H of the culture, however, resulting in a *p*H still within the physiologic range. None of the anesthetics had a significant effect on the osmolality of the supernatants at pharmacologic concentrations (table 1).

Effect of the Anesthetics on Expression Density of the Surface Receptors CD14 and HLA-DR

The influence of the various anesthetics at clinical and pharmacologic concentrations on expression density of the LPS-recognition molecule CD14 is summarized in figure 1. Monocytes isolated from diluted blood cultured in the presence of thiopentone, etomidate, or propofol exhibited a shift from the strongly positive $CD14^{++}$ phenotype to monocytes expressing low levels of CD14, similar to what was observed in time-matched cultures in the absence of anesthetics but stimulated with LPS. None of the anesthetics, either at clinical or pharmacologic concentrations, impaired the LPS-stimulated shift thought to reflect monocyte activation in the coculture assays. In contrast to the substantial changes in the expression density of CD14, only minor effects were observed regarding expression of the major histocom-

Table 1. Dose-dependent Effect of Addition of iv Anesthetics on Osmolality and *p*H of Cultured Whole Blood

	Osmolality (1×)	Osmolality (10×)	*p*H (1×)	*p*H (10×)
Control	293 (292–294)		7.33 (7.31–7.36)	
Thiopentone	294 (293–296)	291 (289–292)	7.35 (7.32–7.38)	7.43 (7.38–7.46)*
Etomidate	294 (293–295)	296 (295–297)	7.35 (7.34–7.36)	7.36 (7.30 –7.37)
Propofol	293 (292–294)	294 (293–295)	7.35 (7.31–7.38)	7.35 (7.32 –7.38)
Ketamine	293 (292–295)	294 (292–295)	7.35 (7.32–7.38)	7.34 (7.30 –7.37)
Midazolam	293 (291–294)	294 (292–295)	7.35 (7.34–7.37)	7.33 (7.30 –7.35)
Fentanyl	293 (293–294)	294 (291–295)	7.35 (7.31–7.38)	7.36 (7.30 –7.38)

The iv anesthetics were added to diluted whole blood cultures at a concentration (*i.e.*, thiopentone: 2.65×10^{-4} M; etomidate: 1.72×10^{-5} M; propofol: 1.57×10^{-4} M; ketamine: 5.89×10^{-5} M; midazolam: 4.30×10^{-6} M; fentanyl: 4.16×10^{-8} M) achievable during induction of anesthesia (1×) or at a pharmacological concentration exceeding that concentration tenfold (10×).

* $P < 0.05$ *versus* culture in the absence of anesthetics.

patibility complex class II molecule HLA-DR. A moderate, albeit significant, decrease of CD14-positive cells expressing HLA-DR compared to time-matched LPS-stimulated controls (97.5% [median]; 94.0–99.4 [25–75 percentile]) was observed in the presence of low concentrations of propofol and LPS (88.7%; 70.4–99.1) and in the presence of supraclinical concentrations of thiopentone and LPS (66.1 [34.8–95.9]%) (table 1).

Effect of Intravenous Anesthetics on Spontaneous Cytokine Release by Cultured Whole Blood

Data are summarized for IL-1β and IL-1ra in figures 2 and 3 and for the functionally antagonistic cytokines TNF-α and IL-10 in figures 4 and 5, respectively. Basal release of cytokines into plasma of whole blood cultured in the absence or presence of the intravenous anesthetics barely exceeded the detection limits of the cytokine enzyme-linked immunosorbent assays, except for the antiinflammatory cytokine IL-1ra (fig. 3). The intravenous anesthetics studied elicited only minor effects at pharmacologic doses on the spontaneous release of either pro- or antiinflammatory cytokines, *i.e.*, a significant increase of IL-1ra was observed in whole blood cultured in the presence of thiopentone (control: 127.1 [86.3–204.1] pg/ml; thiopentone: 396.6 [226.8–576.7] pg/ml; $P < 0.05$) or etomidate (470.0 [216.0–793.1] pg/ml; $P < 0.05$ *versus* control), whereas a consistent statistically significant decrease of the trace amounts of IL-1β released by cultured blood was observed in the presence of etomidate (control: 7.8 [6.4–13.5] pg/ml; etomidate: < 2 [< 2–< 2] pg/ml; $P < 0.05$).

Effect of Intravenous Anesthetics on LPS-stimulated Cytokine Release by Cultured Whole Blood

In contrast to the moderate effects of the studied intravenous anesthetics on basal release of cytokines into plasma, the LPS-stimulated release of cytokines was affected substantially by the presence of various anesthetics. Clinically achievable concentrations of thiopentone (67.6% of control [62.8–76.7]; etomidate (82.3% [78.3–96.5]); and ketamine (83.5% [80.6–88.4]) had a moderate, albeit very consistent, and statistically significant effect on LPS-stimulated IL-1β release, as compared with the time-matched samples of each donor (figure 2A), whereas thiopentone (81.3% [72.1–93.2]) additionally inhibited the physiologic antagonist IL-1ra to a similar extent (figure 3A). A similar consistent increase was observed for IL-10 in the presence of clinically achievable concentrations of etomidate (159.2% [100.0–219.9]; figure 5A), whereas a substantial induction of TNF-α (172.3% [120.5–200.7]) was observed in the presence of propofol (figure 4A).

Pharmacologic concentrations of etomidate (64.4% [57.9–64.9]) and propofol (69.6% of control [59.6–72.3]) produced a moderate decrease, whereas ketamine (53.6% [38.2–57.1]), and most notably thiopentone (23.0% [17.7–37.3]), reduced LPS-stimulated IL-1ra substantially (figure 3B). In contrast to its physiologic inhibitor IL-1ra, IL-1β release was only impaired in the presence of ketamine (14.8% [12.0–18.0]) (figure 2B). Culture in the presence of pharmacologic concentrations of either thiopentone (308.7% [218.9–432.7]) or etomidate (375.6% [243.0–614.3]) increased release of IL-10 significantly (figure 5B), whereas the release of the functionally antagonistic cytokine TNF-α was reduced in the presence of ketamine (46.4% [44.4–56.4]) and most notably in the presence of thiopentone (12.8% [7.6–18.8]) (figure 4B).

Neither the concentrations achievable during induction of anesthesia nor the supraclinical concentrations of the intravenous anesthetics interfered statistically significantly with release of IL-6 (data not shown).

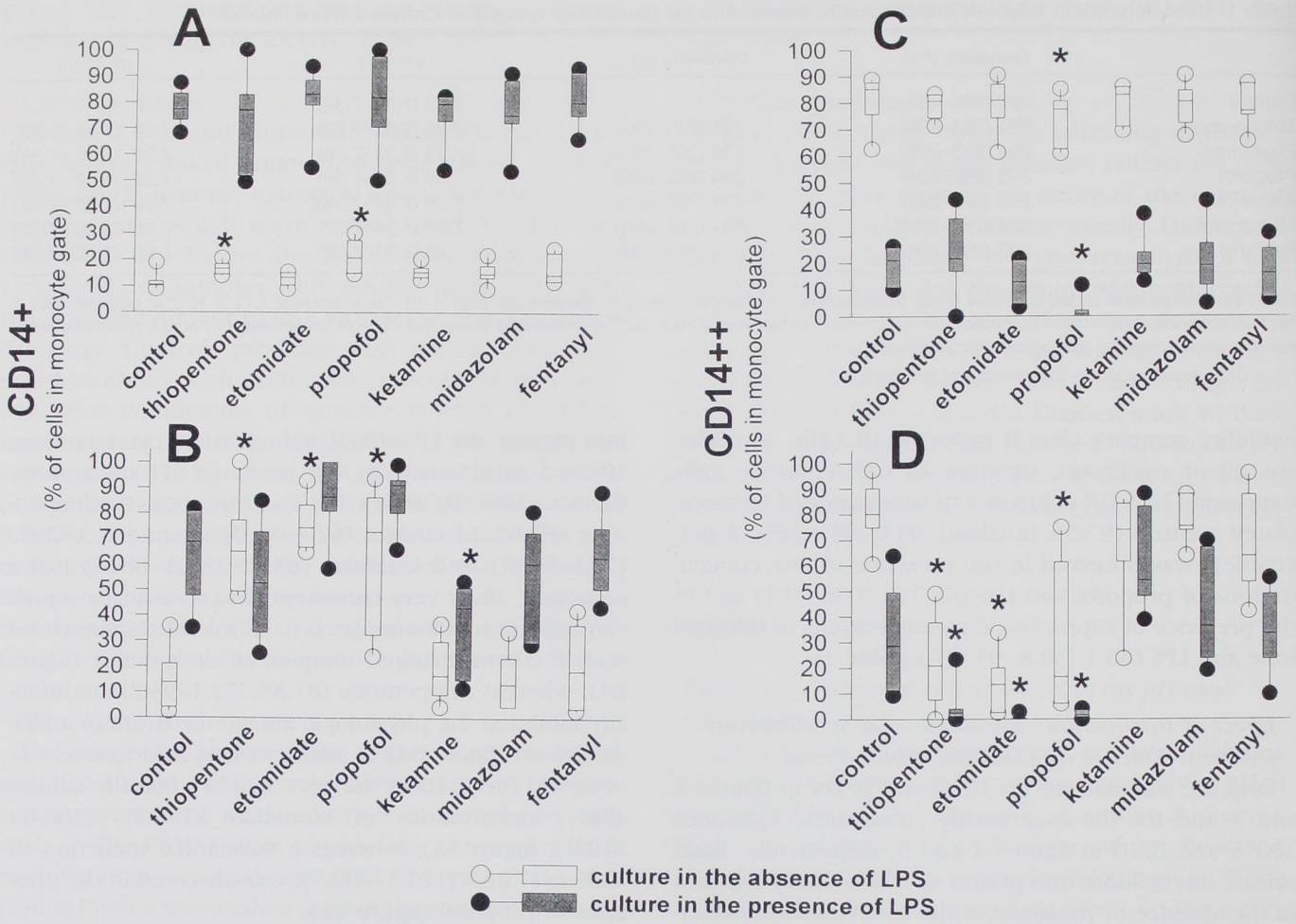

Fig. 1. Influence of intravenous anesthetics on the expression density of the lipopolysaccharide (LPS)-recognition molecule CD14 on monocytes isolated from whole blood cultured in the absence or presence of LPS. Anesthetics were added to the culture system at concentrations achievable during clinical conditions (*A,C*; *i.e.*, thiopentone: $2.65*10^{-4}$ M; etomidate: $1.72*10^{-5}$ M; propofol: $1.57*10^{-4}$ M; ketamine: $5.89*10^{-5}$ M; midazolam: $4.30*10^{-6}$ M; fentanyl: $4.16*10^{-8}$ M) or in pharmacologic doses exceeding these concentrations 10-fold (*B,D*). Peripheral blood mononuclear cells were isolated after 25 h of whole-blood culture by density gradient centrifugation and subjected to fluorescence-activated cell sorter analysis. Expression density of CD14 was analyzed semiquantitatively by flow cytometry with $CD14^{++}$ (*C,D*) reflecting a strongly positive subpopulation of cells in the monocyte gate and $CD14^{+}$ (*A,B*) reflecting an activated phenotype expressing low levels of CD14. For details see materials and methods. Box plots represent the twenty-fifth/seventy-fifth percentile, median, and range. * indicates $P < 0.05$ compared to respective time-matched cultures in the absence of intravenous anesthetics.

Effect of the Additives Benzethonium Chloride and Intralipid on Spontaneous and LPS-stimulated Cytokine Response

None of the additives induced a significant release of cytokines in the absence of LPS (data not shown). Regarding LPS-stimulated cytokine response, the release of TNF-α (control: 3,115 [2,553–3, 328] pg/ml; additive alone: 4,026 [3,031–5,631] pg/ml; ketamine: 1,920 [965–2,393] pg/ml; $P < 0.05$ compared to control and additive alone), IL-1β (control: 2,429 [1,398–3,500] pg/ml; additive alone: 2,073 [1,102–3,108] pg/ml; ketamine: 458 [145–603] pg/ml; $P <$ 0.05 compared to control and additive alone) were not significantly affected by benzethonium chloride, an additive contained in the ketamine preparation used. In contrast, LPS-stimulated concentrations of IL-1ra were not significantly different among control (2,825 [1,205–5,994] pg/ml), additive (2,391 [727–3,807] pg/ml), and ketamine (2,406 [2,102–4,061] pg/ml) conditions. Therefore, the significant decrease in TNF-α and IL-1β concentrations in the presence of ketamine/benzethonium chloride compared to the additive alone confirms the specific effect of ketamine on these cytokines.

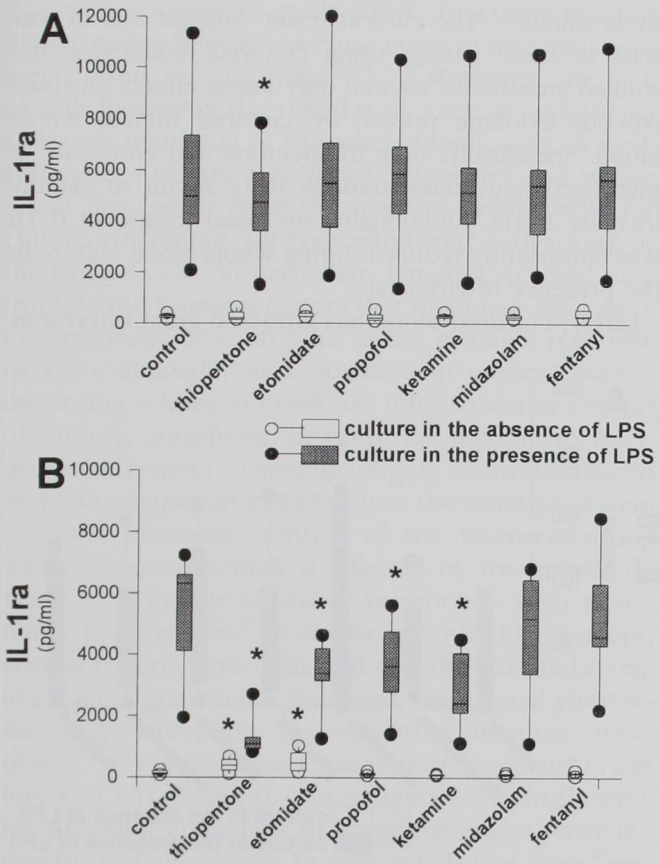

Fig. 3. Dose-dependent effects of intravenous anesthetics on spontaneous and lipopolysaccharide (LPS)-stimulated release of interleukin-1 receptor antagonist (IL-1ra) into cultured blood. Anesthetics were added to the culture system at concentrations achievable during clinical conditions (*A*) or in pharmacologic doses exceeding these concentrations by 10-fold (*B*). Concentrations of IL-1ra, the physiologic antagonist of IL-1, were measured in plasma obtained 25 h after the onset of whole-blood cultures in the absence or presence of intravenous anesthetics. After a 1-h preincubation, half of the wells were additionally stimulated with LPS (1 μg/ml). Box plots represent the twenty-fifth/seventy-fifth percentile, median, and range. * indicates $P < 0.05$ compared to respective time-matched cultures in the absence of intravenous anesthetics.

The soybean oil preparation Intralipid did not significantly affect any of the cytokine concentrations compared to controls, irrespective of the presence or absence of LPS in the culture. The release of TNF-α in the presence of the propofol/soybean oil preparation was increased compared to the additive (control: 3,429 [2,547–4,402] pg/ml; additive alone: 3,040 [2,164–4,147] pg/ml; propofol: 5,772 [4,932–6,003] pg/ml; $P < 0.05$ compared to control and additive alone). The LPS-stimulated IL-10 was significantly increased in the presence of the etomidate/soybean oil preparation (etomidate: 115 [65–128] pg/ml) and by the propofol/soybean oil preparation (propofol: 82 [56–107] pg/ml) compared to control (control: 31 [6.7–52.1] pg/ml) or additive control (Intralipid: 24 [6.6–41.5] pg/ml). Therefore, the observed significant effects of the etomidate and propofol emulsions containing soybean oil on LPS-stimulated TNF-α and IL-10 response are likely to reflect specific effects of the anesthetics.

Discussion

In the current study, we investigated the influence of intravenous anesthetics on spontaneous and LPS-stimu-

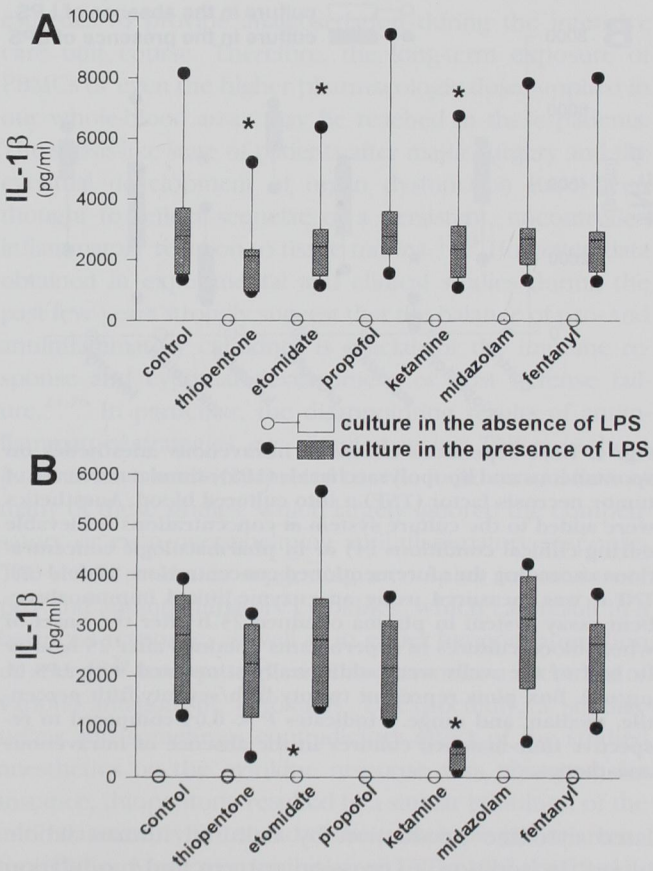

Fig. 2. Dose-dependent effects of intravenous anesthetics on spontaneous and lipopolysaccharide (LPS)-stimulated release of interleukin (IL)-1 into cultured blood. Anesthetics were added to the culture system at concentrations achievable during induction of anesthesia (*A*) or in pharmacologic doses exceeding these concentrations 10-fold (*B*). IL-1 was measured by enzyme-linked immunoabsorbent assay in supernatants obtained at 25 h; after 1-h preincubation, half of the wells were additionally stimulated with LPS (1 μg/ml). Box plots represent the twenty-fifth/seventy-fifth percentile, median, and range. * indicates $P < 0.05$ compared to respective time-matched cultures in the absence of intravenous anesthetics.

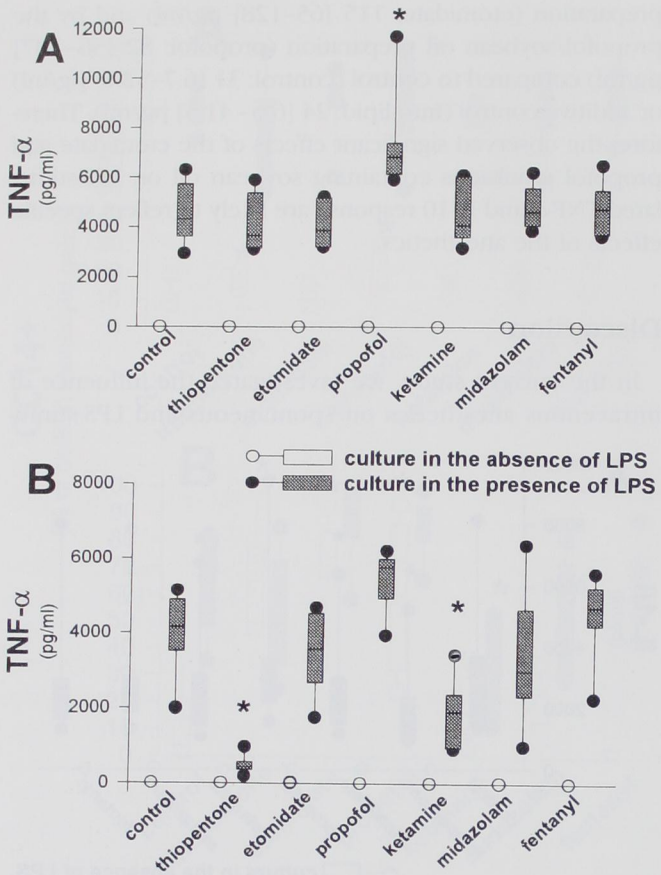

Fig. 4. Dose-dependent effects of intravenous anesthetics on spontaneous and lipopolysaccharide (LPS)-stimulated release of tumor necrosis factor (TNF)-α into cultured blood. Anesthetics were added to the culture system at concentrations achievable during clinical conditions (*A*) or in pharmacologic concentrations exceeding the aforementioned concentrations 10-fold (*B*). TNF-α was measured using an enzyme-linked immunoabsorbent assay system in plasma obtained 25 h after the onset of whole-blood cultures in supernatants obtained after 25 h; after in half of the wells were additionally stimulated with LPS (1 μg/ml). Box plots represent twenty-fifth/seventy-fifth percentile, median, and range. * indicates $P < 0.05$ compared to respective time-matched cultures in the absence of intravenous anesthetics.

lated cytokine production by cultured human whole blood. In addition, expression pattern and modulation by anesthetics of two surface molecules, *i.e.*, the LPS-recognition molecule CD14 and the major histocompatibility complex class II molecule HLA-DR on monocytes, both known to modulate the cytokine response,[16] were studied.

Previous studies using isolated monocytes suggested increased formation of TNF-α when cells were cultured in the presence of propofol, thiopentone, or ketamine, whereas IL-1α was induced mainly by propofol and IL-6 by ketamine.[11] The current results suggest that in contrast to these studies using cultured monocytes, the studied anesthetics elicited only minor effects on spontaneous cytokine release by cultured human whole blood. Specifically, only thiopentone and etomidate at pharmacologic concentrations were found to slightly increase IL-1ra. Additionally, the basal release of IL-1β was consistently reduced during whole-blood culture in the presence of etomidate.

Lately, cultured blood has attracted great interest as

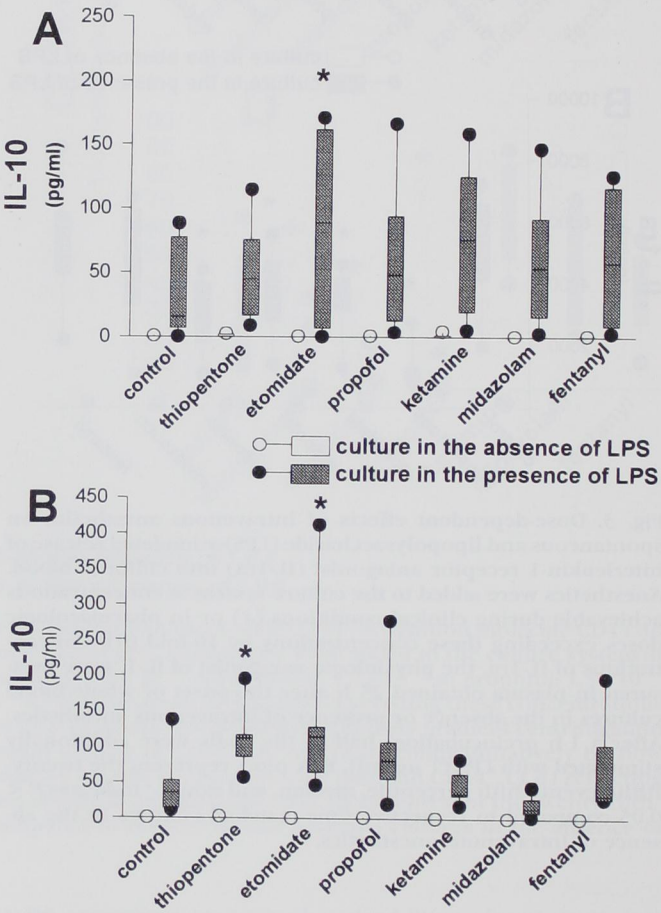

Fig. 5. Dose-dependent effects of intravenous anesthetics on spontaneous and lipopolysaccharide (LPS)-stimulated release of interleukin (IL)-10 into cultured whole blood. Anesthetics were added to the culture system at concentrations achievable during clinical conditions (*A*) or in pharmacologic concentrations exceeding the clinical concentrations 10-fold (*B*). IL-10 was measured in plasma obtained 25 h after the onset of whole-blood culture in the absence or presence of intravenous anesthetics by means of enzyme-linked immunoabsorbent assay. After an 1-h preincubation, half of the wells were additionally stimulated with LPS (1 μg/ml). Box plots represent twenty-fifth/seventy-fifth percentile, median, and range. * indicates $P < 0.05$ compared to respective time-matched cultures in the absence of intravenous anesthetics.

an *ex vivo* model to study PBMC functions in their intact "milieu interne" because many of the regulatory mechanisms remain intact.[12,17,18] However, the lack of cells located in the vessel wall, such as endothelial cells, is a limitation of the whole-blood assay as well. Differences between cultured monocytes and whole blood may result from the need to isolate cells for culture (including adherence-induced activation) or the lack of cells, in particular, lymphocytes and neutrophils and humoral factors that modulate the monocyte response or contribute to the cytokine response *in vivo*. Similarly, data obtained in clinical studies measuring release of cytokines into plasma as a result of surgery, anesthesia, or both, are difficult to interpret with respect to specific effects of anesthetics on immune competent cells because the immune system is under elaborate control of the neuroendocrine stress response, which is affected by the anesthetic plane,[2,3,7,8] or side effects of anesthetics, such as systemic hypotension.[19] Consistent with this concept, several reports have indicated that the anesthetic regimen, *e.g.*, inhalational, balanced, or regional anesthesia, may affect the perioperative immune function.[2,7,8,19] For instance, Crozier *et al.*[8] reported lower levels of circulating IL-6 in patients receiving propofol/alfentanil compared to a control group receiving inhalational anesthesia. In contrast, Taylor *et al.*[20] reported that supplementation of inhalational anesthesia with conventional doses of opioids failed to affect IL-6 response to surgery. Therefore, cultured whole blood seems to be a valuable tool to bridge the gap between studies in isolated cells and immune assays in samples obtained from anesthetized patients to unravel feedback loops operative in the cellular and humoral immune response and their modulation by anesthetics.

In contrast to the lack of effects of the intravenous anesthetics on spontaneous or basal release of the studied cytokines, the ability of PBMCs to mount a cytokine response to gram-negative endotoxin was significantly affected by some of the anesthetics. Interestingly, induction of both cytokines with proinflammatory (*e.g.*, TNF-α and IL-1β) and antiinflammatory profile *in vivo* (*e.g.*, IL-10 or IL-1ra) was modulated by individual anesthetics. Interestingly, ketamine at the higher concentration had a consistent effect on LPS-stimulated cytokine release by cultured whole blood, suggesting a possible antiinflammatory potential of ketamine. These findings may contribute to the beneficial effects of ketamine on LPS toxicity in a mouse model.[21] Furthermore, the inhibitory effect of ketamine on cytokine-mediated mesangial proliferation, a feature of the inflammatory reaction associated with glomerulonephritis[22] that would also be consistent with an antiinflammatory action of ketamine. All other anesthetics that were found to act on the cytokine response produced mixed results with respect to the various functionally antagonistic cytokines but no net shift toward either a primary pro- or an antiinflammatory response.

Evidence suggests that morbidity and mortality associated with major surgery can be mainly attributed to septic complications with host defense failure being a key pathophysiologic feature.[23,24] It is noteworthy that these patients frequently receive continuous infusions of the studied anesthetics to provide analgosedation during the intensive care unit course. Therefore, the long-term exposure of PBMCs or even the higher pharmacologic doses applied in our whole-blood assay may be reached in these patients. The sepsis-like state of patients after major surgery and the eventual development of organ dysfunction have been thought to reflect sequelae of a persistent, uncontrolled inflammatory reaction to tissue trauma.[23,25] However, data obtained in experimental and clinical studies during the past few years strongly suggest that the balance of pro- and antiinflammatory cytokines is crucial for the immune response and eventual development of host defense failure.[24,26] In particular, the disappointing results of antiinflammatory strategies, *e.g.*, directed against TNF-α, in these patients have been interpreted to reflect the central flaw of many of these studies, which largely ignored the compensatory or even overwhelming antiinflammatory response. The recent evidence suggesting a complex interaction of pro- and antiinflammatory cytokines, which can result in a balanced response, as well as in either hyperinflammation or anergy,[24] have prompted us to determine a spectrum of various antagonistic cytokines. Unexpectedly, a simultaneous and sometimes contradictory effect of the studied anesthetics on the cytokine response was observed; for instance, thiopentone resulted in a similar inhibition of the antiinflammatory IL-1ra and of the prototypic proinflammatory TNF-α. Moreover, inhibition of TNF-α was observed in the presence of thiopentone in the assays despite maintained IL-1 response. It is also noteworthy that some of the compounds were found to be inert with respect to expression of surface receptors by PBMCs and the cytokine response. Fentanyl and midazolam, frequently used in combination for long-term sedation, seemingly do not affect any of the studied parameters critical for the host defense on the cellular level.

Evidence suggests that changes in expression of the

CD14 receptor on monocytes produce a phenotypic change that influences the monocytic cytokine pattern during stimulation. Namely, $CD14^{++}$ monocytes have been reported to be primarily responsible for the release of the antiinflammatory IL-10, whereas $CD14^{+}$ monocytes produce primarily proinflammatory cytokines.[15] Consistent with this concept, LPS induced a decrease in CD14 expression density, resulting in a shift to a primarily $CD14^{+}$ phenotype. Interestingly, thiopentone, etomidate, and propofol had a similar effect on CD14 expression density. However, despite this phenotypic change, these anesthetics seem to regulate specific cytokines rather than produce a consistent pro- or antiinflammatory phenotype. Furthermore, their presence results in reprogramming of the cytokine response, whereas anesthetics do not generally downregulate the ability of leukocytes to synthesize cytokines. Nevertheless, in line with the net antiinflammatory effect of ketamine, the shift of the CD14 state of monocytes toward a proinflammatory phenotype was exclusively inhibited in LPS-stimulated whole blood cultured in the presence of ketamine. In contrast, thiopentone substantially inhibited the TNF-α response while inducibility of IL-1β was maintained. Because the cytokine response is highly redundant, the functional significance of inhibition of TNF-α, whereas inducibility of IL-1β, sharing many of the biological functions of TNF-α, is maintained, remains to be determined.

In summary, our results reflect that in contrast to results obtained with cultured cells the studied intravenous anesthetics have only minor effects on basal release of cytokines by leukocytes in a whole blood culture system where many regulatory feedback mechanisms are preserved. However, in contrast to the negligible effects on spontaneous cytokine release thiopentone, etomidate, propofol, and ketamine affected expression of the LPS-recognition molecule CD14 on monocytes, and presence of these anesthetics in the culture system modulated the cytokine response to LPS-stimulation substantially.

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