Propofol inhibits pressure-stimulated macrophage phagocytosis via the GABA\textsubscript{A} receptor and dysregulation of p130cas phosphorylation

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Macrophages are already present within the tissue at the wound site, and phagocytosis of cell debris and contaminated bacteria at the wound site by macrophages is essential for wound repair and prevention of infection (21, 22). This process begins early after injury, although it may persist for days thereafter. Depletion of macrophages, but not neutrophils, from wound sites impairs wound debridement and delays wound healing (31), whereas enhancement of macrophage phagocytosis by administration of heat shock protein 70 accelerates wound healing in mice (29).

The intravenous anesthetic agent propofol is commonly used to induce and maintain anesthesia during surgical or other invasive procedures and to sedate critically ill patients (33, 61). Propofol is also known to alter the functions of immunocompetent cells, such as neutrophils, monocytes, and macrophages (10, 11, 36). Propofol decreases proinflammatory cytokine production and inducible nitric oxide synthase expression in LPS-stimulated mouse macrophage Raw 264.7 cells (10, 11) and increases bacterial accumulation in lung and liver after venous infusion compared with the effects of saline infusion (26). Propofol binds to the \( \beta_2 \)-subunit of the GABA\textsubscript{A} receptor (4, 16), which regulates Cl\textsuperscript{-} channels (32). Some investigators have reported that ion channels, such as those regulating Cl\textsuperscript{-} and Ca\textsuperscript{2+} fluxes, might affect phagocytosis by macrophages and microglia (15, 24). The effects of propofol on macrophage phagocytic activity, therefore, may be potentially important in surgical wound healing.

Inflammation or edema in closed compartments may increase tissue pressure by 5–80 mmHg, and at surgical wound sites, pressure edema may also increase tissue pressures (3, 56). Negative-pressure therapy has been shown to promote wound healing and reduce hospital stay in postsurgical patients (12, 47). Recent reports suggest that mechanical stimuli, such as extracellular pressure and repetitive strain, may alter other aspects of macrophage functions (25, 34). Pressure (40–130 mmHg) increases monocyte migration in a dose-dependent manner and enhances scavenger receptor expression in macrophages (44, 55); increased pressure (40–90 mmHg) has been shown to increase aggregated IgG uptake in mouse macrophage J774.16 cells (34), and extracellular pressure modulates the release of proinflammatory cytokines from human monocyte cells (50). Mechanical strain augments matrix metalloproteinase (MMP)-1 and MMP-3, but not MMP-9, expression in PMA-treated human monocytes or macrophages (63). Therefore, it may be important to understand the effect of extracellular pressure on macrophage functions.

We previously reported that exposure to constant low extracellular pressure (20 mmHg) enhances phagocytosis by human peripheral monocytes and PMA-differentiated THP-1 macrophages through activation of p38 and the phosphatidylinositol

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SURGICAL STRESS AND ANESTHESIA can exert profound effects on immune function (39, 59). The immunosuppressive effects of surgical and anesthetic intervention include impaired T cell proliferation (39), decreased natural killer cell cytotoxicity (43), and altered phagocytic cell function (5, 23). After surgery, the imbalance between pro- and anti-inflammatory cytokines released by monocytes and macrophages may promote dysfunction of innate cellular immunity and increased susceptibility to infection (35, 59). Phagocytosis by alveolar macrophages or neutrophils is decreased during and after major surgery (28).

Macrophages play a particular role in host defense and wound repair (31). Although monocytes and macrophages are recruited from peripheral blood to wound sites over time, tissue macrophages are already present within the tissue at the wound site, and phagocytosis of cell debris and contaminated bacteria at the wound site by macrophages is essential for wound repair and prevention of infection (21, 22). This process begins early after injury, although it may persist for days thereafter. Depletion of macrophages, but not neutrophils, from wound sites impairs wound debridement and delays wound healing (31), whereas enhancement of macrophage phagocytosis by administration of heat shock protein 70 accelerates wound healing in mice (29).
phagocytosis, calculated as the total number of cells with at least one bead relative to the total number of cells counted.

Western blotting. THP-1 macrophages were incubated under ambient or increased pressure (20 mmHg) for 30 min, rinsed once with cold PBS, and lysed with lysis buffer B [50 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10 mM Na pyrophosphate, 50 mM NaF, 10% glycerol, 1% Triton X-100, and 1% dioxycyclic acid (pH 7.6) supplemented with 1 mM PMSF, 10 μg/ml leupeptin, and 10 μg/ml aprotonin]. Protein concentrations in cell lysates were measured using the bicinchoninic acid protein assay reagent (Pierce, Rockford, IL). Equal amounts of protein were loaded in each lane. Cell lysates were resolved under reducing conditions by 10% SDS-PAGE and then transferred to nitrocellulose membranes. The membranes were blocked with 5% BSA in Tris-buffered saline with 0.1% Tween 20 and incubated with antibodies against activated or total forms of protein overnight at 4°C, washed three times with 0.1% Tween 20 + Tris-buffered saline, and then incubated again for 60 min with 2,000:1 peroxidase-conjugated anti-rabbit IgG. The membrane-bound peroxidase activity was detected using ECL Plus Western Blotting Detection kits (Amerham, Arlington Heights, IL). Chemiluminescent images were captured and analyzed using a Kodak Digital Science Image Station 440CF. All blots were studied within the linear range of exposure.

Transfection of small interfering RNA. For inhibition of p130cas protein expression, THP-1 cells were transfected with small interfering RNA (siRNA). The siRNA duplex targeted to human p130cas, 5'-AGGGCTGAGCAGCAGGTAATGTA-3', directed toward the mRNA target 5'-GTGCTGCCAGTGGTTGTAT-3' was designed using Deqor, a web-based tool for the design and quality control of siRNAs and synthesized by Dharmacon (Lafayette, CO). Nontargeted siRNA duplex (NT1) used as a control was purchased from Dharmacon. THP-1 cells (4 × 10⁶ per 60-mm petri dish) were stimulated with PMA (50 ng/ml) for 2 days before transfection. Transfection of duplex siRNAs was performed using Oligofectamine ( Gibco, Gaithersburg, MD) according to the manufacturer’s protocol. At 24–48 h after transfection, cells were used for Western analysis and assays of phagocytosis. Transfection efficacy, measured by transfection of Cy3-conjugated siRNA targeted to luciferase (Upstate, Charlotteville, VA), was 90–97% (55).

Statistical analysis. The significance of differences between groups was calculated by Student’s t-test or paired t-test as appropriate. Bonferroni’s or Sidak’s correction was applied for multiple comparisons where appropriate. The desired level of statistical significance was set a priori at 95% confidence.

RESULTS

In vivo exposure to propofol alters ex vivo macrophage phagocytosis and its response to increased pressure. We previously reported that increased extracellular pressure (20 mmHg above ambient pressure) stimulates serum-opsinized latex bead uptake by primary isolated monocytes from healthy volunteers as well as PMA-differentiated human monocyte THP-1 cells (THP-1 macrophages) (51–53). Several reports suggest that anesthetic agents and surgical stress may affect phagocytic cell function (5, 23, 28) and that tissue pressure may be increased within surgical wounds or at surgical sites postoperatively (56). Therefore, in a pilot study, we compared the effects of increased extracellular pressure on the phagocytosis of primary monocytes isolated from the peripheral venous blood of normal healthy volunteers and from the peripheral venous blood of four randomly chosen patients receiving anesthesia. Exposure to pressure increased phagocytosis in monocytes from healthy volunteers (22.9 ± 3.3 vs. 31.8 ± 4.7%, n = 8, P < 0.005; Fig. 1A), consistent with our previous...
observations (51, 52). However, pressure failed to increase phagocytosis in monocytes isolated from surgical patients (Fig. 1B). Further analysis revealed that two patients had received anesthetics including propofol, a commonly used intravenous anesthetic agent, and two patients had been anesthetized without propofol. Indeed, the monocytes from the two patients non-propofol-anesthetized patients displayed increased phagocytosis in response to pressure, similar to monocytes from healthy volunteers, whereas the monocytes from the two propofol-anesthetized patients exhibited decreased phagocytosis in response to extracellular pressure (Fig. 1B).

To confirm this observation, we sampled monocytes from an additional seven propofol-anesthetized patients and three non-propofol-anesthetized patients and obtained similar results (Fig. 1C). Pooling these samples for statistical analysis revealed that pressure statistically significantly increased phagocytosis in monocytes from non-propofol-anesthetized patients (36.54 ± 3.54 vs. 49.63 ± 3.27% \( n = 5 \), \( P < 0.02 \)), just as we observed in the healthy volunteers. However, in monocytes obtained from propofol-anesthetized patients, pressure not only did not increase, but it actually significantly decreased, phagocytosis (44.25 ± 4.58 vs. 31.39 ± 4.03% \( n = 9 \), \( P < 0.001 \)).

Similar effects of in vitro and in vivo propofol exposure on macrophages. Next, we examined whether propofol modulates macrophage phagocytosis in vitro in primary isolated peripheral monocytes obtained from healthy volunteers and in THP-1 macrophages. Initially, to define the optimal condition for in vitro propofol treatment, we tested the exposure time to propofol and the propofol dose using THP-1 macrophages, in which we previously demonstrated a response to pressure similar to that of primary monocytes (51, 52). THP-1 macrophages were pretreated for 15 min–4 h with 10 \( \mu \)g/ml propofol or the equivalent amount of Intralipid, the vehicle in which propofol is delivered clinically (Fig. 2A). Pretreatment with Intralipid or propofol increased basal phagocytosis in a time-dependent manner that reached a plateau at 2 h. Pressure increased phagocytosis in Intralipid-treated cells at all time points tested. In propofol-treated cells, inhibition of phagocytosis by pressure was observed after 30 min, and this effect lasted \( \geq 4 \) h after in vitro propofol treatment. Our observations in primary monocytes are also consistent with the idea that the propofol effect may last for \( \geq 4 \) h, because the blood monocyte isolation and preparation procedure takes \( \geq 3–4 \) h after the blood is drawn, and we observed persistent changes 4 h later in propofol-anesthetized patients.

Next, THP-1 macrophages were pretreated with various doses of propofol (2.5–20 \( \mu \)g/ml) or Intralipid for 30 min (Fig. 2B). At lower concentrations, Intralipid did not affect basal phagocytosis and pressure increased phagocytosis, whereas propofol increased basal phagocytosis and pressure reversed the propofol-stimulated phagocytosis. Optimal propofol and pressure effects were observed at 10 \( \mu \)g/ml propofol. A higher dose of Intralipid (20 \( \mu \)g/ml Intralipid) increased phagocytosis in Intralipid-treated cells up to a plateau at 2 h. Similar effects of in vitro and in vivo propofol exposure on macrophages were observed in primary peripheral monocytes isolated from surgical patients (study I). Primary human peripheral monocytes were isolated from 4 randomly selected surgical patients (pt 1, pt 2, pt 3, pt 4) who received general anesthesia, and cells were incubated with serum-opsonized fluorescence-labeled latex beads (5 beads/10^6 cells) under ambient pressure or increased pressure (20 mmHg: solid bar) for 2 h. Number of intracellular latex beads was counted under ambient pressure or increased pressure for 2 h, washed with PBS, and then fixed. Pressure increased phagocytosis in monocytes from non-propofol-anesthetized patients, but pressure decreased phagocytosis in monocytes from propofol-infused patients.
Values are means ± SE. B: propofol dose response. THP-1 macrophages were pretreated with various doses of propofol (2.5–20 µg/ml) or corresponding concentrations of Intralipid for 30 min and then incubated with serum-opsonized fluorescence-labeled latex beads (5 beads/cell) under ambient pressure (control) or increased pressure for 2 h. Values are means ± SE.

Fig. 2. Time-course and dose-response studies. A: time course of exposure to propofol. THP-1 macrophages were pretreated with propofol (Prop, 10 µg/ml) or an equivalent concentration of Intralipid (IntraL) as vehicle control for 15 min–4 h and then incubated with serum-opsonized fluorescence-labeled latex beads (5 beads/cell) under ambient pressure or increased pressure for 2 h. Values are means ± SE. B: propofol dose response. THP-1 macrophages were pretreated with various doses of propofol (2.5–20 µg/ml) or corresponding concentrations of Intralipid for 30 min and then incubated with serum-opsonized fluorescence-labeled latex beads (5 beads/cell) under ambient pressure (control) or increased pressure for 2 h. Values are means ± SE.

dose of Intralipid (a vehicle control equivalent to 20 µg/ml propofol) tended to also increase basal phagocytosis, but pressure exerted a further stimulatory effect in cells treated with this concentration of Intralipid. In contrast, in cells treated with 20 µg/ml propofol, phagocytosis did not increase with pressure, although whether phagocytosis was actually inhibited was difficult to discern in the presence of the increased baseline.

Picrotoxin also cancelled the pressure effect in propofol-treated monocytes, as well as in propofol-treated THP-1 macrophages. Furthermore, our previous studies (51, 52) demonstrated a similar response of THP-1 macrophages and primary isolated monocytes from healthy volunteers to extracellular pressure with regard to increased phagocytosis, and this pressure-stimulated phagocytosis is mediated similarly by activation of the p38 MAPK and PI3K-Akt pathways in primary monocytes and THP-1 macrophages. On the basis of these results, we used THP-1 macrophages as a model system for further analysis.

In subsequent studies, we pretreated monocytes or THP-1 macrophages with 10 µg/ml propofol for 30 min, because 10 µg/ml is similar to pharmacologically relevant concentrations during anesthesia (8, 14) and the propofol effect was not observed until ≥30 min after propofol exposure. Pressure significantly increased phagocytosis in cells treated with the Intralipid vehicle control (Fig. 3, A and B), similar to cells evaluated without experimental pharmacological treatment (Fig. 1A). Pretreatment with propofol significantly increased basal phagocytosis by primary monocytes (39.70 ± 10.03 vs. 50.47 ± 7.70%, n = 3, P < 0.05) and THP-1 macrophages (17.82 ± 1.44 vs. 22.82 ± 1.07%, n = 10, P < 0.001), but phagocytosis by primary monocytes (Fig. 3A) and THP-1 macrophages (Fig. 3B) treated with propofol was significantly reduced by pressure [39.37 ± 7.67% (n = 3, P < 0.05) and 18.42 ± 0.71% (n = 10, P < 0.01), respectively].

$\text{GABA}_A$ receptor may mediate the effect of propofol on pressure-stimulated phagocytosis but does not contribute to modulation of phagocytosis by propofol under ambient pressure. Propofol binds to the $\text{GABA}_A$ receptor $\beta_2$-subunit and exerts at least some of its actions via the $\text{GABA}_A$ receptor (16). Gillette and Dacheux (19) demonstrated that $\text{GABA}_A$ receptors are activated by pressure in rabbit retina, and pressure application might contribute to activation of the $\text{GABA}_A$ receptor. We used the $\text{GABA}_A$ receptor antagonists picrotoxin (a non-competitive antagonist) and SR-95531 (a competitive antagonist) to examine whether the $\text{GABA}_A$ receptor mediates the propofol effect on macrophage phagocytosis. Primary isolated monocytes (Fig. 4A) or THP-1 macrophages (Fig. 4B) were preincubated with 2 µM picrotoxin or THP-1 macrophages were incubated with 2 µM SR-95531 (Fig. 4C) for 30 min before pretreatment with Intralipid or propofol. Neither picrotoxin nor SR-95531 affected basal phagocytosis at ambient pressure or stimulation of phagocytosis by propofol at ambient pressure. However, picrotoxin (Fig. 4A and B) and SR-95531 (Fig. 4C) each prevented the inhibition of phagocytosis by pressure in propofol-treated cells.

To further validate these results, THP-1 macrophages were similarly pretreated with the competitive antagonist SR-95531 for 30 min, and SR-95531 was removed before stimulation with Intralipid or propofol or pressure. As shown in Fig. 4D, phagocytosis in THP-1 macrophages pretreated with SR-95531, which was then removed, resembled that observed in untreated intact THP-1 macrophages. Phagocytosis was increased by pressure or propofol alone, and pressure inhibited phagocytosis in propofol-stimulated cells from which SR-95531 had been removed. These data suggest that propofol may stimulate phagocytosis at ambient pressure independently of the $\text{GABA}_A$ receptor but that the $\text{GABA}_A$ receptor may mediate reversal of the pressure effect on phagocytosis by propofol.
Propofol does not act via the signal pathways previously reported to mediate the pressure effect on phagocytosis. Previous studies demonstrated that decreasing FAK and ERK activation and increasing the activation of p38 and the PI3K-Akt-p70S6k pathway contribute to the stimulation of macrophage phagocytosis by extracellular pressure (51–53). We therefore examined the effect of propofol on these signals. Propofol did not affect FAK, p38, or Akt phosphorylation under ambient or increased pressure conditions (data not shown). In contrast, pretreatment with propofol significantly increased ERK phosphorylation compared with cells treated with Intralipid at ambient pressures ($P < 0.05$, $n = 8$), whereas exposure to pressure inhibited ERK phosphorylation in Intralipid- and propofol-treated THP-1 macrophages ($P < 0.02$, $n = 8$; Fig. 5). Since our previous study demonstrated that inhibition of ERK activation by pressure is accompanied by increased THP-1 macrophage phagocytosis by pressure (52), it seems unlikely that activation of ERK at ambient pressure contributes to increased phagocytosis at ambient pressure by propofol or that modulation of ERK contributes to the propofol effect in pressure-stimulated macrophages. These data suggest that FAK, p38, ERK, and Akt may not mediate the modulation of macrophage phagocytosis by propofol under ambient or increased pressure conditions.

$p130^\text{cas}$ may mediate propofol- and pressure-stimulated phagocytosis. $p130^\text{cas}$ (a Crk-associated substrate) is a docking protein involved in regulation of the actin cytoskeleton and cell migration (1, 20, 45, 48). Some studies suggest that $p130^\text{cas}$ may be involved in bacterial or apoptotic cell phagocytosis by macrophages (6, 18, 49). We therefore examined whether $p130^\text{cas}$ phosphorylation might contribute to the stimulation of phagocytosis by extracellular pressure and the reversal of this effect by propofol. Exposure to increased pressure or pretreatment with propofol significantly inhibited $p130^\text{cas}$ phosphorylation in THP-1 macrophages. However, the combination of propofol and pressure increased $p130^\text{cas}$ phosphorylation compared with propofol alone (Fig. 6A). The inverse parallels between phagocytosis and $p130^\text{cas}$ phosphorylation in all four conditions raised the possibility that modulation of $p130^\text{cas}$ phosphorylation by pressure or propofol might contribute to the effects of these stimuli on macrophage phagocytosis.

To further evaluate the role of $p130^\text{cas}$ in mediating pressure-stimulated macrophage phagocytosis and the effect of propofol on macrophage phagocytosis, $p130^\text{cas}$ protein expression was inhibited by transfection with specific siRNA targeted to $p130^\text{cas}$. Transfection with $p130^\text{cas}$ siRNA reduced total $p130^\text{cas}$ protein by $60.1 \pm 5.6\%$ ($n = 5$, $P < 0.001$) compared with cells transfected with NT1 control siRNA (Fig. 6B). Cells transfected with NT1 or $p130^\text{cas}$ siRNA were preincubated with the vehicle control Intralipid or propofol before the phagocytosis assay. In NT1-transfected THP-1 macrophages treated with Intralipid, pressure significantly increased phagocytosis ($n = 9$, $P < 0.01$), and basal phagocytosis was also significantly increased in cells transfected with $p130^\text{cas}$ siRNA compared with cells transfected with NT1 ($n = 9$, $P < 0.05$, by Student’s $t$-test with Bonferroni’s or Sidak’s correction), but pressure did not further increase phagocytosis in cells transfected with $p130^\text{cas}$ siRNA (Fig. 6C). Propofol increased basal phagocytosis and pressure reversed phagocytosis in propofol-treated cells transfected with NT1 siRNA, similar to the effects of these stimuli in control THP-1 cells and normal primary human monocytes. Phagocytosis was significantly increased by $p130^\text{cas}$ reduction in THP-1 cells treated with Intralipid at ambient pressure. However, phagocytosis in cells transfected with $p130^\text{cas}$ siRNA was neither increased by propofol nor reversed by pressure (Fig. 6C). Surveys of the distribution of...
cells with different numbers of beads in these experiments demonstrated that the number of bioavailable beads was not a limiting factor (not shown). These data suggest that pressure and/or propofol may increase macrophage phagocytosis by inhibiting p130cas phosphorylation.

Since a specific pharmacological inhibitor for p130cas was not available, we reduced p130cas with specifically targeted siRNA. Because basal phosphorylation was increased by p130cas reduction but there was no further increase with the combination of propofol and pressure, we might have concluded that p130cas is required for the effects of propofol and pressure on phagocytosis or that the reduction in p130cas maximally stimulated phagocytosis by an unrelated pathway so that no further modulation of phagocytosis by propofol or pressure was possible. To address this issue, we examined the effects of stimulation of phagocytosis by LPS (5 μg/ml) in THP-1 macrophages in which p130cas had been reduced by transfection with p130cas siRNA. Despite similar p130cas reduction, LPS stimulated phagocytosis (data not shown), suggesting that the cells had not been maximally stimulated by cas reduction and supporting the alternate hypothesis that cas mediates the effects of propofol and pressure on phagocytosis.

Fig. 4. GABAA receptor antagonist studies. A: effect of picrotoxin (PTX), a noncompetitive inhibitor, on phagocytosis modulated by propofol in primary isolated monocytes. Primary isolated monocytes pretreated with or without 2 μM picrotoxin for 30 min were treated with 10 μg/ml propofol or Intralipid and then incubated with serum-opsonized fluorescence-labeled latex beads (5 beads/cell) under ambient or increased pressure for 2 h. Values are means ± SE. B: effect of picrotoxin on phagocytosis modulated by propofol in THP-1 macrophages. THP-1 macrophages pretreated with or without 2 μM picrotoxin were treated with or without Intralipid or propofol. Pressure increased phagocytosis in Intralipid-treated or untreated THP-1 macrophages pretreated with or without picrotoxin. Picrotoxin did not affect basal or pressure-stimulated phagocytosis in untreated or Intralipid-treated THP-1 macrophages or propofol-stimulated phagocytosis at ambient pressure. Picrotoxin abrogated the effect of pressure in propofol-treated cells. *P < 0.05 vs. corresponding control. #P < 0.05 vs. Intralipid alone. C: effect of SR-95531 (SR), a competitive inhibitor, on phagocytosis modulated by propofol. Pressure increased phagocytosis in Intralipid-treated THP-1 macrophages pretreated with or without 2 μM SR-95531. SR-95531 did not affect basal or propofol-stimulated phagocytosis. SR-95531 prevented the effect of pressure in propofol-treated cells. *P < 0.05 vs. corresponding control. #P < 0.05 vs. Intralipid alone. D: effect of pretreatment with SR-95531 followed by removal of the agent on phagocytosis modulated by propofol. THP-1 macrophages were pretreated with 2 μM SR-95531 for 30 min, SR-95531 was washed away, and the cells were allowed to recover for 1 h. Response of these cells to pressure and propofol was similar to that of THP-1 cells that were not exposed to SR-95531. Values are means ± SE (n = 6). *P < 0.02 vs. corresponding control. #P < 0.001 vs. Intralipid alone.
Modulation of p130cas phosphorylation by pressure and propofol appears independent of the GABA<sub>A</sub> receptor. Our results suggest that the effect of propofol on macrophage phagocytosis may be mediated through GABA<sub>A</sub> receptor activation and reduction of p130cas phosphorylation. To examine whether inhibition of p130cas phosphorylation by propofol requires GABA<sub>A</sub> receptor activation, we evaluated the effects of Intralipid, propofol, and pressure on p130cas phosphorylation in THP-1 macrophages treated with the GABA<sub>A</sub> receptor antagonist picrotoxin. As described above, exposure to pressure or pretreatment with propofol significantly inhibited p130cas phosphorylation, and pressure increased p130cas phosphorylation in propofol-treated cells. Picrotoxin also inhibited p130cas phosphorylation in Intralipid (vehicle)-treated THP-1 macrophages at ambient pressure, but pressure did not affect p130cas phosphorylation in Intralipid-treated THP-1 macrophages (Fig. 7, left). In propofol-treated cells, there was no further inhibition of p130cas phosphorylation by picrotoxin, and exposure to pressure increased p130cas phosphorylation in picrotoxin-treated cells (Fig. 7, right). These data suggest that propofol increases basal and reverses pressure-stimulated macrophage phagocytosis through inhibition of p130cas phosphorylation independently of the GABA<sub>A</sub> receptor activation that is also separately required for propofol to reverse the pressure effect.

DISCUSSION

Mononuclear phagocytes, such as monocytes and macrophages, are the first line of host defense against infection and inflammation and play pivotal roles in wound repair (2, 31). Recruitment of macrophages into wound sites is a critical event for the early stage of wound healing (21, 22). Increasing the recruitment of monocytes or macrophages accelerates wound healing (22, 60). Inhibition of macrophage phagocytosis with antimacrophage serum also slows wound healing (31), whereas administration of heat shock protein 70, which stimulates macrophage phagocytosis (60), accelerates wound healing (29). These reports suggest that macrophage phagocytosis is essential for normal wound healing.

Although circulating monocytes are recruited to the surgical wound, tissue macrophages existing skin and organs targeted by the surgical procedure might be exposed to propofol during anesthesia and surgery (21, 31, 62). Leibovich and Ross (31) reported that elimination of local macrophages at the wound site impairs the wound-healing process, and impaired macrophage phagocytic function also leads to a defect in wound healing (54). Furthermore, macrophages might be activated at the wound site, where a large amount of the intracellular content is released into the extracellular space (29), and these macrophages produce cytokines, growth factors, and chemokines, which promote further recruitment of immune cells (7, 41). These tissue macrophages may play important roles in wound healing immediately after surgery.

Propofol is most widely used to induce and maintain anesthesia during surgical or other invasive procedures, for sedation in critically ill patients, and for improved outcomes by protecting neurological tissue against ischemia in neurosurgical settings (33, 61). Propofol has been proposed to decrease lipid peroxidation and activate the immune system (13). Conversely, propofol has also been reported to inhibit immune cell functions, such as LPS-induced cytokine release and the oxidative stress response (10). Propofol also attenuates Kupffer cell activation and LPS-induced hepatocellular damage (57, 58). The effect of propofol on phagocytosis has not been clear. Some investigators have reported that propofol inhibits phagocytosis in neutrophils and macrophages in vitro (11, 30), and others described no effect of propofol on human leukocyte phagocytosis (42).

The present study investigated the effect of the commonly used anesthetic agent propofol on macrophage phagocytosis. Increased extracellular pressure stimulated phagocytosis in monocytes isolated from healthy volunteers, as reported previously (51, 52), and similarly stimulated phagocytosis in monocytes from non-propofol-anesthetized surgical patients. In contrast, exposure to increased extracellular pressure reduced phagocytosis in monocytes from propofol-anesthetized surgical patients. Furthermore, ex vivo pretreatment with propofol increased basal phagocytosis and reversed the effects of pressure on phagocytosis in monocytes isolated from healthy volunteers and non-propofol-anesthetized patients. Mechanistic studies have demonstrated that exposure to pressure or propofol increases phagocytosis by inhibiting p130cas phosphorylation and that the reversal of pressure-stimulated macrophage phagocytosis by propofol is mediated by GABA<sub>A</sub> receptors. Moreover, downregulation of p130cas phosphorylation contributed to increased macrophage phagocytosis by extracellular pressure or propofol treatment, and the stimulation of p130cas phosphorylation by pressure might mediate reversal of phagocytosis in propofol-treated cells.

Fig. 5. Effect of propofol on ERK activation. Top: typical Western blot probed for phosphorylated ERK and then stripped and reprobed for total ERK as a loading control. Bottom: summary of densitometric results. Values are means ± SE (n = 8), expressed as ratio of phosphorylated ERK (pERK) to total ERK (tERK), normalized to Intralipid-treated control values. Pressure inhibited ERK phosphorylation in Intralipid- and propofol-treated THP-1 macrophages. Propofol increased ERK phosphorylation. *P < 0.02 vs. corresponding control. #P < 0.05 vs. Intralipid-treated control.
In our initial study (Fig. 1), we evaluated the effect of extracellular pressure on phagocytosis in monocytes obtained from patients who received general anesthesia with or without propofol. Pressure increased phagocytosis in monocytes from non-propofol-anesthetized patients, similar to the effects of pressure on monocytes from healthy volunteers, but pressure did not have this effect in propofol-anesthetized patients. The effects of propofol are sufficient to explain our findings, and the observation that the response to pressure was similar in monocytes from non-propofol-anesthetized patients and monocytes from healthy unanesthetized volunteers suggests that at least some other anesthetic agents might not exert a similar effect on pressure-stimulated phagocytosis. It remains conceivable that some intravenous anesthetic agent other than those administered to our non-propofol-anesthetized patients might also have a propofol-like effect on macrophage phagocytosis.

However, a comprehensive survey of anesthetic and sedating agents is beyond the scope of the present study.

Chen et al. (11) described inhibition of macrophage phagocytosis at 6 and 24 h after treatment with propofol. However, close scrutiny of their report also demonstrates increased phagocytic activity after a shorter 1-h treatment period, consistent with our present observation. Although monocytes migrate into surgical sites and differentiate into macrophages over time, tissue macrophages are resident within the surgical site at the time of surgery and begin to interact with contaminating bacteria or necrotic debris from the time of initial incision. We studied the effects of shorter-term propofol treatment, because we were interested in macrophage function in the midst of a typical surgical procedure, and the duration of most surgical procedures is less than the 6- to 24-h periods studied by some other groups. Whether pressure and propofol affect monocyte

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**Fig. 6.** Studies of p130cas and pressure. A: effect of pressure or propofol on p130cas phosphorylation. Top: typical Western blot probed for phosphorylated p130cas and then stripped and reprobed for total p130cas as a loading control. Bottom: summary of densitometric results. Values are means ± SE (n = 7), expressed as ratio of phosphorylated p130cas (p-p130cas) to total p130cas (t-p130cas), normalized to Intralipid-treated control values. p130cas phosphorylation was inhibited by pressure or propofol. Pressure increased p130cas phosphorylation in propofol-treated cells. *P < 0.02 vs. Intralipid-treated controls. #P < 0.05 vs. propofol alone.

B: inhibition of p130cas protein expression by p130cas small interfering RNA (siRNA). Top: typical Western blot probed for total p130cas and reprobed for tubulin as a loading control. Bottom: summary of densitometric results. Values are means ± SE (n = 5), expressed as ratio of total p130cas to tubulin, normalized to results from cells transfected with nontargeting (NT1) siRNA. Transfection with p130cas siRNA reduced total p130cas protein expression 60%. *P < 0.001.

C: effect of pressure and propofol on phagocytosis in THP-1 macrophages transfected with p130cas siRNA. Values are means ± SE (n = 9 pooled from 3 separate experiments, each done in triplicate and yielding similar results). Pressure or propofol treatment increased phagocytosis in THP-1 macrophages. Pressure also reversed phagocytosis in NT1-transfected and propofol-treated cells. Transfection with p130cas siRNA increased basal phagocytosis and abrogated effects of pressure and propofol. *P < 0.05 vs. NT1-transfected and Intralipid-treated control cells; #P < 0.05 vs. NT1-transfected propofol-treated cells (by Student’s t-test with Bonferroni’s or Sidak’s correction for multiple comparison).
Clphagocytosis by propofol was prevented by GABA<sub>A</sub> receptors. Indeed, reversal of pressure-stimulated macrophage basal phagocytosis or stimulation of basal phagocytosis by toxin antagonists. However, these antagonists did not affect p130cas phosphorylation in non-picrotoxin- and picrotoxin-treated cells. Significantly different from corresponding control: *P < 0.05; †P < 0.005.

We previously reported that p38 activation and/or inhibition of pressure-stimulated THP-1 macrophages. However, our previous study suggests that inhibition of ERK phosphorylation by pressure is accompanied by pressure-stimulated THP-1 macrophage phagocytosis (53). Furthermore, ERK phosphorylation was decreased by exposure to pressure in propofol-treated cells as well as in untreated cells. Thus activation of ERK by propofol is not likely to mediate the propofol effect on monocyte/macrophage phagocytosis under ambient and increased pressure. Pretreatment with propofol also did not alter FAK, p38, or Akt phosphorylation in THP-1 macrophages, suggesting that propofol is likely to act independently of these signals.

The docking protein p130cas is an adaptor that promotes protein-protein interaction, facilitates focal adhesion complex formation along with FAK and Src, and mediates integrin signal transduction, leading to migration, proliferation, and survival of various cell types (17, 45, 48). p130cas is required for Salmonella typhimurium invasion into host cells (49), and the secreted virulent protein of Yersinia, YopH, binds to p130cas as a substrate (6). Glucocorticoids enhance macrophage phagocytosis of apoptotic cells by downregulating p130cas expression (18), which would be expected to also reduce phosphorylated p130cas. Consistent with this finding, the inhibition of p130cas phosphorylation by exposure to increased extracellular pressure or pretreatment with propofol was also associated with increased phagocytosis in human peripheral monocytes and THP-1 macrophages (Fig. 6A and Fig. 3, A and B), and inhibition of p130cas protein expression by p130cas-specific siRNA also increased basal phagocytosis in our studies (Fig. 6, B and C). Although p130cas has been reported to play a role as a mechanotransducer for cell deformation (17, 46), the role of p130cas in mechanical stimulation-induced phagocytosis in monocytes or macrophages has not
been examined. Here, we demonstrated that exposure to extracellular pressure or the anesthetic agent propofol inhibited p130cas phosphorylation, leading in each case to enhanced phagocytosis.

We also observed reduced p130cas phosphorylation in THP-1 macrophages treated with the GABA_A receptor antagonist picrotoxin, which did not affect basal macrophage phagocytosis. Propofol and picrotoxin bind to GABA_A receptors. Picrotoxin reduces GABA_A receptor currents (38), whereas propofol increases GABA_A receptor currents (16). Activation of p130cas is also regulated in a system without ion channels (46). Inhibition of p130cas phosphorylation might be associated with GABA_A receptor gating, which may induce actin cytoskeletal reorganization. However, if GABA_A receptor currents are reduced by picrotoxin, macrophage phagocytosis is unaffected by this agent, even though it also reduces p130cas phosphorylation. Ince et al. (24) reported that ionic current changes are involved in human macrophage phagocytosis. The direction of ionic current changes might be another important factor for macrophage phagocytosis. Our data also showed that exposure to pressure did not inhibit p130cas phosphorylation further in picrotoxin-treated cells, but pressure augmented p130cas phosphorylation in cells treated with propofol and cells treated with picrotoxin + propofol. These results suggest that regulation of macrophage phagocytosis by pressure requires at least tonic levels of the GABA_A receptor currents but is independently influenced by p130cas phosphorylation. Propofol appears to modulate the effect via its effect on p130cas phosphorylation.

In conclusion, the commonly used anesthetic agent propofol stimulates macrophage phagocytosis but reverses pressure-stimulated macrophage phagocytosis through activation of GABA_A receptors and inhibition of p130cas phosphorylation. Whether these effects are beneficial or detrimental likely depends on the clinical situation, inasmuch as inflammatory activity may help patients resolve disease states or cause further disease in pathological hyperinflammatory or septic conditions. However, these results demonstrate a mechanism for an immunomodulatory effect of propofol and suggest potential novel targets for interventions to modulate phagocytosis by mimicking the propofol pathway or by blocking it in propofol-treated patients.

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