ANESTHESIOLOGY

# Sevoflurane Exerts Protective Effects in Murine Peritonitis-induced Sepsis *via* Hypoxia-inducible Factor 1α/Adenosine A2B Receptor Signaling

Kristian-Christos Ngamsri, M.D., Friederike Fabian, M.D., Anika Fuhr, M.Sc., Jutta Gamper-Tsigaras, M.Sc., Andreas Straub, M.D., David Fecher, M.Sc., Maria Steinke, Ph.D., Heike Walles, Ph.D., Jörg Reutershan, M.D., Franziska M. Konrad, M.D.

*Anesthesiology 2021; 135:136–50*

## EDITOR'S PERSPECTIVE

### What We Already Know about This Topic

- Sepsis is a major cause of mortality in critically ill intensive care unit patients because of host inflammatory injury.
- Although inhaled sevoflurane is reported to have anti-inflammatory effects, its role in laboratory models is not well characterized.

### What This Article Tells Us That Is New

- In two murine peritonitis-induced sepsis models, sevoflurane reduced peritoneal lavage neutrophil counts by lower adhesion molecule expression, reduced cytokines, and protein extravasation. The mechanism of these reported protective effects was due to the expression of hypoxia-inducible factor 1α and adenosine A2B receptor in the intestine, liver, and lung.

## ABSTRACT

**Background:** Sepsis is one of the leading causes of mortality in intensive care units, and sedation in the intensive care unit during sepsis is usually performed intravenously. The inhalative anesthetic sevoflurane has been shown to elicit protective effects in various inflammatory studies, but its role in peritonitis-induced sepsis remains elusive. The hypothesis was that sevoflurane controls the neutrophil infiltration by stabilization of hypoxia-inducible factor 1α and elevated adenosine A2B receptor expression.

**Methods:** In mouse models of zymosan- and fecal-induced peritonitis, male mice were anesthetized with sevoflurane (2 volume percent, 30 min) after the onset of inflammation. Control animals received the solvent saline. The neutrophil counts and adhesion molecules on neutrophils in the peritoneal lavage of wild-type, adenosine A2B receptor $-^{/}-$, and chimeric animals were determined by flow cytometry 4 h after stimulation. Cytokines and protein release were determined in the lavage. Further, the adenosine A2B receptor and its transcription factor hypoxia-inducible factor 1α were evaluated by real-time polymerase chain reaction and Western blot analysis 4 h after stimulation.

**Results:** Sevoflurane reduced the neutrophil counts in the peritoneal lavage (mean ± SD, 25 ± 17 × $10^5$ *vs.* 12 ± 7 × $10^5$ neutrophils; $P = 0.004$; n = 19/17) by lower expression of various adhesion molecules on neutrophils of wild-type animals but not of adenosine A2B receptor –/– animals. The cytokines concentration (means ± SD, tumor necrosis factor α [pg/ml], 523 ± 227 *vs.* 281 ± 101; $P = 0.002$; n = 9/9) and protein extravasation (mean ± SD [mg/ml], 1.4 ± 0.3 *vs.* 0.8 ± 0.4; $P = 0.002$; n = 12/11) were also lower after sevoflurane only in the wild-type mice. Chimeric mice showed the required expression of the adenosine A2B receptor on the hematopoietic and nonhematopoietic compartments for the protective effects of the anesthetic. Sevoflurane induced the expression of hypoxia-inducible factor 1α and adenosine A2B receptor in the intestine, liver, and lung.

**Conclusions:** Sevoflurane exerts various protective effects in two murine peritonitis-induced sepsis models. These protective effects were linked with a functional adenosine A2B receptor.

(Anesthesiology 2021; 135:136–50)

Sepsis is one of the leading causes of mortality and critical illness worldwide. Despite decades of intensive research, the underlying mechanisms remain elusive.[1] In the early stage of sepsis, neutrophils are the first cells of the innate immune system to be recruited to the site of infection.[2] These cells are necessary for the clearance of the pathogen, but their excessive infiltration into the tissue leads to destruction and impairs organ function.[3–5]

Patients with severe sepsis are treated in the intensive care unit (ICU) and require sedation, which is usually performed with the intravenous anesthetic propofol. In a model of polymicrobial peritonitis, propofol enhanced morbidity and mortality in rats.[6] Moreover, sevoflurane, a volatile anesthetic mainly used in the operation theater, can



This article is featured in "This Month in Anesthesiology," page A1. This article is accompanied by an editorial on p. 15. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org). This article has a visual abstract available in the online version.

Submitted for publication July 13, 2020. Accepted for publication March 2, 2021. Published online first on April 29, 2021. From the Department of Anesthesiology and Intensive Care Medicine, University Hospital of Tübingen, Tübingen, Germany (K.-C.N., F.F., A.F., J.G.-T., F.M.K.); the Department of Anesthesiology and Intensive Care Medicine, Hospital of Ravensburg, Ravensburg, Germany (A.S.); the Department of Tissue Engineering and Regenerative Medicine, University Hospital Würzburg, Würzburg, Germany (D.F., M.S.); the Core Facility Tissue Engineering, University of Magdeburg, Magdeburg, Germany (H.W.); and the Department of Anesthesiology and Intensive Care Medicine, Hospital of Bayreuth, Bayreuth, Germany (J.R.).

 Anesthesiology 2021; 135:136–50. DOI: 10.1097/ALN.0000000000003788

be applied to ICU units.[7] Sevoflurane has been shown to elicit protective effects in terms of hepatic ischemia–reperfusion injury[8] and acute pulmonary inflammation.[9–11] In a septic model, sevoflurane reduced mortality,[12] but the underlying mechanism has not been investigated thus far. A link between the protective effects of sevoflurane and adenosine A2B receptor is supposed.[8] Therefore, adenosine A2B receptor is known to be crucial in terms of anti-inflammatory effects in acute inflammation, influencing neutrophil migration and microvascular permeability.[13] Patients in the ICU have been shown to occasionally have reduced ligand affinity and distribution of adenosine receptors.[14] The aim of our study was to investigate the detailed effects of sevoflurane in two peritonitis models and the link between the anesthetic and functional adenosine A2B receptor with the underlying mechanism. The primary goal of our study was to examine the effects of sevoflurane on the migratory behavior of neutrophils. The role of the hypoxia-inducible factor 1α/adenosine A2B receptor signaling pathway in this context served as a secondary goal. We hypothesized that sevoflurane had protective effects *via* hypoxia-inducible factor 1α/adenosine A2B receptor signaling. A link between sevoflurane and adenosine A2B receptor would allow analyses of the adenosine A2B receptor distribution in patients who would potentially benefit from the therapeutic use of sevoflurane in sepsis. Recent literature on inflammation has suggested the identification of subgroups of patients for a specific therapy.[15]

## Materials and Methods

### Animals

We used adenosine A2B receptor gene-deficient mice (adenosine A2B receptor −/−) and corresponding wild-type mice (C57BL/6) from Charles River Laboratories (Germany). The mice were male and between 8 and 12 weeks old. All animal protocols were approved by the Animal Care and Use Committee of the University of Tübingen (Tübingen, Germany) and the institutional animal protection board of the University of Würzburg (Würzburg, Germany). The small intestinal submucosal scaffold was generated from jejunal explants of piglets as described previously.[16]

### Peritonitis Models and Application

All experiments were performed in deeply anesthetized mice (xylazine at 100 mg/kg body weight; ketamine at 10 mg/kg body weight), and all animals bled to death, as described previously.[17] The mice inhaled sevoflurane in a custom-made chamber at 2 volume percent with an additional oxygen supply for 30 min breathed spontaneously but lost consciousness. To detect the optimal time point for the administration of anesthetic sevoflurane, we performed a time curve. Sevoflurane was applied simultaneously 1 and 3 h after zymosan (fig. 1A). Because the protective effects of the anesthetic were greatest 1 h after zymosan installation, we chose this time point for all subsequent experiments, including fecal-induced peritonitis experiments as described previously.[17]

### *In Vivo* Migration Assay

Lungs and liver samples were homogenized. Cell counts of blood, peritoneal lavage, lungs, and liver samples were determined, and the samples were stained with a fluorescent antibody mix consisting of CD45 (Becton Dickinson, USA), 7/4-fluorescein isothiocyanate (AbD Serotec, USA), and Gr-1 (Lymphocyte Culture, USA) to detect neutrophils. A detailed description of the gating process is described in Supplemental Digital Content 1, fig. 1A (http://links.lww.com/ALN/C603). The samples were measured with a FACSCanto II flow cytometer (Becton Dickinson), and data analysis was performed by using FlowJo software (version 7.8.2; Ashland, USA). For the analyses of the expression of the adhesion molecules on neutrophils, additional staining was carried out with CD11a-phycoerythrin, CD11b-phycoerythrin, CD31-phycoerythrin, CD44-phycoerythrin, CD45-peridinin-chlorophyll-protein, CD162-phycoerythrin (all from Becton Dickinson), and CD54-phycoerythrin (Molecular Probes, USA).

### Chemokine Release

The release of keratinocyte-derived chemokine, macrophage-inflammatory protein-2, tumor necrosis factor α, and interleukin-6 was measured in the peritoneal lavage of mice by enzyme-linked immunosorbent assays (R&D Systems, USA) according to the manufacturer's protocol.

### Gene Expression

Total RNA was isolated from the murine intestine, lungs, and liver by using pegGOLD TriFast (Peqlab, USA). cDNA synthesis was performed by using a Bio-Rad iScript kit (Bio-Rad, USA) according to the manufacturer's directions. Gene expression of murine adenosine A2B receptor and the transcription factor hypoxia-inducible factor 1α was evaluated by using the following primers: adenosine A2B receptor, 5′-gcattacagacccccaccaa-3′ and 5′-tttatacctgag-cgggacgc-3′; and hypoxia-inducible factor 1α, 5′-atgttct-gcccaccctgt-3′ and 5′-gagagcgcggaaaactct-3′. The 18s gene served as the housekeeping gene (5′-gtaacccgttgaac-cccatt-3′ and 5′-ccatccaatcggtagtagcg-3′).

### Western Blot

The organs were removed and prepared for Western blot analysis 4 h after zymosan administration. Protein concentration was determined according to the standard protocol of the protein assay kit (Pierce; Thermo Fisher, USA). After the protein concentration was equalized, the samples were loaded on sodium dodecyl sulfate–polyacrylamide gels.

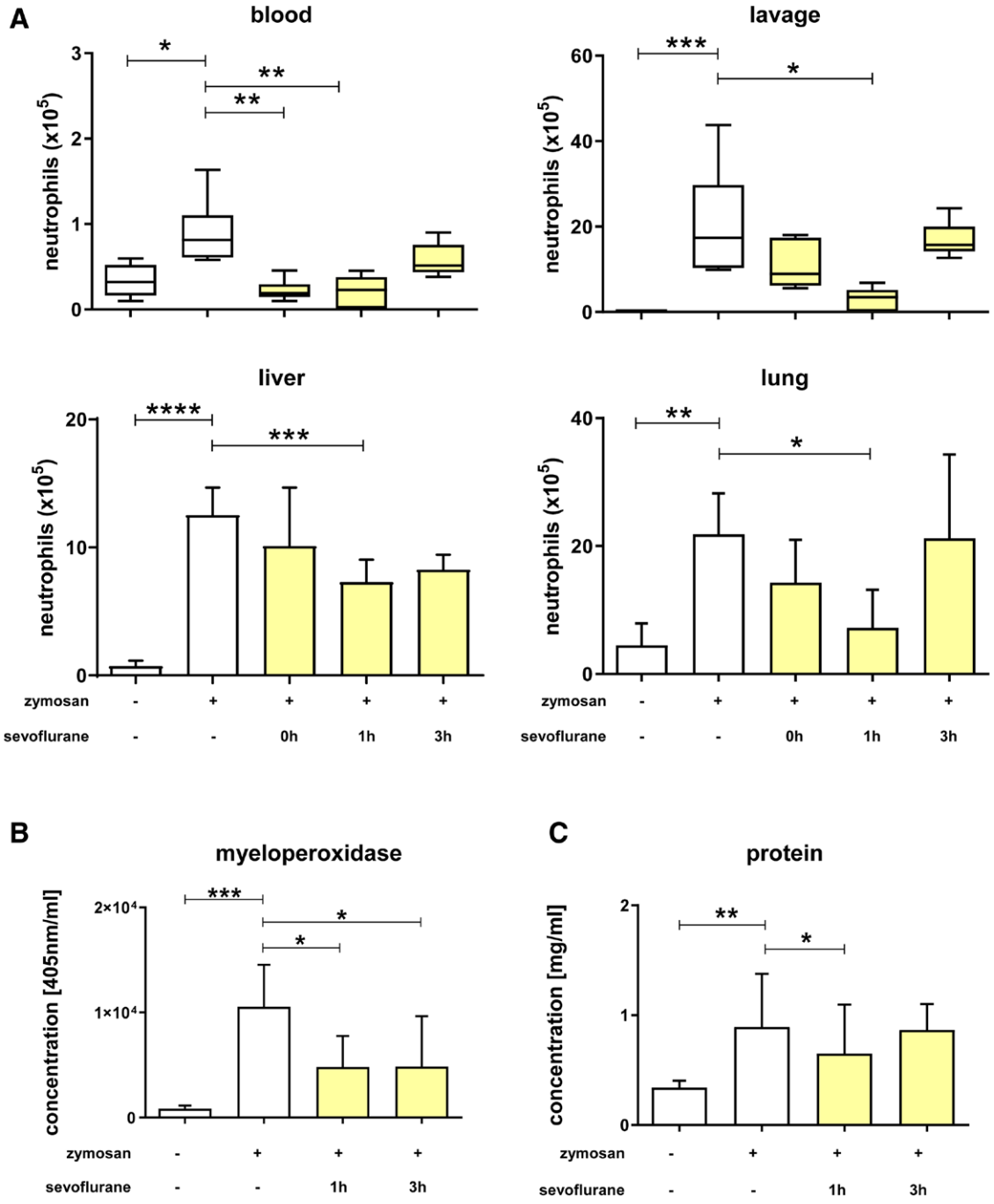


**Fig. 1.** Time dependency of the anti-inflammatory effects of sevoflurane. (*A*) Wild-type animals inhaled sevoflurane simultaneous with the induction of inflammation by zymosan or 1 to 3 h afterward. Neutrophil migration was assessed by flow cytometry in the blood, peritoneal lavage, liver, and lung (all values n = 6). (*B* and *C*) Myeloperoxidase, an enzyme released from activated neutrophils, was determined in peritoneal lavage (*B*; for all values n = 6), and the protein concentration (*C*) was determined as a marker for capillary leakage (all values n = 6). The data are presented as the mean ± SD or as the median and interquartile range. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Statistical analyses were performed by one-way ANOVA and Bonferroni test or Kruskal–Wallis test.

After the proteins were blotted on polyvinylidene difluoride membranes, anti-adenosine $A_{2B}$-R (H-40; Santa Cruz, USA) and anti-hypoxia-inducible factor 1α (ab1; Abcam, United Kingdom) were utilized. The rabbit monoclonal anti–glyceraldehyde 3-phosphate dehydrogenase antibody was used as a housekeeping protein (Santa Cruz).

## Microvascular Leakage

Protein extravasation into the peritoneal lavage as a marker for capillary leakage was determined 4 h after the onset of inflammation by using a bicinchoninic protein assay kit according to the standard protocol (Pierce; Thermo Fisher).

## Generation of Chimeric Mice

Chimeric mice that expressed adenosine A2B receptor on either hematopoietic (adenosine A2B receptor blood) or nonhematopoietic cells (adenosine A2B receptor tissue) were generated by transferring bone marrow between wild-type and adenosine A2B receptor −/− mice as previously described.[18]

## Immunohistochemical Neutrophil Detection

With a Vectastain ABC kit, neutrophil accumulation in the intestine, lung, and liver tissue was visualized by immunohistochemistry (Vector Laboratories, Germany) as previously described.[17] Tissue slides were processed with a Leitz DM IRB microscope (Leica, Germany) and analyzed with AxioVision, version 4.8.2 (Carl Zeiss, Germany).

## *In Vitro* Neutrophil Migration

We performed an *in vitro* transmigration assay of human neutrophils through a monolayer of CaCo-2 cells (ATCC HTB-37, USA). Human epithelial cells were cultivated on inserts of a Transwell system (Costar, USA) until reaching confluence. Epithelial cells were seeded on the bottom side of the inserts so that cells migrated—comparable to the *in vivo* migration—from the basolateral side through the epithelial monolayer. Neutrophils from whole human blood were isolated. The cells were treated with sevoflurane vaporization of 2 volume percent continuously in a custom-made chamber for 30 min. Neutrophil migration through a monolayer of epithelial cells was initiated by the chemoattractant formyl-methionyl-leucyl-phenylalanine (20 ng/ml; Sigma-Aldrich, USA). After 1 h, migrated neutrophils in the lower chamber were quantified by the determination of myeloperoxidase (absorption length, 405 nm).

To verify our *in vivo* findings, we evaluated the effects of sevoflurane after adenosine A2B receptor inhibition with a specific antagonist, PSB1115 (Sigma–Aldrich). Human neutrophils and CaCo-2 cells were pretreated with the adenosine A2B receptor antagonist, and neutrophils were applied to the inserts. Neutrophil transmigration through the basolateral side of the intestinal epithelial monolayer was initiated by formyl-methionyl-leucyl-phenylalanine. To test our hypothesis that the protective effects of sevoflurane treatment depend on adenosine A2B receptor/hypoxia-inducible factor 1α signaling, we additionally depleted adenosine A2B receptor by small interfering RNA (20 nm; OriGene, USA) and evaluated the effects of overexpression of adenosine A2B receptor by a human cDNA plasmid (4 μg; OriGene). The effects of hypoxia-inducible factor 1α depletion on neutrophil migration were determined by human small interfering RNA hypoxia-inducible factor 1α (Santa Cruz). Nontargeting small interfering RNA (OriGene) and small interfering RNA control (Santa Cruz) were used as controls.

Furthermore, we used an advanced model of the human intestinal barrier to study the effects of sevoflurane on neutrophil transmigration. This model is based on a porcine decellularized small intestinal submucosal scaffold. In this biologic scaffold, the architecture of the intestinal extracellular matrix is preserved. Human intestinal epithelial cells (CaCo-2) were cultured on the bottom side of the scaffold as described previously.[19] Confluence was controlled by the permeability of fluorescein-dextran. Compared to Transwell-based models, in which neutrophils migrate through synthetic pores, neutrophils have to migrate through the extracellular matrix mostly composed of collagen and elastin, which mimics the situation *in vivo*. The inflammation-induced effects of formyl-methionyl-leucyl-phenylalanine on the accumulation of intracellular cyclic adenosine monophosphate (cAMP) in human epithelial intestinal cells were investigated by enzyme-linked immunosorbent assays following the manufacturer's instructions (Enzo Biosciences, Germany).

## Statistical Analysis

Continuous variables are reported as the mean ± SD or median and interquartile range according to their distribution. The distribution was evaluated by investigating kurtosis, skewness, Q-Q plots, and histograms. The required number of animals needed for the experiments was based on our previous experience with this design. No *a priori* statistical power calculation was conducted. For all the animal experiments, the mice were randomly assigned to the groups and used in alternating sequential order, and experiments were performed at the same time point. Additionally, blinding of the experimenters took place in the animal experiments to avoid subjective bias. There were no missing data, and all animals were included in further analyses. However, in some *in vitro* experiments, sample sizes were adapted according to the observed effect strengths and based on previous experiences. No *P* value adjustments were made for these interim analyses of the data. Independent-sample *t* tests were used to compare numerical variables that were approximately normally distributed, whereas Mann–Whitney tests were used to evaluate skewed variables. For comparisons between more than two groups, one-way ANOVA was performed and adjusted by Bonferroni correction for multiple comparisons. Residuals were inspected; in the case of severe deviation from a normal distribution even after log transformation, nonparametric methods were used (Kruskal–Wallis test). All statistical tests were two-tailed, and the significance level was set at $P \leq 0.05$ (corrected for multiple testing). Statistical analysis

was performed using GraphPad Prism version 8.4.2 for Windows (GraphPad Software, USA).

## Results

### Time-dependent Anti-inflammatory Effects of Sevoflurane

We determined neutrophil accumulation in the circulation, peritoneal lavage, and liver and lung tissue 4 h after zymosan-induced peritonitis by flow cytometry (fig. 1A; Supplemental Digital Content 2, table 1A, http://links.lww.com/ALN/C604). The anesthetic sevoflurane was applied simultaneously with the induction of peritonitis and 1 and 3 h after zymosan administration. In the circulation, neutrophilia was similar to baseline neutrophil counts without inflammation after the application of sevoflurane simultaneously with and 1 h after the induction of inflammation. In the peritoneal lavage, neutrophil influx was lower 1 h after zymosan administration. At this time point, a reduction in neutrophil migration into the liver and lung was also observed. To verify our findings, we determined myeloperoxidase, which is an enzyme released upon neutrophil activation and is associated with leukocyte activity, in the peritoneal lavage (fig. 1B; Supplemental Digital Content 2, table 1B, http://links.lww.com/ALN/C604). In accordance with our flow-cytometric findings, myeloperoxidase was reduced 1 and 3 h after zymosan. The application of sevoflurane diminished the microvascular permeability when the anesthetic was applied 1 h after inflammation (fig. 1C; Supplemental Digital Content 2, table 1C, http://links.lww.com/ALN/C604). For all subsequent experiments, we chose sevoflurane application 1 h after the induction of zymosan.

### The Protective Effects of Sevoflurane Depend on Functional Adenosine A2B Receptor Signaling

Zymosan-induced peritonitis was induced in wild-type and adenosine A2B receptor −/− animals, and the effects of sevoflurane on neutrophil migration were evaluated. Confirming our previous results and demonstrating reproducibility, the application of the anesthetic reduced neutrophil accumulation in the circulation and neutrophil migration into the peritoneal lavage and lung and liver tissues in the wild-type animals. This effect was not observed in the adenosine A2B receptor −/− animals (fig. 2A; Supplemental Digital Content 3, table 2A, http://links.lww.com/ALN/C605). To visualize the effects of sevoflurane on neutrophil migration, we performed immunohistochemical detection of neutrophils in all three organs (fig. 2B). Neutrophils were marked with a specific antibody so that they appeared brown in the tissue. Sevoflurane reduced the detected neutrophils in the lung, liver, and intestine in the wild-type animals but had no effect on neutrophil influx into the organs of the adenosine A2B receptor −/− animals. The concentration of myeloperoxidase affirmed this finding with a reduction after sevoflurane in the wild-type mice and no effect in the adenosine A2B receptor −/− mice (fig. 2C; Supplemental Digital Content 3, table 2B, http://links.lww.com/ALN/C605). Capillary leakage was also lower in the wild-type mice after the inhalation of sevoflurane (fig. 2D; Supplemental Digital Content 3, table 2C, http://links.lww.com/ALN/C605). The anesthetic failed to influence microvascular permeability in the adenosine A2B receptor −/− animals.

Because the release of cytokines triggers neutrophil migration, we determined the concentration of the two main neutrophil chemoattractants in the mice in peritoneal lavage: keratinocyte-derived chemokine and macrophage-inflammatory protein-2, which resemble human interleukin-8. Both chemoattractants were lower in the wild-type animals after sevoflurane administration (fig. 2E; Supplemental Digital Content 3, table 2D, http://links.lww.com/ALN/C605). In addition, the cytokines tumor necrosis factor α and interleukin-6 were diminished by the administration of sevoflurane in the wild-type animals. Confirming our previous finding, sevoflurane had no effects in the adenosine A2B receptor −/− animals in this setting.

To increase the clinical impact of our study, we evaluated the effects of sevoflurane in a fecal-induced model of peritonitis. The application of the anesthetic 1 h after inflammation reduced neutrophil migration into the peritoneal lavage and both organs (fig. 2F; Supplemental Digital Content 3, table 2E, http://links.lww.com/ALN/C605). This finding was confirmed by the determination of myeloperoxidase (fig. 2G; Supplemental Digital Content 3, table 2F, http://links.lww.com/ALN/C605), where sevoflurane also reduced the release. Microvascular permeability was also lower after the animals inhaled sevoflurane, highlighting the protective properties of sevoflurane in sepsis (fig. 2H; Supplemental Digital Content 3, table 2G, http://links.lww.com/ALN/C605).

### The Influence of Sevoflurane on Adhesion Molecules on Neutrophils

We determined the expression of specific adhesion molecules on neutrophils. The investigated adhesion molecules all play distinct roles in the different steps of neutrophil migration, and the expression patterns change during migration.[20]

We investigated the expression of CD44, CD31, CD162, CD54, CD11b, and CD11a. Sevoflurane diminished the expression of CD44 and CD31 on intravascular neutrophils in the wild-type animals (fig. 3A; Supplemental Digital Content 4, table 3A, http://links.lww.com/ALN/C606). In these animals, neutrophils in the peritoneal lavage showed lower expression of CD162, CD54, CD44, and CD31 on their surfaces after inhalation of sevoflurane (fig. 3B; Supplemental Digital Content 4, table 3B, http://links.lww.com/ALN/C606). In the livers of the wild-type

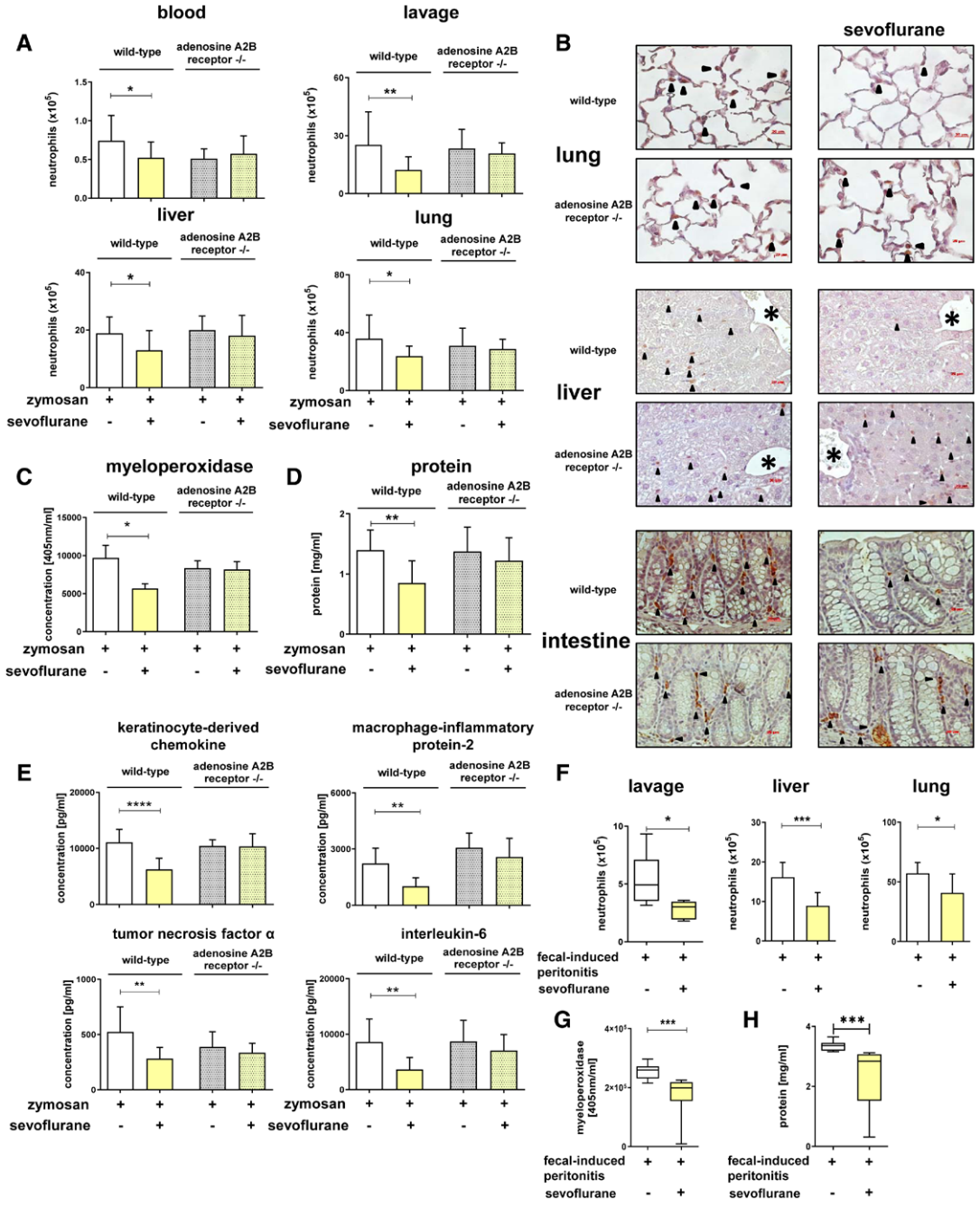


**Fig. 2.** Influence of the therapeutic use of sevoflurane on neutrophil migration and cytokine release in wild-type and adenosine A2B receptor knockout mice. (*A*) Neutrophil accumulation into the indicated compartments was assessed in the wild-type and adenosine A2B receptor –/– mice after zymosan-induced peritonitis (blood: n = 15/17/13/13; lavage: n = 19/17/10/9; liver: n = 13/19/17/13; and lung: n = 14/12/12/15). (*B*) Histologic slides of the lung, liver, and intestine were taken (one slide for each tissue from each animal; n = 4 mice), and neutrophils are marked with a specific antibody, appear *brown*, and are additionally marked with *arrows*. The *left column* of slides received only zymosan, whereas the *right column* additionally obtained sevoflurane. The *asterisk* represents a vein. (*C*) Myeloperoxidase (n = 9/8/8/9) and (*D*) protein concentrations were determined in peritoneal lavage (n = 12/11/12/10). (*E*) Zymosan-induced release of the cytokines keratinocyte-derived chemokine (n = 9/10/12/10), macrophage-inflammatory protein (n = 8/11/10/12), tumor necrosis factor α (n = 9/9/13/11), and interleukin-6 (n = 14/12/12/15) was determined by enzyme-linked immunosorbent assays. (*F*) Inflammation was induced by fecal injection, and the migration of neutrophils into the peritoneal lavage (n = 8/8), liver (n = 8/8), and lung (n = 8/8) was determined. (*G* and *H*) Myeloperoxidase (*G*; n = 11/8) and protein (*H*) concentrations were measured in peritoneal lavage (n = 8/8). The data are presented as the mean ± SD or as the median and interquartile range. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. Statistical analyses were performed by one-way ANOVA and Bonferroni test and two-group analyses by unpaired *t* tests or Mann–Whitney tests.

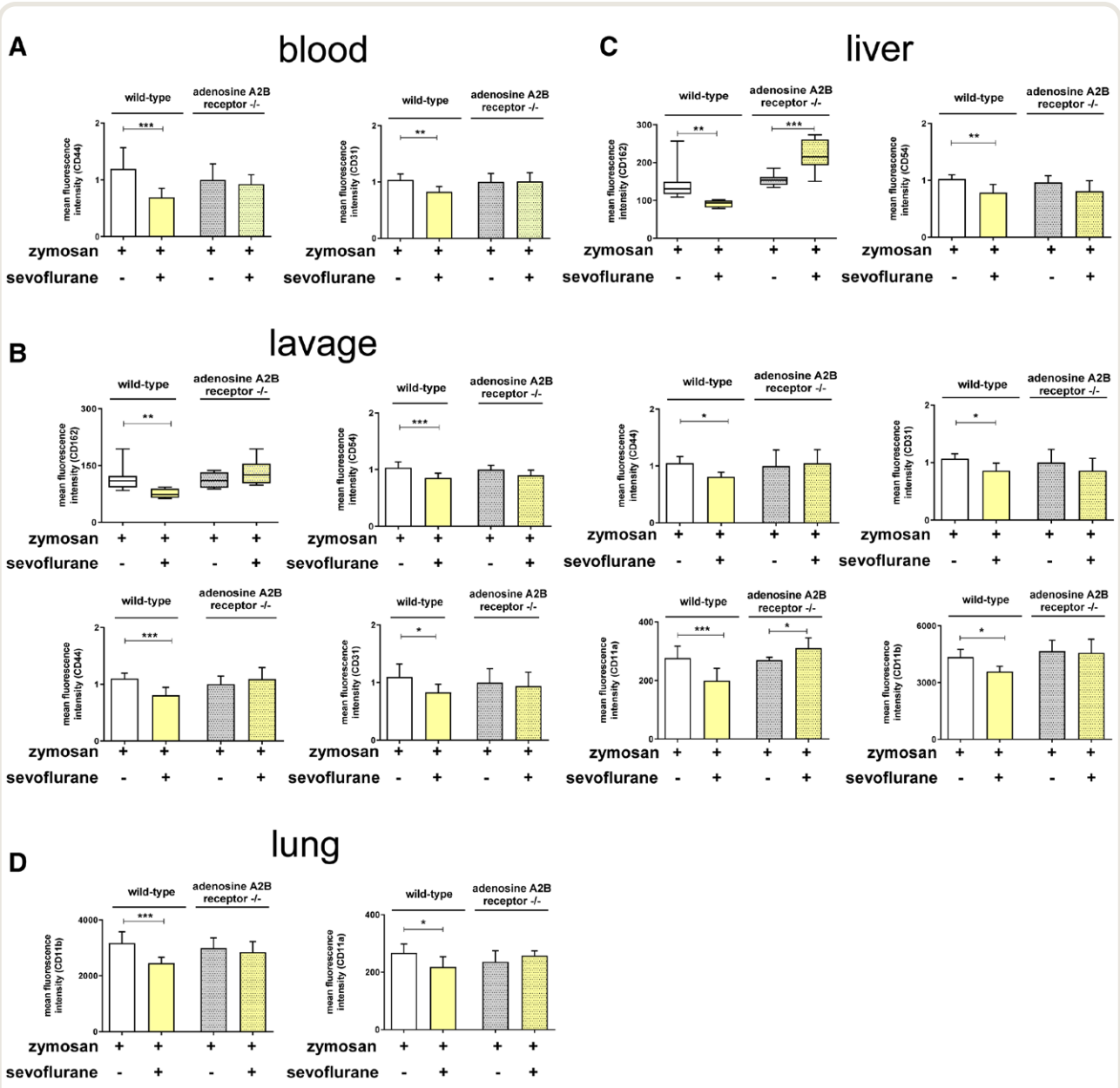


**Fig. 3.** Influence of sevoflurane on the expression of adhesion molecules on neutrophils. Wild-type and adenosine A2B receptor –/– mice were treated with zymosan and sevoflurane, and the surface expression of CD31 (platelet endothelial cell adhesion molecule; pecam-1), CD44 (homing cell adhesion molecule; HCAM), CD162 (p-selectin glycoprotein ligand-1; PSGL-1), CD54 (intercellular adhesion molecule 1; ICAM-1), CD11b (macrophage-1 antigen; MAC-1), and CD11a (lymphocyte function-associated antigen 1; LFA-1) on neutrophils was assessed (n ≥ 8). Sevoflurane-induced differences in the adhesion molecules on neutrophils in the blood (*A*, CD44: wild-type: n = 8/11; adenosine A2B receptor –/–: n = 8/9; CD31: wild-type: n = 10/8; adenosine A2B receptor –/–: n = 9/8), peritoneal lavage (*B*, CD162: wild-type: n = 9/9; adenosine A2B receptor –/–: n = 8/7; CD54: wild-type: n = 8/7; adenosine A2B receptor –/–: n = 8/8; CD44: wild-type: n = 8/10; adenosine A2B receptor –/–: n = 8/8; CD31: wild-type: n = 9/8; adenosine A2B receptor –/–: n = 8/8), liver (*C*, CD162: wild-type: n = 8/8; adenosine A2B receptor –/–: n = 8/7; CD54: wild-type: n = 8/8; adenosine A2B receptor –/–: n = 7/7; CD44: wild-type: n = 8/8; adenosine A2B receptor –/–: n = 8/7; CD31: wild-type: n = 9/8; adenosine A2B receptor –/–: n = 8/8; CD11a: wild-type: n = 9/8; adenosine A2B receptor –/–: n = 8/8; CD31: wild-type: n = 8/8; adenosine A2B receptor –/–: n = 8/7), and lung (*D*, CD11a: wild-type: n = 8/8; adenosine A2B receptor –/–: n = 8/7; CD31: wild-type: n = 8/8; adenosine A2B receptor –/–: n = 8/8) were determined. The data are presented as the mean ± SD. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Statistical analyses were performed by one-way ANOVA and Bonferroni test or Kruskal–Wallis test.

animals, CD162, CD54, CD44, CD31, CD11a, and CD11b were reduced on neutrophils (fig. 3C; Supplemental Digital Content 4, table 3C, http://links.lww.com/ALN/C606). In the lung, an effect of sevoflurane on the expression of CD11b and CD11a was detectable (fig. 3D; Supplemental Digital Content 4, table 3D, http://links.lww.com/ALN/C606). According to our finding that the effect of sevoflurane depends on functional adenosine A2B receptor, the expression of adhesion molecules on neutrophils was not affected in the adenosine A2B receptor −/− animals.

### Sevoflurane Increases the Expression of Adenosine A2B Receptor and Hypoxia-inducible Factor 1α during Acute Peritonitis

Next, we investigated the impact of sevoflurane on the expression of adenosine A2B receptor. The gene expression of adenosine A2B receptor in the intestine, liver, and lung was greater after the application of sevoflurane (fig. 4A; Supplemental Digital Content 5, table 4A, http://links.lww.com/ALN/C607). Western blots confirmed these results at the protein concentration (fig. 4B; Supplemental Digital Content 6, fig. 2A, http://links.lww.com/ALN/C608; Supplemental Digital Content 5, table 4B, http://links.lww.com/ALN/C607). Under inflammatory conditions, hypoxia-inducible factor 1α has been shown to be a transcription factor of adenosine A2B receptor. We investigated the impact of sevoflurane on this transcription factor in our setting (fig. 4C; Supplemental Digital Content 5, table 4C, http://links.lww.com/ALN/C607). The therapeutic use of sevoflurane enhanced the gene and protein expression of hypoxia-inducible factor 1α in all three organs (fig. 4D; Supplemental Digital Content 6, fig. 2B, http://links.lww.com/ALN/C608; Supplemental Digital Content 5, table 4D, http://links.lww.com/ALN/C607), explaining our findings on the impact of sevoflurane on adenosine A2B receptor.

### The Role of Adenosine A2B Receptor in Hematopoietic and Nonhematopoietic Tissue

To investigate whether adenosine A2B receptor on hematopoietic or nonhematopoietic cells is crucial for the protective effects of sevoflurane, we determined the effects on neutrophil migration in chimeric mice (fig. 5A). Adenosine A2B receptor expression in the different tissues of the chimeric mice was confirmed by real-time polymerase chain reaction (Supplemental Digital Content 1, fig. 1B, http://links.lww.com/ALN/C603). Neutrophil influx was determined in the lavage, liver, and lung samples of the chimeric mice (fig. 5A), in which sevoflurane did not show any protective effects. The anesthetic had no effect on the release of myeloperoxidase in either chimeric mouse (fig. 5B). In addition, a functional adenosine A2B receptor was necessary in both compartments to prevent capillary leakage by sevoflurane (fig. 5C).

### The Protective Effects of Sevoflurane *In Vitro* Depend on a Functional Adenosine A2B Receptor

To further verify our *in vivo* findings, we determined the effect of sevoflurane on the migration of human neutrophils through a monolayer of human intestinal epithelium in a Transwell system *in vitro*. Migration was reduced by the anesthetic, and the blockade of adenosine A2B receptor by a specific antagonist reversed this finding (fig. 6A; Supplemental Digital Content 5, table 4E, http://links.lww.com/ALN/C607). The elevation of adenosine A2B receptor expression by a plasmid did not show a synergistic effect when it was combined with sevoflurane in terms of neutrophil migration through an epithelial monolayer (fig. 6B; Supplemental Digital Content 5, table 4F, http://links.lww.com/ALN/C607). Adenosine A2B receptor depletion by small interfering RNA abolished the protective effects of the anesthetic on neutrophil migration (fig. 6C; Supplemental Digital Content 5, table 4G, http://links.lww.com/ALN/C607), further confirming our *in vivo* results. The impact of sevoflurane on the migration of human neutrophils through intestinal tissue was further investigated by the use of a three-dimensional human intestinal tissue model based on porcine decellularized small intestinal submucosal scaffolds.[19] This migration was again lowered by sevoflurane (fig. 6D; Supplemental Digital Content 5, table 4H, http://links.lww.com/ALN/C607).

We determined the impact of sevoflurane on the release of the intracellular signaling protein cAMP, a component of the downstream signaling pathway of adenosine A2B receptor. The anesthetic elevated the release of cAMP, further confirming our *in vivo* findings on the impact of sevoflurane on the expression of adenosine A2B receptor (fig. 6E; Supplemental Digital Content 5, table 4I, http://links.lww.com/ALN/C607).

After depletion of the transcription factor hypoxia-inducible factor 1α by small interfering RNA, a Transwell migration assay of human neutrophils through a monolayer of human intestinal cells was performed. Hypoxia-inducible factor 1α depletion abolished the anti-inflammatory effects of sevoflurane on neutrophil migration (fig. 6, F and G; Supplemental Digital Content 5, tables 4J and 4K, http://links.lww.com/ALN/C607). A schematic overview of the effects of the anesthetic in our model of acute inflammation is shown (fig. 6H).

## Discussion

We investigated the detailed effect of anesthetic sevoflurane in the acute phase of zymosan- and polymicrobial-induced peritonitis. We detected reduced neutrophil migration into the peritoneal lavage, liver, and lung with altered expression of adhesion molecules on neutrophils and anti-inflammatory effects of the therapeutic use of sevoflurane on microvascular permeability.

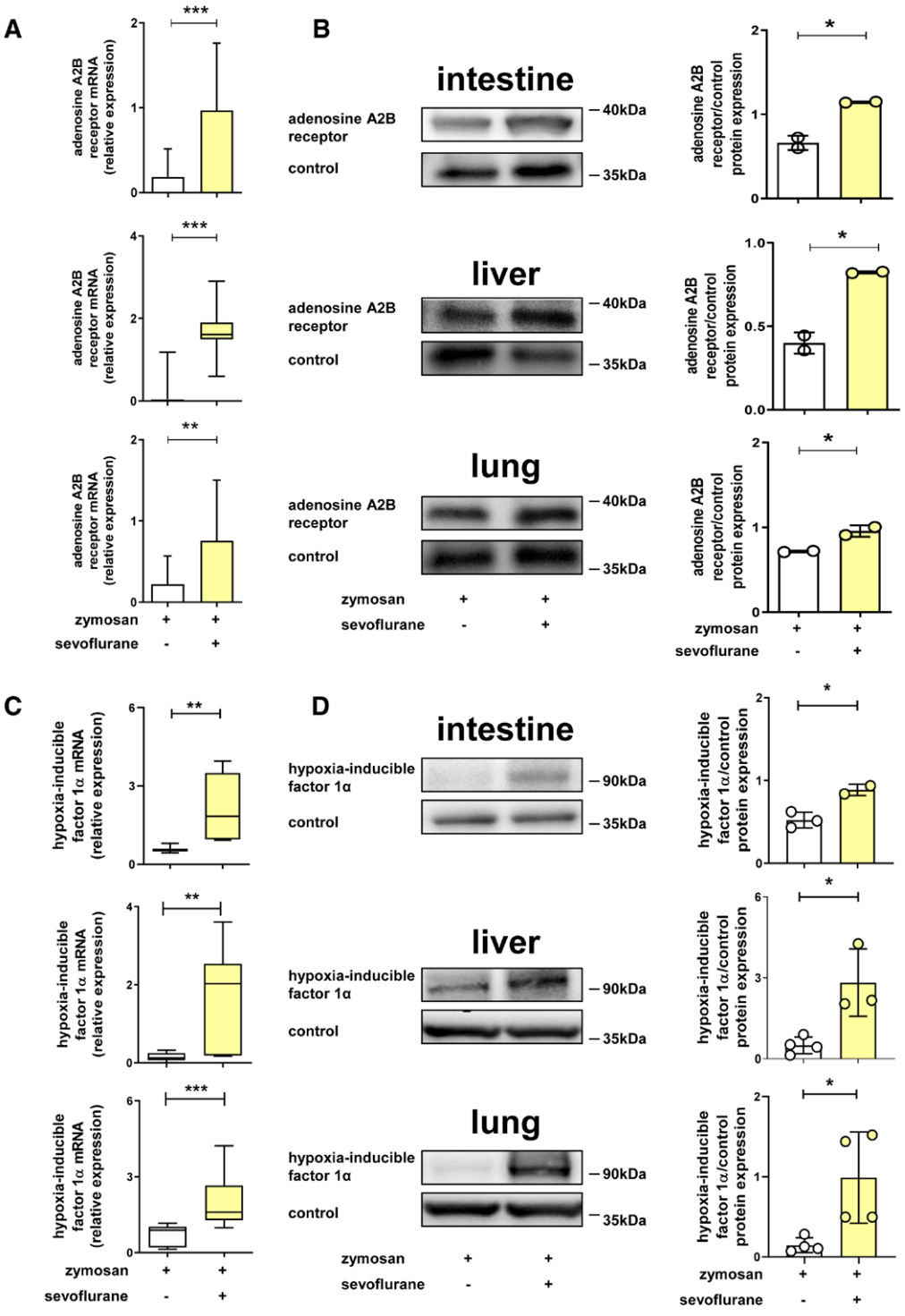


**Fig. 4.** Sevoflurane increased adenosine A2B receptor and hypoxia-inducible factor 1α expression during acute peritoneal inflammation. (*A* and *B*) All wild-type animals had zymosan-induced peritonitis, and the influence of sevoflurane on the gene (*A*, interstine: n = 30/30; liver: n = 30/23; lung: n = 30/24) and protein expression (*B*, interstine: n = 2/2; liver: n = 2/2; lung: n = 2/2) of adenosine A2B receptor was determined. (*C* and *D*) The effects of sevoflurane on hypoxia-inducible factor 1α gene (*C*, interstine: n = 10/7; liver: n = 10/7; lung: n = 11/9) and protein expression (*D*, interstine: n = 3/2; liver: n = 4/3; lung: n = 4/4) in the intestine, liver, and lung tissue of wild-type animals were evaluated by real-time polymerase chain reaction and Western blots. Glycerinaldehyde-3-phosphate dehydrogenase served as control protein. The data are presented as the mean ± SD or as the median and interquartile range. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Statistical analyses were performed by unpaired *t* tests or Mann–Whitney tests.

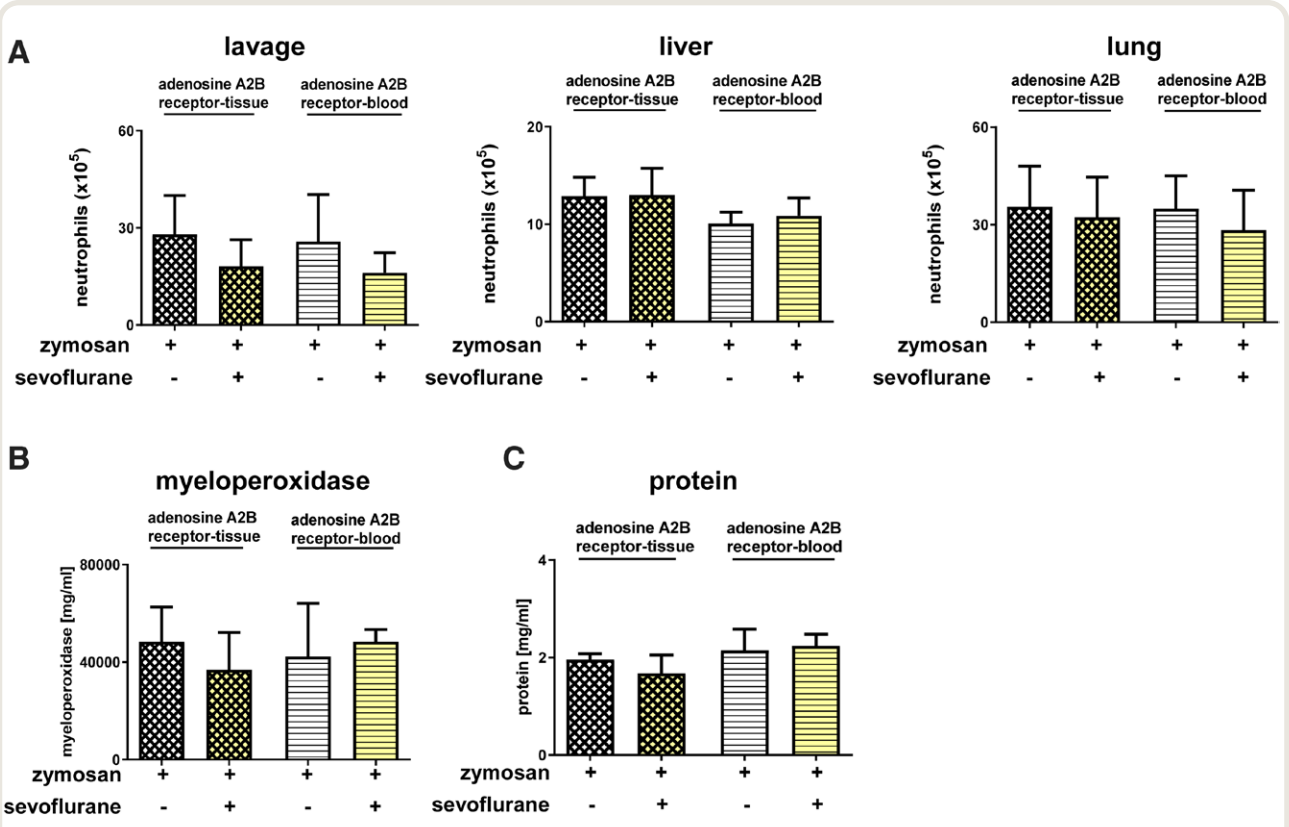


**Fig. 5.** The expression of adenosine A2B receptor on hematopoietic and nonhematopoietic cells is necessary for the anti-inflammatory effects of sevoflurane. (*A*) Chimeric mice with adenosine A2B receptor only on nonhematopoietic cells were generated by transplanting bone marrow from adenosine A2B receptor –/– mice to wild-type mice (adenosine A2B receptor-tissue); chimeric mice with adenosine A2B receptor only on hematopoietic cells were received by transplanting bone marrow from wild-type to adenosine A2B receptor –/– mice (adenosine A2B receptor-blood). Inflammation was induced by zymosan, and the migration of neutrophils into the peritoneal lavage, liver, and lung was determined (all values n = 6). (*B* and *C*) Myeloperoxidase (*B*, all values n = 6) and protein concentrations (*C*, all values n = 6) were measured in peritoneal lavage. The data are presented as the mean ± SD, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Statistical analyses were performed by one-way ANOVA and Bonferroni test.

In a model of cecal ligation and puncture, the inhalation of sevoflurane and the intravenous application of the primary sevoflurane metabolite improved survival and reduced tissue damage. Cellular infiltration, histology, or capillary leakage was not assessed in this study.[12] Lucchinetti *et al.*[21] investigated the effects of sevoflurane in a model of ischemia–reperfusion injury. The authors detected a downregulation of CD11b, which is considered an activation marker of leukocytes, on neutrophils. Similarly, in the current study, the expression of CD11b on neutrophils was reduced in both organs—liver and lung. We detected a significant reduction in adhesion molecules on neutrophils in all investigated compartments and organs (circulation, peritoneal lavage, liver, and lung tissue) in the wild-type animals after sevoflurane treatment, indicating the pivotal effect of the anesthetic on neutrophils. Because peritonitis and subsequent sepsis are major problems in ICUs, we tried to increase the clinical impact of our study by administering sevoflurane after the onset of inflammation so that the anesthetic was used as a therapeutic.

Extracellular adenosine signaling has anti-inflammatory functions in the acute phase of diverse diseases, such as acute lung injury and intestinal inflammation.[22] During inflammation, extracellular adenosine predominantly stems from the breakdown of precursor nucleotides such as adenosine triphosphate.[23] Experimental data showed that a reduced extracellular adenosine concentration exacerbated the inflammatory response during acute pulmonary inflammation.[24] Patients in ICUs may have altered adenosine receptor expression or ligand affinity.[14,25] Additional studies on these adenosine receptors in diverse clinical settings are needed to further define their exact role, expression, and function in different states of inflammation and critical illnesses. The findings of this study may provide a molecular link between the protective effects of sevoflurane and functional adenosine A2B receptor during peritonitis-induced sepsis. Similarly, Kiers *et al.*[26] observed an increased

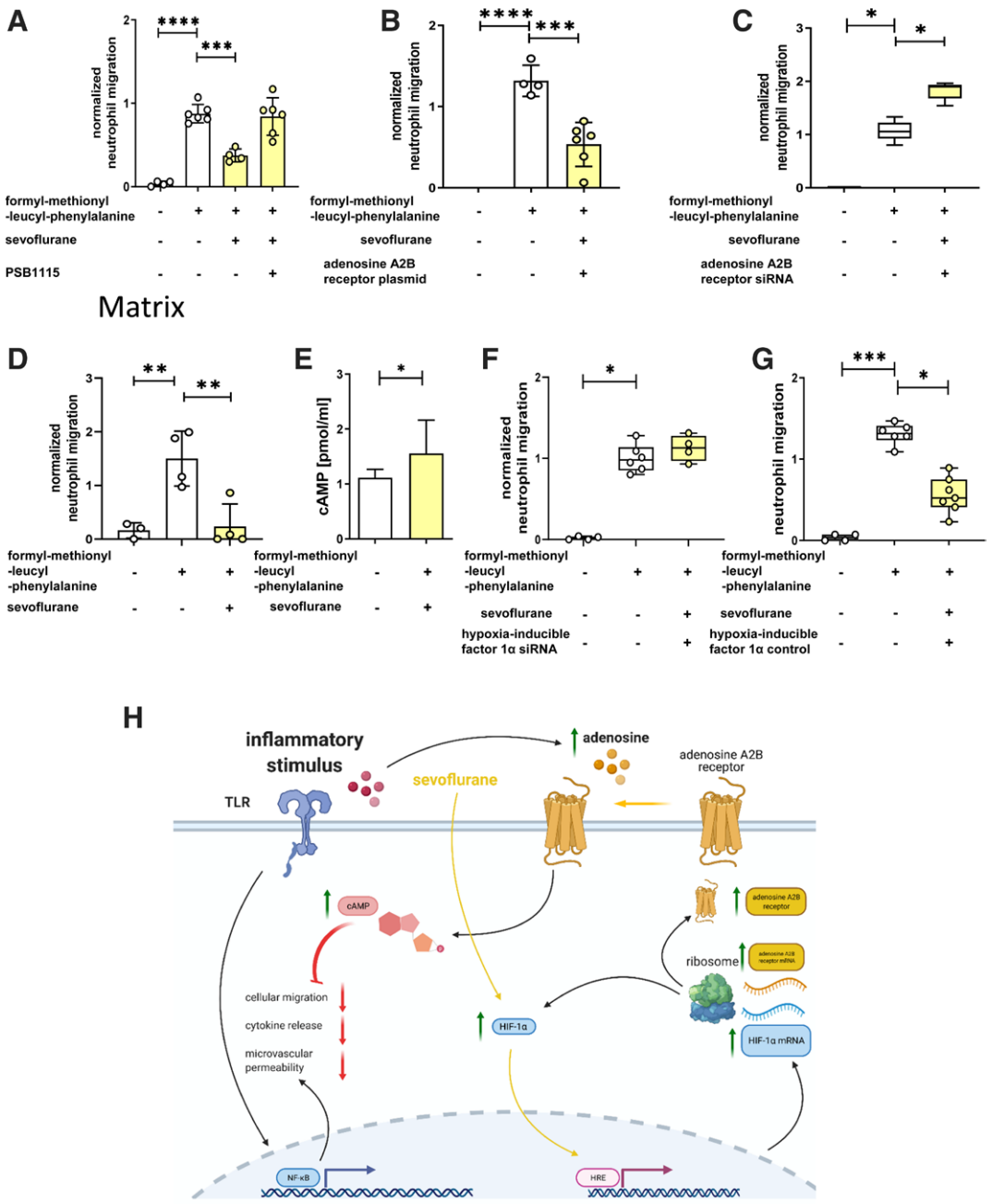


**Fig. 6.** *In vitro* effects of sevoflurane on human neutrophils. (*A*) Human neutrophils were isolated and treated with sevoflurane with a specific adenosine A2B receptor antagonist (PSB1115), and migration through a monolayer of human intestinal epithelial cells (CaCo-2; n = 4/6/4/4) initiated by the chemoattractant formyl-methionyl-leucyl-phenylalanine was assessed. (*B* and *C*) *In vitro* migration of human neutrophils was performed after upregulation of adenosine A2B receptor with a plasmid (*B*, n = 4/4/6) and after adenosine A2B receptor depletion by small interfering RNA (*C*, n = 4/4/6). (*D*) The effects of sevoflurane on neutrophil migration through a three-dimensional tissue model based on small intestinal submucosal scaffolds were assessed (n = 2 control group; n = 4 treatment groups). The number of control groups without inflammation was kept low, because we focused on the inflammatory groups. (*E*) The concentration of the intracellular signaling protein cyclic adenosine monophosphate (cAMP) after sevoflurane treatment in human intestinal epithelial cells was evaluated by enzyme-linked immunosorbent assays (n = 6/6). (*F* and *G*) Furthermore, we detected the effects of the anesthetic on neutrophil migration after the depletion of hypoxia-inducible factor 1α by small interfering RNA (*F*, n = 4/6/4) and the control small interfering RNA (*G*, n = 4/6/7). The data are presented as the mean ± SD or as the median and interquartile range. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. Statistical analyses were performed by one-way ANOVA and Bonferroni test and Kruskal–Wallis test. Data from two-group analyses were derived from unpaired data by *t* tests or Mann–Whitney tests. (*H*) Schematic overview of the effects of sevoflurane on hypoxia-inducible factor 1α and adenosine A2B receptor expression during acute inflammation.

extracellular adenosine concentration, reduced endotoxin-related inflammation, and augmented adenosine A2B receptor expression in a murine model of short-term hypoxia. The authors added experiments with healthy volunteers in an endotoxemia analysis, in which short-term hypoxia also elevated adenosine expression and reduced inflammatory cytokine concentrations. Therefore, inflammation is linked with hypoxemic conditions.[27] In humans, sevoflurane has been shown to reduce cytokine expression during lung resection and cardiopulmonary bypass surgeries.[28,29] For a few years, sevoflurane has been available for application in ICUs by using the AnaConDa device. Jerath *et al.*[30] indicated in their meta-analysis that sevoflurane may promote faster weaning and extubation of ICU patients, showing beneficial aspects for the use of this anesthetic in the ICU.

Adenosine A2B receptor is well known to be protective in terms of acute lung inflammation and liver ischemia.[8,18,31] In cecal ligation and puncture-induced peritonitis, the loss of adenosine A2B receptor enhanced chemokine release and dampened survival.[13] Inflammation increases the expression of adenosine A2B receptor, most likely as part of natural defense mechanisms, but an increase in adenosine A2B receptor stimulation by a specific agonist dampens acute inflammation even further.[18,32,33] Furthermore, it seems that the activation of adenosine A2B receptor on regulatory T cells can induce protective effects during acute inflammation.[34] Specific inhibition of equilibrative nucleoside receptor 2, a cellular adenosine transporter, reduced the uptake of adenosine and raised adenosine expression, which induced protective effects during acute pulmonary inflammation through adenosine A2B receptor.[35]

Various experimental studies in mice have shown that BAY60-6583, the most examined activator of adenosine A2B receptor, has protective effects in different models of inflammation.[36,37] Unfortunately, to the best of our knowledge, there are no clinical data available.[38] Adenosine itself has a short half-life and undesirable side effects, such as atrial fibrillation.[39] In contrast, various hypoxia-inducible factor–prolyl hydroxylase inhibitors, which prevent the degradation of hypoxia-inducible factor and induce a transient increase in hypoxia-inducible factor–related genes, exist. These hypoxia-inducible factor–prolyl hydroxylase inhibitors are currently under investigation in clinical trials. Elevated hypoxia-inducible factor–related genes can increase renal erythropoietin. In chronic kidney disease–related anemia, hypoxia-inducible factor inhibitors were used and successfully passed phase 3 of clinical development.[40,41]

The inflammatory process is linked with hypoxia and hypoxic mechanisms.[27] In inflammatory colitis, hypoxia-inducible factor 1α was elevated,[42] and the mucosa became hypoxic.[43] Hypoxia-inducible factor 2α, an immunomodulatory isoform of the transcription factor, demonstrated cardioprotective effects in a cardiac ischemia–reperfusion model.[44] To the best of our knowledge, there is no connection between hypoxia-inducible factor 2α and adenosine A2B receptor signaling. Therefore, we focused our experiments on hypoxia-inducible factor 1α/adenosine A2B receptor signaling.

Our findings detected a link between sevoflurane and hypoxia-inducible factor 1α/adenosine A2B receptor signaling, but the possible direct mechanism remains elusive. In a recently published study, sevoflurane reduced the immune response by decreasing cytokine expression *via* elevated activation of signal transducer and activator of transcription 3 signaling in a hepatic ischemia–reperfusion model in rats.[45] Signal transducer and activator of transcription 3 is involved in inflammation-induced hypoxia-inducible factor 1α gene expression.[46] Based on these studies and our findings, it is tempting to speculate that sevoflurane induced elevated gene expression and stabilization of hypoxia-inducible factor 1α, providing the observed beneficial effects during peritonitis-related sepsis *via* activation of signal transducer and activator of transcription 3 signaling. Further studies are needed to prove exactly how the anesthetic sevoflurane contributes to the stabilization of hypoxia-inducible factor 1α.

In this study, we utilized only male mice, and this study did not highlight possible sex-specific differences. As limitations of our study, the potential effects of the other adenosine receptors, adenosine A1 receptor, adenosine A2A receptor, and adenosine A3 receptor, were not considered. Furthermore, it would be interesting to investigate the effects of sevoflurane on the activation and stabilization of hypoxia-inducible factor 2α in future projects.

## Conclusions

The presented results demonstrate a protective role of the anesthetic sevoflurane in two murine peritonitis-induced sepsis models. Sevoflurane reduced the release of inflammatory cytokines, neutrophil migration, and protein release in two different peritonitis models. Functional adenosine A2B receptor and hypoxia-inducible factor 1α appear to be involved in these effects. Although the exact mechanism of sevoflurane in mitigating inflammation related to sepsis is still not understood, these findings further support the idea that clinical sevoflurane administration should be considered for sepsis and systemic inflammation.

## Acknowledgments

Adenosine A2B receptor gene–deficient mice were kindly provided by Katya Ravid, Ph.D. (Department of Biochemistry, School of Medicine, Boston University, Boston, Massachusetts). The authors thank Lina Maria Serna Higuita, M.D. (Institute of Clinical Epidemiology and Applied Biometry, University of Tübingen, Tübingen, Germany), for statistical counseling and work-up of the results.

## Research Support

Supported by the Else Kröner-Fresenius-Stiftung 2014_A148 (to Dr. Konrad) and German Research Foundation grant No. KO 4280/2-1 (to Dr. Konrad).

## Competing Interests

Dr. Straub has indicated the following financial relationships: speaker honoraria and travel support from CSL Behring (Hattersheim, Germany); speaker honoraria from Aspen Germany (Munich, Germany); and speaker honoraria from Schöchl Medical Education (Mattsee, Austria). The other authors declare no competing interests.

## Correspondence

Address correspondence to Dr. Konrad: University of Tübingen, Hoppe-Seyler-Strasse 3, 72076 Tübingen, Germany. franziska.konrad@uni-tuebingen.de. 

## References

1. Iskander KN, Osuchowski MF, Stearns-Kurosawa DJ, Kurosawa S, Stepien D, Valentine C, Remick DG: Sepsis: Multiple abnormalities, heterogeneous responses, and evolving understanding. Physiol Rev 2013; 93:1247–88
2. Kolaczkowska E, Kubes P: Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol 2013; 13:159–75
3. Luo L, Zhang S, Wang Y, Rahman M, Syk I, Zhang E, Thorlacius H: Proinflammatory role of neutrophil extracellular traps in abdominal sepsis. Am J Physiol Lung Cell Mol Physiol 2014; 307:L586–96
4. Bhowmick R, Clark S, Bonventre JV, Leong JM, McCormick BA: Cytosolic phospholipase A2α promotes pulmonary inflammation and systemic disease during *Streptococcus pneumoniae* infection. Infect Immun 2017; 85:e00280-17
5. Bhowmick R, Maung N, Hurley BP, Ghanem EB, Gronert K, McCormick BA, Leong JM: Systemic disease during *Streptococcus pneumoniae* acute lung infection requires 12-lipoxygenase–dependent inflammation. J Immunol 2013; 191:5115–23
6. Schläpfer M, Piegeler T, Dull RO, Schwartz DE, Mao M, Bonini MG, Z'Graggen BR, Beck-Schimmer B, Minshall RD: Propofol increases morbidity and mortality in a rat model of sepsis. Crit Care 2015; 19:45
7. Farrell R, Oomen G, Carey P: A technical review of the history, development and performance of the anaesthetic conserving device "AnaConDa" for delivering volatile anaesthetic in intensive and post-operative critical care. J Clin Monit Comput 2018; 32:595–604
8. Granja TF, Köhler D, Schad J, de Oliveira CB, Konrad F, Hoch-Gutbrod M, Streienberger A, Rosenberger P, Straub A: Adenosine receptor Adora2b plays a mechanistic role in the protective effect of the volatile anesthetic sevoflurane during liver ischemia/reperfusion. ANESTHESIOLOGY 2016; 125:547–60
9. Shen QY, Wu L, Wei CS, Zhou YN, Wu HM: Sevoflurane prevents airway remodeling via down-regulation of VEGF and TGF-β1 in mice with OVA-induced chronic airway inflammation. Inflammation 2019; 42:1015–22
10. Wang L, Zha B, Shen Q, Zou H, Cheng C, Wu H, Liu R: Sevoflurane inhibits the Th2 response and NLRP3 expression in murine allergic airway inflammation. J Immunol Res 2018; 2018:9021037
11. Yang Y, Wang WF, Li YH, Li LS, Guo X, Liu R: Sevoflurane attenuates ventilator-induced lung injury by regulating c-PLA2 expression. Mol Med Rep 2018; 18:2923–8
12. Herrmann IK, Castellon M, Schwartz DE, Hasler M, Urner M, Hu G, Minshall RD, Beck-Schimmer B: Volatile anesthetics improve survival after cecal ligation and puncture. ANESTHESIOLOGY 2013; 119:901–6
13. Csóka B, Németh ZH, Rosenberger P, Eltzschig HK, Spolarics Z, Pacher P, Selmeczy Z, Koscsó B, Himer L, Vizi ES, Blackburn MR, Deitch EA, Haskó G: A2B adenosine receptors protect against sepsis-induced mortality by dampening excessive inflammation. J Immunol 2010; 185:542–50
14. Kreth S, Kaufmann I, Ledderose C, Luchting B, Thiel M: Reduced ligand affinity leads to an impaired function of the adenosine A2A receptor of human granulocytes in sepsis. J Cell Mol Med 2009; 13:985–94
15. Dushianthan A, Grocott MP, Postle AD, Cusack R: Acute respiratory distress syndrome and acute lung injury. Postgrad Med J 2011; 87:612–22
16. Pusch J, Votteler M, Göhler S, Engl J, Hampel M, Walles H, Schenke-Layland K: The physiological performance of a three-dimensional model that mimics the microenvironment of the small intestine. Biomaterials 2011; 32:7469–78
17. Ngamsri KC, Jans C, Putri RA, Schindler K, Gamper-Tsigaras J, Eggstein C, Köhler D, Konrad FM: Inhibition of CXCR4 and CXCR7 is protective in acute peritoneal inflammation. Front Immunol 2020; 11:407
18. Konrad FM, Witte E, Vollmer I, Stark S, Reutershan J: Adenosine receptor A2b on hematopoietic cells mediates LPS-induced migration of PMNs into the lung interstitium. Am J Physiol Lung Cell Mol Physiol 2012; 303:L425–38
19. Schweinlin M, Rossi A, Lodes N, Lotz C, Hackenberg S, Steinke M, Walles H, Groeber F: Human barrier

models for the *in vitro* assessment of drug delivery. Drug Deliv Transl Res 2017; 7:217–27
20. Konrad FM, Wohlert J, Gamper-Tsigaras J, Ngamsri KC, Reutershan J: How adhesion molecule patterns change while neutrophils traffic through the lung during inflammation. Mediators Inflamm 2019; 2019:1208086
21. Lucchinetti E, Ambrosio S, Aguirre J, Herrmann P, Härter L, Keel M, Meier T, Zaugg M: Sevoflurane inhalation at sedative concentrations provides endothelial protection against ischemia–reperfusion injury in humans. Anesthesiology 2007; 106:262–8
22. Eltzschig HK, Sitkovsky MV, Robson SC: Purinergic signaling during inflammation. N Engl J Med 2013; 368:1260
23. Eltzschig HK: Extracellular adenosine signaling in molecular medicine. J Mol Med (Berl) 2013; 91:141–6
24. Eckle T, Füllbier L, Wehrmann M, Khoury J, Mittelbronn M, Ibla J, Rosenberger P, Eltzschig HK: Identification of ectonucleotidases CD39 and CD73 in innate protection during acute lung injury. J Immunol 2007; 178:8127–37
25. Gaubert M, Marlinge M, Kerbaul F, Resseguier N, Laine M, Cautella J, Cordier C, Colomb B, Kipson N, Thuny F, Mottola G, Fenouillet E, Ruf J, Paganelli F, Guieu R, Bonello L: Adenosine plasma level and A2A receptor expression in patients with cardiogenic shock. Crit Care Med 2018; 46:e874–80
26. Kiers D, Wielockx B, Peters E, van Eijk LT, Gerretsen J, John A, Janssen E, Groeneveld R, Peters M, Damen L, Meneses AM, Krüger A, Langereis JD, Zomer AL, Blackburn MR, Joosten LA, Netea MG, Riksen NP, van der Hoeven JG, Scheffer GJ, Eltzschig HK, Pickkers P, Kox M: Short-term hypoxia dampens inflammation *in vivo* via enhanced adenosine release and adenosine 2B receptor stimulation. EBioMedicine 2018; 33:144–56
27. Eltzschig HK, Carmeliet P: Hypoxia and inflammation. N Engl J Med 2011; 364:656–65
28. Kortekaas KA, van der Baan A, Aarts LP, Palmen M, Cobbaert CM, Verhagen JC, Engbers FH, Klautz RJ, Lindeman JH: Cardiospecific sevoflurane treatment quenches inflammation but does not attenuate myocardial cell damage markers: A proof-of-concept study in patients undergoing mitral valve repair. Br J Anaesth 2014; 112:1005–14
29. de la Gala F, Pineiro P, Reyes A, Vara E, Olmedilla L, Cruz P, Garutti I: Postoperative pulmonary complications, pulmonary and systemic inflammatory responses after lung resection surgery with prolonged one-lung ventilation: Randomized controlled trial comparing intravenous and inhalational anaesthesia. Br J Anaesth 2017; 119:655–63
30. Jerath A, Panckhurst J, Parotto M, Lightfoot N, Wasowicz M, Ferguson ND, Steel A, Beattie WS: Safety and efficacy of volatile anesthetic agents compared with standard intravenous midazolam/propofol sedation in ventilated critical care patients: A meta-analysis and systematic review of prospective trials. Anesth Analg 2017; 124:1190–9
31. Koscsó B, Trepakov A, Csóka B, Németh ZH, Pacher P, Eltzschig HK, Haskó G: Stimulation of A2B adenosine receptors protects against trauma-hemorrhagic shock-induced lung injury. Purinergic Signal 2013; 9:427–32
32. Eckle T, Faigle M, Grenz A, Laucher S, Thompson LF, Eltzschig HK: A2B adenosine receptor dampens hypoxia-induced vascular leak. Blood 2008; 111:2024–35
33. Eckle T, Grenz A, Laucher S, Eltzschig HK: A2B adenosine receptor signaling attenuates acute lung injury by enhancing alveolar fluid clearance in mice. J Clin Invest 2008; 118:3301–15
34. Ehrentraut H, Clambey ET, McNamee EN, Brodsky KS, Ehrentraut SF, Poth JM, Riegel AK, Westrich JA, Colgan SP, Eltzschig HK: CD73+ regulatory T cells contribute to adenosine-mediated resolution of acute lung injury. FASEB J 2013; 27:2207–19
35. Eckle T, Hughes K, Ehrentraut H, Brodsky KS, Rosenberger P, Choi DS, Ravid K, Weng T, Xia Y, Blackburn MR, Eltzschig HK: Crosstalk between the equilibrative nucleoside transporter ENT2 and alveolar Adora2b adenosine receptors dampens acute lung injury. FASEB J 2013; 27:3078–89
36. Aherne CM, Saeedi B, Collins CB, Masterson JC, McNamee EN, Perrenoud L, Rapp CR, Curtis VF, Bayless A, Fletcher A, Glover LE, Evans CM, Jedlicka P, Furuta GT, de Zoeten EF, Colgan SP, Eltzschig HK: Epithelial-specific A2B adenosine receptor signaling protects the colonic epithelial barrier during acute colitis. Mucosal Immunol 2015; 8:1324–38
37. Borg N, Alter C, Görldt N, Jacoby C, Ding Z, Steckel B, Quast C, Bönner F, Friebe D, Temme S, Flögel U, Schrader J: CD73 on T cells orchestrates cardiac wound healing after myocardial infarction by purinergic metabolic reprogramming. Circulation 2017; 136:297–313
38. Jacobson KA, Tosh DK, Jain S, Gao ZG: Historical and current adenosine receptor agonists in preclinical and clinical development. Front Cell Neurosci 2019; 13:124
39. Kanei Y, Hanon S, Van-Tosh A, Schweitzer P: Adenosine-induced atrial fibrillation during pharmacologic stress testing: Report of eight cases and review of the literature. Int J Cardiol 2008; 129:e15–7
40. Pergola PE, Spinowitz BS, Hartman CS, Maroni BJ, Haase VH: Vadadustat, a novel oral HIF stabilizer, provides effective anemia treatment in nondialysis-dependent chronic kidney disease. Kidney Int 2016; 90:1115–22
41. Akizawa T, Tsubakihara Y, Nangaku M, Endo Y, Nakajima H, Kohno T, Imai Y, Kawase N, Hara K, Lepore J, Cobitz A: Effects of daprodustat, a novel hypoxia-inducible factor prolyl hydroxylase inhibitor

on anemia management in Japanese hemodialysis subjects. Am J Nephrol 2017; 45:127–35
42. Giatromanolaki A, Sivridis E, Maltezos E, Papazoglou D, Simopoulos C, Gatter KC, Harris AL, Koukourakis MI: Hypoxia inducible factor 1α and 2α overexpression in inflammatory bowel disease. J Clin Pathol 2003; 56:209–13
43. Karhausen J, Furuta GT, Tomaszewski JE, Johnson RS, Colgan SP, Haase VH: Epithelial hypoxia-inducible factor-1 is protective in murine experimental colitis. J Clin Invest 2004; 114:1098–106
44. Koeppen M, Lee JW, Seo SW, Brodsky KS, Kreth S, Yang IV, Buttrick PM, Eckle T, Eltzschig HK: Hypoxia-inducible factor 2-α–dependent induction of amphiregulin dampens myocardial ischemia–reperfusion injury. Nat Commun 2018; 9:816
45. Sima LJ, Ma XW: Effect of sevoflurane on hepatic ischemia–reperfusion injury in rats via JAK2-STAT3 pathway. Eur Rev Med Pharmacol Sci 2019; 23:1350–6
46. Xu Q, Briggs J, Park S, Niu G, Kortylewski M, Zhang S, Gritsko T, Turkson J, Kay H, Semenza GL, Cheng JQ, Jove R, Yu H: Targeting Stat3 blocks both HIF-1 and VEGF expression induced by multiple oncogenic growth signaling pathways. Oncogene 2005; 24:5552–60

## ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

# Hanaoka Style: A Blend of East and West That's Off the Hook

Seishu Hanaoka (1760 to 1835), a prominent Japanese physician, famously demonstrated his ingenuity when treating a boy with an errant fishhook (*lower right*) lodged in his throat. Unlike his medical peers, Hanaoka avoided tugging on the fishline dangling from the patient's mouth. Instead, by stringing abacus beads onto the line, Hanaoka created a beaded rod to advance the hook and dislodge it from traumatized tissue. Most contemporary Japanese physicians practiced traditional medicine, *kanpo*, which used herbs to treat disharmony. So-called "Hanaoka Style" blended *kanpo* with *rangaku*, or Western medicine, which emphasized the assessment of organs to discern the cause of illness. As in the fishhook incident, the brilliant Hanaoka always taught his students to individualize patient therapy to alleviate suffering. In 1804, he would administer the world's first recorded general anesthetic by mixing the plants monkshood and moonflower to make *Mafutsusan*. In this herbal preparation, aconitine from monkshood potentiated the sedative effect of moonflower, an anticholinergic. Once again, Seishu Hanaoka reimagined existing medical knowledge with his signature style. (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology.)

*Melissa L. Coleman, M.D., Penn State College of Medicine, Hershey, Pennsylvania, and Jane S. Moon, M.D., University of California, Los Angeles, California.*