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ORIGINAL ARTICLE

Isoflurane inhibits neutrophil recruitment in the cutaneous Arthus reaction model

Carla Carbo · Koichi Yuki · Melanie Demers · Denisa D. Wagner · Motomu Shimaoka

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Abstract
Purpose Neutrophil recruitment to the inflammatory sites is regulated by a variety of adhesion molecules including β2 integrins. The dependency of neutrophil recruitment on β2 integrins is variable in different tissues, but has not yet been verified in the cutaneous passive reverse Arthus reaction. We examined this question and also evaluated the impact of isoflurane on neutrophil recruitment to the skin because we previously showed in vitro that isoflurane binds and inhibits β2 integrins.

Methods The dependency on β2 integrins in neutrophil recruitment to the skin in the Arthus reaction was examined using αL, αM and β2 knockout mice. Then, we evaluated the effect of isoflurane on neutrophil recruitment to the skin. In addition, the effects of isoflurane on neutrophil binding to intercellular adhesion molecule-1 (ICAM-1), one of the β2 integrin ligands, were studied in vitro using cell adhesion assays.
Results Neutrophil recruitment to the skin in the Arthus reaction model was totally dependent on β2 integrins, as β2 knockout mice completely abolished it. However, the defect of only one of the β2 integrins was not sufficient to abolish neutrophil recruitment. Isoflurane reduced neutrophil recruitment to the skin by approximately 90 %. Also, isoflurane inhibited neutrophil adhesion to β2 integrin ligand ICAM-1.
Conclusions We demonstrated that (1) neutrophil recruitment to the skin was totally dependent on β2 integrins, and (2) isoflurane significantly impaired neutrophil recruitment. Based on the previous studies on the contribution of other adhesion molecules in neutrophil recruitment, it is likely that isoflurane at least partially affects on β2 integrins in this model.

Keywords β2 Integrin · Reverse passive Arthus reaction · Skin · Isoflurane · Neutrophil recruitment

C. Carbo and K. Yuki contributed equally.

C. Carbo · K. Yuki · M. Demers · D. D. Wagner · M. Shimaoka
Immune Disease Institute, Boston, MA, USA

C. Carbo · M. Demers · D. D. Wagner · M. Shimaoka
Program in Cellular and Molecular Medicine,
Children's Hospital Boston, Boston, MA, USA

C. Carbo · M. Demers · D. D. Wagner
Department of Pediatrics, Harvard Medical School,
Boston, MA, USA

K. Yuki (✉)
Department of Anesthesiology, Perioperative and Pain Medicine,
Children's Hospital Boston, 300 Longwood Avenue,
Boston, MA 02115, USA
e-mail: yuki.koichi@gmail.com

K. Yuki · M. Shimaoka
Department of Anaesthesia, Harvard Medical School,
Boston, MA, USA

M. Shimaoka (✉)
Department of Molecular Pathobiology and Cell Adhesion
Biology, Mie University, Graduate School of Medicine,
2-174 Edobashi, Tsu, Mie 514-8507, Japan
e-mail: shimaoka@doc.medic.mie-u.ac.jp

Introduction

While general anesthesia has been used for more than 150 years, the mechanism of volatile anesthetics still has not been delineated [1, 2]. Interestingly, these mysterious molecules have been reported to affect not only neuronal cells [3, 4], but also leukocytes [5]. Acute inflammation

due to tissue damage and/or pathogen invasion is a hallmark feature in surgery. Leukocytes are recruited to the inflammatory sites to remove dead cells, cellular debris and pathogens [6]. This will not only enhance tissue repair and wound healing, but also reduce the perioperative infection. Therefore, it is important to know whether or not volatile anesthetics will alter the behavior of leukocytes, particularly that of the first defense cells neutrophils in the setting of tissue damage and/or pathogen invasion [7, 8]. The off-target effect of anesthetics could potentially pose an impact on patients' outcome [9].

Neutrophils undergo several steps using various adhesion molecules to reach inflammatory sites [7, 10]. Initially, selectins help them to tether to and roll on the endothelium. Multiple chemoattractants are secreted from the wounded tissues to guide them to their destination. Chemokines, chemoattractants (such as C5a) and proinflammatory cytokines such as (TNF-α) activate adhesion molecules on neutrophils including β2 integrins. These events cause neutrophil arrest on the endothelium and subsequent intravascular crawling to transmigration sites. They then transmigrate through the endothelium to reach the inflammatory sites.

β2 integrins consist of noncovalently associated α- and β-subunits. Among four β2 integrins (αLβ2, αMβ2, αXβ2 and αDβ2), αLβ2 and αMβ2 are predominantly expressed on neutrophils [11]. αLβ2 is involved in neutrophil arrest and transmigration, and αMβ2 in intravascular crawling and transmigration [10]. The importance of β2 integrins is illustrated by the rare genetic disorder leukocyte adhesion deficiency (LAD) type-1, the loss of expression of the β2 integrins [12–14]. In LAD-1, which is characterized by recurrent severe soft tissue infections and chronic periodontitis, neutrophil mobilization into the extracellular, inflammatory sites is profoundly impaired [14, 15]. Previously de Rossi et al. [16] demonstrated in vitro that isoflurane attenuated the activation of β2 integrins, suggesting that this may impact on neutrophil recruitment. We also demonstrated in vitro that isoflurane blocks the binding of αLβ2 and αMβ2 to the ligand intercellular adhesion molecule-1 (ICAM-1) [17–19]. In addition, our nuclear magnetic resonance, X-ray crystallography, and photolabeling experiments found that isoflurane directly binds to αLβ2 [17–19]. However, the effect of volatile anesthetics on β2 integrins in vivo remains to be determined.

Although the importance of β2 integrins in neutrophil recruitment is well appreciated, the degree of dependency on them is variable in different tissues and organs [20, 21]. The recruitment to the peritoneal cavity and the lung is not completely dependent on β2 integrins. On the other hand, the recruitment to the skin is suggested to be predominantly dependent on β2 integrins in some models [20, 21]. In this study, we evaluate the degree of β2 dependency in neutrophil recruitment to the skin in the reverse passive Arthus reaction, a well-established model for neutrophil study. We also evaluated the effect of isoflurane, a commonly used volatile anesthetic on neutrophil recruitment in this system.

Materials and methods

Mice

Six- to 8-week-old C57BL/6J wild-type mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). αL, αM and β2 knockout mice of C57BL/6J background were purchased from the Jackson Laboratory, bred and housed in our animal facility. Six- to 8-week-old knockout mice were used for the experiments. The phenotypes of αL, αM, and β2 knockout mice were shown in Table 1. Experimental procedures were approved by the Animal Care and Use Committees of the Immune Disease Institute and Harvard Medical School (Boston, MA, USA).

Reverse passive Arthus reaction

First, mice were placed in the induction chamber and briefly anesthetized with isoflurane using an isoflurane vaporizer (VetQuip; New South Wales, Australia). Then, mice were taken out from the induction chamber to induce the reverse passive Arthus reaction. The backs of the mice were shaved over a large area. The reverse passive Arthus reaction was elicited by intravenous injection of 1 % BSA (75 μg protein/g mouse in PBS) immediately followed by intradermal injection of anti-BSA antibody 20 μL each (5 μg/μL in PBS) (Sigma) at two sites of the center of the back as previously described [22]. Nonimmunizing rabbit IgG was injected as a control. Once mice woke up from anesthesia, mice were divided into two groups. One group

Table 1 Reported phenotype of integrin knockout mice

Knockout mice	Phenotype
αL knockout mice	Leukocytosis, no gross abnormalities in growth and development, no increased rate of spontaneous infection [15], reduced T cell proliferation and natural killer cell cytotoxicity [45]
αM knockout mice	No gross abnormalities in growth and development, spontaneous bacterial abscesses of salivary glands (1 %) [46]
β2 knockout mice	Leukocytosis, lymphadenopathy, splenomegaly, increased immunoglobulin levels, severe defect in T cell proliferation, spontaneous skin ulceration (95 %) [20]

of mice was placed into the induction chamber again and exposed to isoflurane 2 %. Soon after, mice were taken out from the induction chamber and placed on the nose cone to be continuously exposed to isoflurane for the duration of the experiment (2 or 4 h). Mice were placed flat on their stomachs on a warm pad (37 °C) with ocular ointment applied to their eyes. Another group of mice was not exposed to isoflurane. After 2 or 4 h, mice were killed. A 6 mm skin biopsy was taken from the site where anti-BSA antibody was injected, and weighed to assess edema formation. In addition, 100 μL Evans Blue (1 %) was injected into some mice to visualize the inflammatory area.

Determination of myeloperoxidase (MPO) activity

Myeloperoxidase is a peroxidase enzyme that is most abundantly expressed in the granules of neutrophils [23, 24]. It is one of the most abundant proteins in neutrophils, accounting for about 5 % of the cell weight [24]. It is also expressed in the granules of monocytes, but the MPO content of each monocyte is about 1/45–1/50 of that of neutrophil [25]. The measurement of MPO of the skin is a well-established method to estimate neutrophil content in the cutaneous inflammation [25–27]. Briefly, the dermis of 6 mm skin biopsies was blended in 50 mmol/L potassium phosphate buffer, centrifuged, resuspended, and sonicated in potassium phosphate buffer supplemented with 50 mmol/L hexadecyltrimethylammonium bromide (Sigma). After centrifugation of the cell lysates, MPO activity was assessed in the supernatant by adding tetramethylbenzidine and by absorbance reading at 450 nm after stopping the reaction with 0.3 N sulfuric acid.

Preparation of mouse neutrophils

Mouse neutrophils were isolated from bone marrow of wild-type (WT) mice using negative sorting with MACS separation columns (Miltenyl Biotec, Auburn, CA) as previously described [26, 28]. Briefly, after red blood cell lysis, bone marrow cells were incubated for 10 min at 4 °C with anti-CD45R antibody (10 μg/mL), anti-CD5 antibody (10 μg/mL), anti-CD8 antibody (5 μg/mL), and anti-CD4 antibody (5 μg/mL) (all from BD Biosciences, Franklin Lakes, NJ). After washing twice, cells were incubated with anti-rat IgG antibody coupled to magnetic beads (Miltenyi Biotec). The cells were run through a magnetic column. The eluted neutrophils were stained with anti Gr-1 antibody (BD Biosciences, San Jose, CA), and the percentage of Gr-1 positive cells was determined by flow cytometry. Only samples with >90 % Gr-1 positive cells were used for adhesion studies.

Flow cytometry

Surface expressions of various integrins on neutrophils were evaluated using flow cytometry. Neutrophils were incubated for 30 min at room temperature with the following rat anti-mouse antibodies; anti-αL, anti-αM, anti-αX, anti-β1, anti-β2, anti-L-selectin antibodies (BD Biosciences, San Jose, CA, USA). After washing, samples were subject to flow cytometry analysis using FACScan (BD Bioscience, San Jose, CA, USA). Data are shown as mean ± SEM.

Neutrophil adhesion assay

Neutrophil adhesion assay using a U-bottom-well plate was performed as follows. U-bottom 96-well plates were coated with mouse ICAM-1-Fc (15 μg/mL; R&D Systems, Minneapolis, MN, USA) at 4 °C overnight and then blocked with HEPES buffered saline (HBS) containing 2 % bovine serum albumin (BSA) for 6 h at room temperature. The plate was washed with Tris-buffered saline containing Tween-20. 50 μL each of HBS containing either 2 mM Ca^{2+}/Mg^{2+} (final assay concentration 1 mM Ca^{2+}/Mg^{2+}) or 2 mM Mg^{2+} (final assay concentration 1 mM Mg^{2+}) was aliquoted to each well. Mouse neutrophils were incubated with anti-Fc blocking antibody, labeled with 2′,7′-bis-(carboxyethyl)-5 (and -6)-carboxyfluorescein acetoxymethyl ester (Invitrogen, Carlsbad, CA, USA) in L-15 medium (Cambrex Bioscience, East Rutherford, NJ, USA) containing 2.5 % fetal bovine serum, washed, and resuspended in HBS at the density of 1.0×10^5 cells/50 μL. 50 μL of cell suspensions were added to each well and incubated at room temperature for 15 min with mock, isoflurane (2 %) or αLβ2 and αMβ2 antagonist called compound #4 (10 μM) [29]. Isoflurane was administered to the cells in an airtight closed chamber using isoflurane vaporizer. The concentration of isoflurane was measured using Ultima infrared spectroscopy (Datex Instrument Corp., Helsinki, Finland). Following incubation, wells were washed, and fluorescence intensity of emission was measured using a fluorescence microplate reader at excitation of 485 nm and emission of 538 nm (Fluoroskan Ascent; Thermo Scientific, Waltham, MA, USA). Binding percentage was calculated as [fluorescence intensity of emission following wash/input fluorescence intensity of emission] × 100 %.

Statistical analysis

Results were expressed as described in figures. One-way analysis of variance (ANOVA) with Tukey's post hoc was performed, and results were considered to be statistically significant when $p < 0.05$.

Results

Neutrophil recruitment to the skin in the reverse passive Arthus reaction model is β2-integrin dependent

The previous study of the rat reverse passive Arthus reaction model by Rote et al. [26] demonstrated that neutrophil recruitment to the skin was not totally, but significantly inhibited by the administration of either β2 antagonist or both αLβ2 and αMβ2 antagonists. This study certainly demonstrated that β2 integrins play a central role in cutaneous neutrophil recruitment in this model, but did not rule out the possibility that β2 integrin independent pathway may exist.

β2 integrins on neutrophils were predominantly αLβ2 and αMβ2 (data not shown), as compatible with the previous report [11]. To assess if neutrophil recruitment to the skin is purely β2 integrins dependent, we evaluated neutrophil recruitment to the skin using αL, αM and β2 knockout mice. Mice were subjected to the reverse passive Arthus reaction for 4 h, and skin inflammation was evaluated by measuring both weight and MPO activity of the punch-biopsied skin. Neutrophils dominate the initial leukocyte influx to sites of acute inflammation [30, 31]. This event is followed by the influx of monocytes. Monocytes infiltrate into inflamed tissue several hours after the onset of inflammation [31]. In the reverse Arthus reaction model, neutrophils are the major cellular constituent during the first 12 h and peaks at 4 h, while monocytes predominate between 15 and 24 h [32]. In addition, the MPO is predominantly expressed in neutrophils as described in the "Materials and methods", and the MPO measurement is considered as a good surrogate of infilitrated neutrophils at our experimental time frame. β2 knockout mice showed the complete inhibition of neutrophil recruitment to the skin and no edema formation (Fig. 1a). However, αL and αM knockout mice did not show any reduction in neutrophil recruitment to the skin. These results demonstrated that neutrophil recruitment to the skin in this model was totally β2 integrin-dependent, and inhibited only when both αLβ2 and αMβ2 integrins were blocked (Fig. 1b, c).

Isoflurane inhibits neutrophil recruitment to the skin in the reverse passive Arthus reaction

Because the neutrophil recruitment to the skin in the Arthus reaction is completely β2 integrin dependent, we examined neutrophil recruitment to the skin in the presence of isoflurane, which blocked αLβ2 and αMβ2 in vitro [17]. Following the injections for the reverse passive Arthus reaction, mice were assigned to the group exposed or not exposed to isoflurane. Neutrophil recruitment and edema formation at the inflammatory site were evaluated at 2 and 4 h. At 2 h, there was almost no neutrophil recruitment in the inflammatory site in mice exposed to isoflurane (89.2 % reduction), while in non-anesthetized mice neutrophil recruitment was observed to a great extent (Fig. 2a). At 4 h, neutrophil recruitment increased in both groups, but was still significantly lower in the isoflurane group (86.9 % reduction) (Fig. 2a). At 2 h, there was less edema formation in the anesthetized mice, which remained the same at 4 h (Fig. 2b). Isoflurane significantly decreased vascular permeability compared to mice not exposed to anesthesia (Fig. 3), supporting the less edema formation in the isoflurane group. This result is compatible with the previous in vitro data of isoflurane as an αLβ2 and αMβ2 antagonist.

Isoflurane inhibits β2 integrin-mediated neutrophil adhesion to ICAM-1

Among various ligands for β2 integrins, ICAM-1 plays a major role in neutrophil recruitment [33]. Sequential binding of αLβ2 and αMβ2 to ICAM-1 helps neutrophils to capture and stably adhere to the endothelium [34], which is a requisite step for neutrophil recruitment. To see if isoflurane impairs the binding of β2 integrins on neutrophils to ICAM-1, we tested the binding of neutrophils to ICAM-1 in both resting (Mg^{2+}/Ca^{2+}) and stimulatory (Mg^{2+}) conditions with or without isoflurane [12]. The binding of β2 integrins to ICAM-1 increases in a stimulatory condition [35]. As expected, neutrophils bound to ICAM-1 more in Mg^{2+} than in Mg^{2+}/Ca^{2+} in the absence of isoflurane (Fig. 4). Isoflurane inhibited cell adhesion of neutrophils to ICAM-1 in the presence of Mg^{2+} to the comparable level with an αLβ2/αMβ2 antagonist, called compound #4 (Fig. 4).

Discussion

In this study we demonstrated in the reverse passive Arthus reaction model that (1) neutrophil recruitment to the skin is completely dependent on β2 integrins, (2) isoflurane significantly attenuates neutrophil recruitment, and (3) isoflurane attenuates the binding of neutrophils to ICAM-1.

The reverse passive Arthus reaction occurs in response to the immune complex mediated injury. Once the Arthus reaction occurs, neutrophils will be recruited to the skin with the help of cooperative actions of adhesion molecules. The identification of critical adhesion molecule is important to help to modulate the neutrophil recruitment process. The contribution of various adhesion molecules in neutrophil recruitment to the skin has been investigated using knockout mice or blocking antibodies. L-selectin knockout mice demonstrated approximately 33–34 % reduction of neutrophil recruitment compared with the wild-type mice

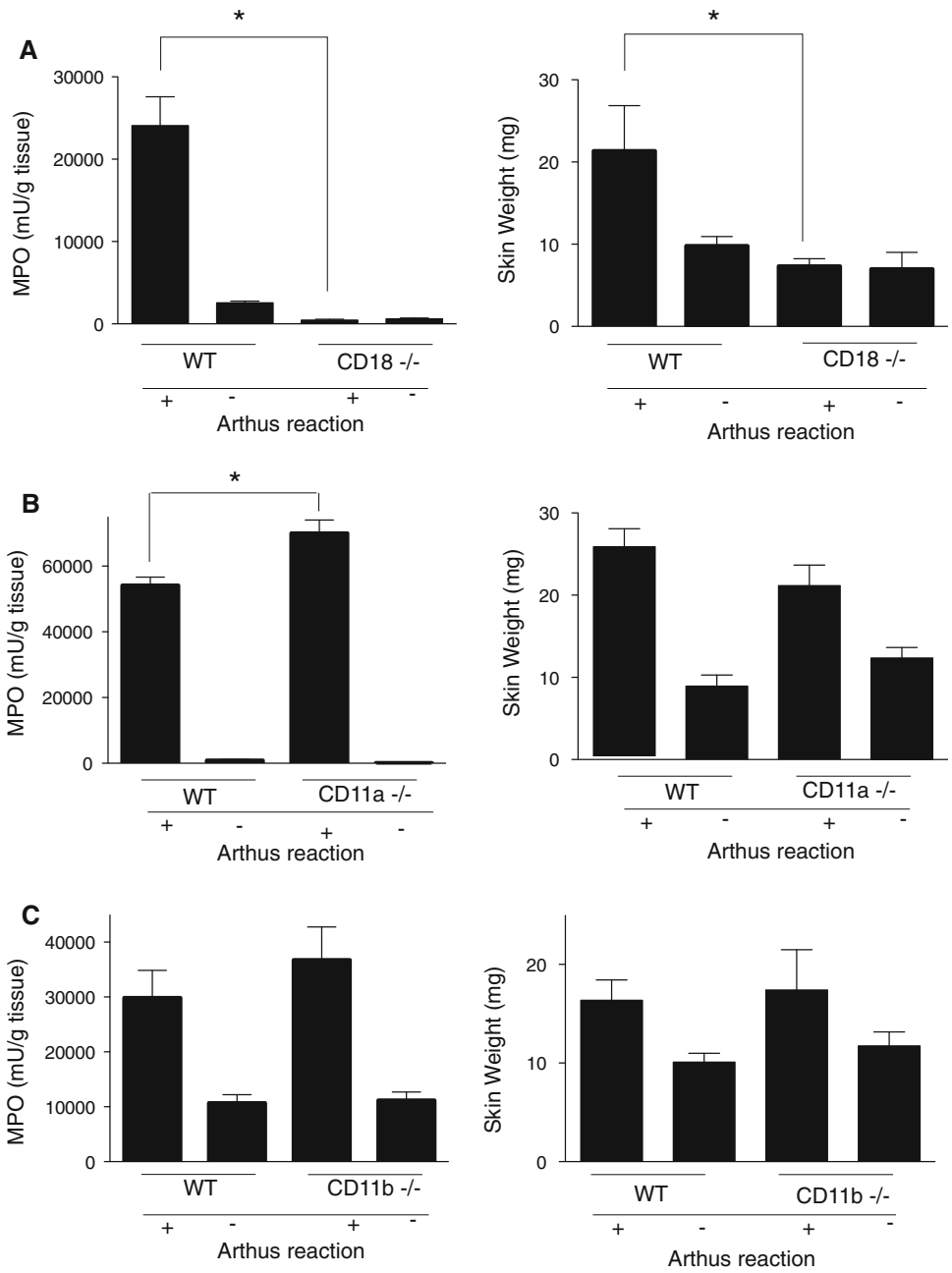

Fig. 1 Neutrophil recruitment was inhibited in $\beta 2^{-/-}$ mice, but not $\alpha L^{-/-}$ mice or $\alpha M^{-/-}$ mice. Neutrophil recruitment and skin weight of punch-biopsied skin from mice with or without the reverse passive Arthus reaction were examined in WT, $\beta 2^{-/-}$ (**a**), $\alpha L^{-/-}$ (**b**) and $\alpha M^{-/-}$ mice (**c**). Neutrophil recruitment was assessed by myeloperoxidase activity level (MPO). Data represent mean ± standard deviation of MPO or skin weight of 4–6 mice. $*p < 0.05$

[36]. E-selectin blocking antibody did not affect the degree of neutrophil recruitment [37]. P-selectin blocking antibody reduced neutrophil recruitment by 32 % [37]. Also, blocking P-selectin glycoprotein ligand-1, a ligand to all three selectins [38, 39] reduced only by 38 % [37]. The contribution of ICAM-1 was evaluated using ICAM-1 knockout mice [36]. ICAM-1 knockout mice showed 43–54 % reduction of neutrophil recruitment. Interestingly, the double knockout of L-selectin and ICAM-1 showed only 54 % reduction, which is almost comparable to the result of ICAM-1 knockout mice. This suggests that ICAM-1 (i.e. binding to $\beta 2$ integrins) plays a significant role in neutrophil recruitment over selectin. Also, it is easily speculated that that there are other ligands to $\beta 2$ integrins other than ICAM-l because $\beta 2$ integrins knockout mice abolished neutrophil recruitment completely, while ICAM-1 knockout mice inhibited only by about 50 %. For example, fibrinogen is one of the ligands to $\alpha M\beta 2$ and considered to be an important player in leukocyte migration [40]. Our results suggest that $\beta 2$ integrins are critical adhesion molecules in neutrophil recruitment to the skin in this model. Isoflurane reduced neutrophil recruitment by approximately 90 %. In the context of predominant role of $\beta 2$ integrins in neutrophil recruitment to the skin, the

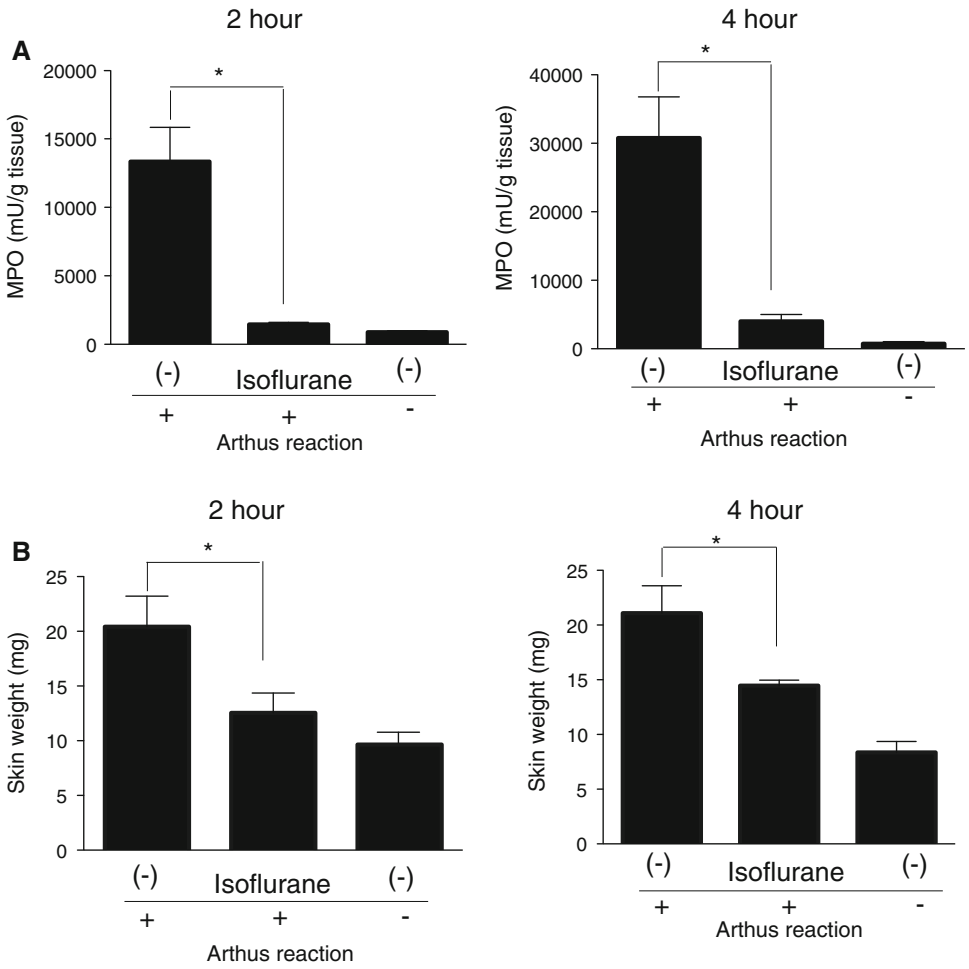

Fig. 2 Isoflurane inhibits neutrophil recruitment and reduces edema in the reverse passive Arthus reaction model. Neutrophil recruitment (**a**) and skin weight (**b**) of punch-biopsied skin were examined using mice exposed or not exposed to isoflurane. Neutrophil recruitment was assessed by myeloperoxidase activity level (MPO). Data represent mean ± standard deviation of MPO or skin weight of 7–9 mice. $*p < 0.05$

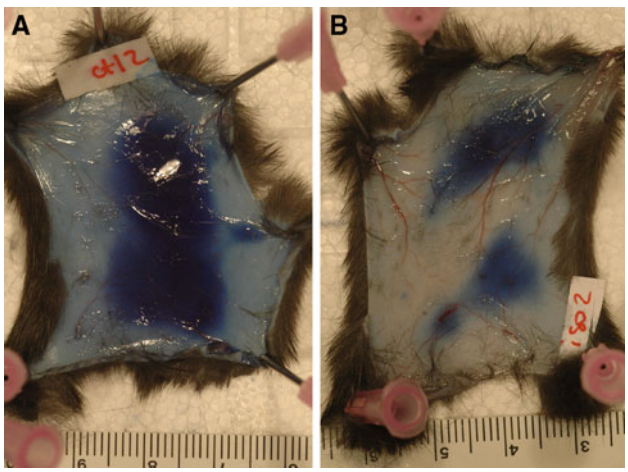

Fig. 3 Isoflurane inhibits vascular permeability in the reverse passive Arthus reaction model. Evans Blue was injected to show areas of inflammation on mouse skin after the reverse passive Arthus reaction. Mice **a** not exposed to isoflurane and **b** exposed to isoflurane

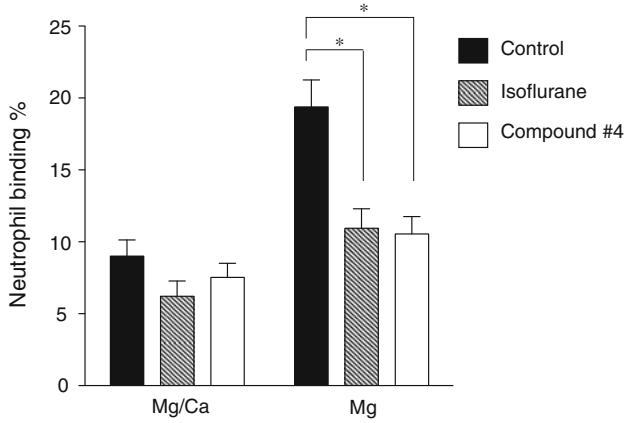

Fig. 4 Isoflurane inhibits neutrophil adhesion to ICAM-1. Neutrophil binding to 96-well plates coated with ICAM-1 was examined in the presence of mock, isoflurane (2 %), or αLβ2 and αMβ2 antagonist compound #4 (1 μM). Data are expressed as mean ± SE of six independent experiments. $*p < 0.01$ versus mock-treated samples in Mg condition. An αLβ2 and αMβ2 antagonist known as compound #4 [29] was used as a positive control for the inhibition

significant inhibition by isoflurane is considered to be at least partly due to the impairment of the function of β2 integrins.

As shown in Fig. 1, both αL and αM knockout mice did not show any reduction in neutrophil recruitment to the skin. This observation is in line with the previous report by

Rote et al. [26]. Similarly, the dermal inflammation induced by interleukin-1 and lipopolysaccharide (LPS) was inhibited by combined administration of αL and αM antibodies, but each of these antibodies alone had minimal effect [41]. These observations may indicate that αLβ2 and αMβ2 can independently mediate neutrophil recruitment in the cutaneous inflammation. Notably, there was a statistically significant increase of neutrophil recruitment in αL knockout mice relative to wild-type mice. Although not statistically significant, there is also a trend of increased neutrophil recruitment in αM knockout mice. As previously described by Ding et al. [15] there is no increased compensatory expression of αMβ2 in αL knockout mice and αLβ2 in αM knockout mice. The increased neutrophil recruitment in αL knockout mice over wild-type mice may reflect the underlying leukocytosis in αL knockout mice. αM knockout mice are reported to have reduction of apoptosis in neutrophils accumulating in the inflamed tissues [42]. This may explain the increased neutrophil accumulation in αM knockout mice.

In the resting condition, β2 integrin α- and β-cytoplasmic tails associate with each other and constrain itself in an inactive form, which has a low affinity to its ligands [35]. Intracellular signaling pathways are activated by various mediators, impinge on the cytoplasmic domains of the integrin and induce it into an active conformation, which has a high affinity to its ligands. All biological integrin ligands are multivalent, and can also contribute to integrin clustering, which will further increase ligand binding capability. Therefore, isoflurane can impairs the function of β2 integrins in various ways; (1) by directly binding to and inhibiting αLβ2 and αMβ2, (2) inhibiting intracellular signaling pathways, (3) impairing clustering. Our result of the reverse passive Arthus reaction strongly suggests that isoflurane impairs the function of β2 integrins, but whether or not isoflurane directly binds and inhibits β2 integrins in vivo still remains to be clarified. We previously used photoactivatable isoflurane called azi-isoflurane to identify isoflurane binding sites on αLβ2 protein [18]. Because this compound is very difficult to produce and is limited in amount, in vivo application has not been tested yet. In the future, using photoactivatable isoflurane in vivo may help to clarify the aforementioned questions.

Surgical infections and postoperative sepsis are among the most serious complications after surgery [43]. The loss or reduction of β2 integrin expression is associated with recurrent soft tissue infections and periodontitis as illustrated in LAD-1, and functional alternation of β2 integrin by isoflurane may pose clinical implication. There are several clinical studies that suggest a potential contribution of volatile anesthetics to immune disorders. For example, in long-term alcoholic patients, isoflurane anesthesia was associated with increased postoperative tracheobronchitis and pneumonia compared to propofol anesthesia [9]. More cases of recurrent breast cancer were observed in patients who underwent resection of cancer with sevoflurane anesthesia versus local anesthesia [44]. The number of patients enrolled in these studies is small, and it is important to evaluate the significance of various anesthetics on immune functions and outcomes by large, randomized controlled studies in the future. However, it has to be kept in mind that clinical outcome studies themselves do not address the underlying mechanism of anesthetics induced immunomodulation. Therefore, it is extremely important to address the mechanism of isoflurane-induced immunomodulation. In the long run, the investigation into this question will help us to understand the still unresolved mechanism of volatile anesthetics as well as redesigning immunomodulation-free anesthetics.

In conclusion, we showed that isoflurane blocked neutrophil recruitment in vivo, and suggested that the impairment of β2 integrin function is at least partly responsible. Further study will enhance our understanding the mechanism of this observation and clinical implication.

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References

1. Arhem P, Klement G, Nilsson J. Mechanisms of anesthesia: towards integrating network, cellular, and molecular level modeling. Neuropsychopharmacology. 2003;28(Suppl 1):S40–7.
2. Eckenhoff RG. Promiscuous ligands and attractive cavities: how do the inhaled anesthetics work? Mol Interv. 2001;1(5):258–68.
3. Franks NP. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat Rev Neurosci. 2008;9(5):370–86.
4. Hemmings HC Jr, Akabas MH, Goldstein PA, Trudell JR, Orser BA, Harrison NL. Emerging molecular mechanisms of general anesthetic action. Trends Pharmacol Sci. 2005;26(10):503–10.
5. Kurosawa S, Kato M. Anesthetics, immune cells, and immune responses. J Anesth. 2008;22(3):263–77.
6. Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med. 1999;341(10):738–46.
7. Williams MR, Azcutia V, Newton G, Alcaide P, Luscinskas FW. Emerging mechanisms of neutrophil recruitment across endothelium. Trends Immunol. 2011;32(10):461–9.
8. Smith JA. Neutrophils, host defense, and inflammation: a double-edged sword. J Leukoc Biol. 1994;56(6):672–86.
9. Von Dossow V, Baur S, Sander M, Tonnesen H, Marks C, Paschen C, Berger G, Spies CD. Propofol increased the interleukin-6 to interleukin-10 ratio more than isoflurane after surgery in long-term alcoholic patients. J Int Med Res. 2007;35(3):395–405.
10. Phillipson M, Kubes P. The neutrophil in vascular inflammation. Nat Med. 2011;17(11):1381–90.
11. Alon R, Feigelson S. From rolling to arrest on blood vessels: leukocyte tap dancing on endothelial integrin ligands and chemokines at sub-second contacts. Semin Immunol. 2002;14(2):93–104.

12. Hogg N, Stewart MP, Scarth SL, Newton R, Shaw JM, Law SK, Klein N. A novel leukocyte adhesion deficiency caused by expressed but nonfunctional beta2 integrins Mac-1 and LFA-1. J Clin Invest. 1999;103(1):97–106.
13. Fischer A, Lisowska-Grospierre B, Anderson DC, Springer TA. Leukocyte adhesion deficiency: molecular basis and functional consequences. Immunodefic Rev. 1988;1(1):39–54.
14. Etzioni A. Defects in the leukocyte adhesion cascade. Clin Rev Allergy Immunol. 2010;38(1):54–60.
15. Ding ZM, Babensee JE, Simon SI, Lu H, Perrard JL, Bullard DC, Dai XY, Bromley SK, Dustin ML, Entman ML, Smith CW, Ballantyne CM. Relative contribution of LFA-1 and Mac-1 to neutrophil adhesion and migration. J Immunol. 1999;163(9): 5029–38.
16. de Rossi LW, Horn NA, Buhre W, Gass F, Hutschenreuter G, Rossaint R. The effect of isoflurane on neutrophil selectin and beta(2)-integrin activation in vitro. Anesth Analg. 2002;95(3): 583–7 (table of contents).
17. Yuki K, Astrof NS, Bracken C, Yoo R, Silkworth W, Soriano SG, Shimaoka M. The volatile anesthetic isoflurane perturbs conformational activation of integrin LFA-1 by binding to the allosteric regulatory cavity. FASEB J. 2008;22(12):4109–16.
18. Yuki K, Bu W, Xi J, Sen M, Shimaoka M, Eckenhoff RG. Isoflurane binds and stabilizes a closed conformation of the leukocyte function-associated antigen-1. FASEB J. 2012.
19. Zhang H, Astrof NS, Liu JH, Wang JH, Shimaoka M. Crystal structure of isoflurane bound to integrin LFA-1 supports a unified mechanism of volatile anesthetic action in the immune and central nervous systems. FASEB J. 2009;23(8):2735–40.
20. Scharffetter-Kochanek K, Lu H, Norman K, van Nood N, Munoz F, Grabbe S, McArthur M, Lorenzo I, Kaplan S, Ley K, Smith CW, Montgomery CA, Rich S, Beaudet AL. Spontaneous skin ulceration and defective T cell function in CD18 null mice. J Exp Med. 1998;188(1):119–31.
21. Mizgerd JP, Kubo H, Kutkoski GJ, Bhagwan SD, Scharffetter-Kochanek K, Beaudet AL, Doerschuk CM. Neutrophil emigration in the skin, lungs, and peritoneum: different requirements for CD11/CD18 revealed by CD18-deficient mice. J Exp Med. 1997;186(8):1357–64.
22. Goerge T, Ho-Tin-Noe B, Carbo C, Benarafa C, Remold-O'Donnell E, Zhao BQ, Cifuni SM, Wagner DD. Inflammation induces hemorrhage in thrombocytopenia. Blood. 2008;111(10): 4958–64.
23. Klebanoff SJ. Myeloperoxidase: friend and foe. J Leukoc Biol. 2005;77(5):598–625.
24. Schultz J, Kaminker K. Myeloperoxidase of the leucocyte of normal human blood. I. Content and localization. Arch Biochem Biophys. 1962;96:465–7.
25. Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol. 1982;78(3): 206–9.
26. Rote WE, Dempsey E, Maki S, Vlasuk GP, Moyle M. The role of CD11/CD18 integrins in the reverse passive Arthus reaction in rat dermal tissue. J Leukoc Biol. 1996;59(2):254–61.
27. Benarafa C, Priebe GP, Remold-O'Donnell E. The neutrophil serine protease inhibitor serpinb1 preserves lung defense functions in *Pseudomonas aeruginosa* infection. J Exp Med. 2007; 204(8):1901–9.
28. Bergmeier W, Goerge T, Wang HW, Crittenden JR, Baldwin AC, Cifuni SM, Housman DE, Graybiel AM, Wagner DD. Mice lacking the signaling molecule CalDAG-GEFI represent a model for leukocyte adhesion deficiency type III. J Clin Invest. 2007; 117(6):1699–707.
29. Shimaoka M, Salas A, Yang W, Weitz-Schmidt G, Springer TA. Small molecule integrin antagonists that bind to the beta2 subunit I-like domain and activate signals in one direction and block them in the other. Immunity. 2003;19(3):391–402.
30. Soehnlein O, Zernecke A, Eriksson EE, Rothfuchs AG, Pham CT, Herwald H, Bidzhekov K, Rottenberg ME, Weber C, Lindbom L. Neutrophil secretion products pave the way for inflammatory monocytes. Blood. 2008;112(4):1461–71.
31. Soehnlein O, Lindbom L, Weber C. Mechanisms underlying neutrophil-mediated monocyte recruitment. Blood. 2009;114(21): 4613–23.
32. Smith RJ, Iden SS, Rohloff NA. A model of Arthus pleurisy: modulation by various pharmacologic and therapeutic agents. Clin Immunol Immunopathol. 1983;26(1):24–34.
33. Smith CW, Marlin SD, Rothlein R, Toman C, Anderson DC. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. J Clin Invest. 1989;83(6):2008–17.
34. Hentzen ER, Neelamegham S, Kansas GS, Benanti JA, McIntire LV, Smith CW, Simon SI. Sequential binding of CD11a/CD18 and CD11b/CD18 defines neutrophil capture and stable adhesion to intercellular adhesion molecule-1. Blood. 2000;95(3):911–20.
35. Shimaoka M, Springer TA. Therapeutic antagonists and conformational regulation of integrin function. Nat Rev Drug Discov. 2003;2(9):703–16.
36. Kaburagi Y, Hasegawa M, Nagaoka T, Shimada Y, Hamaguchi Y, Komura K, Saito E, Yanaba K, Takehara K, Kadono T, Steeber DA, Tedder TF, Sato S. The cutaneous reverse Arthus reaction requires intercellular adhesion molecule 1 and L-selectin expression. J Immunol. 2002;168(6):2970–8.
37. Yanaba K, Komura K, Horikawa M, Matsushita Y, Takehara K, Sato S. P-selectin glycoprotein ligand-1 is required for the development of cutaneous vasculitis induced by immune complex deposition. J Leukoc Biol. 2004;76(2):374–82.
38. Guyer DA, Moore KL, Lynam EB, Schammel CM, Rogelj S, McEver RP, Sklar LA. P-selectin glycoprotein ligand-1 (PSGL-1) is a ligand for L-selectin in neutrophil aggregation. Blood. 1996; 88(7):2415–21.
39. Asa D, Raycroft L, Ma L, Aeed PA, Kaytes PS, Elhammer AP, Geng JG. The P-selectin glycoprotein ligand functions as a common human leukocyte ligand for P- and E-selectins. J Biol Chem. 1995;270(19):11662–70.
40. Forsyth CB, Solovjov DA, Ugarova TP, Plow EF. Integrin alpha(M)beta(2)-mediated cell migration to fibrinogen and its recognition peptides. J Exp Med. 2001;193(10):1123–33.
41. Issekutz AC, Issekutz TB. The contribution of LFA-1 (CD11a/CD18) and MAC-1 (CD11b/CD18) to the in vivo migration of polymorphonuclear leucocytes to inflammatory reactions in the rat. Immunology. 1992;76(4):655–61.
42. Mayadas TN, Cullere X. Neutrophil beta2 integrins: moderators of life or death decisions. Trends Immunol. 2005;26(7):388–95.
43. Bratzler DW, Hunt DR. The surgical infection prevention and surgical care improvement projects: national initiatives to improve outcomes for patients having surgery. Clin Infect Dis. 2006;43(3):322–30.
44. Exadaktylos AK, Buggy DJ, Moriarty DC, Mascha E, Sessler DI. Can anesthetic technique for primary breast cancer surgery affect recurrence or metastasis? Anesthesiology. 2006;105(4):660–4.
45. Schmits R, Kundig TM, Baker DM, Shumaker G, Simard JJ, Duncan G, Wakeham A, Shahinian A, van der Heiden A, Bachmann MF, Ohashi PS, Mak TW, Hickstein DD. LFA-1-deficient mice show normal CTL responses to virus but fail to reject immunogenic tumor. J Exp Med. 1996;183(4):1415–26.
46. Lu H, Smith CW, Perrard J, Bullard D, Tang L, Shappell SB, Entman ML, Beaudet AL, Ballantyne CM. LFA-1 is sufficient in mediating neutrophil emigration in Mac-1-deficient mice. J Clin Invest. 1997;99(6):1340–50.