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A monoclonal antibody derived by inoculation of human umbilical vein endothelial cells is a potential inhibitor of acetylcholine receptor-linked vasorelaxation

Seung Hyo Jung[1,*], Yunkyoung Ryu[1,*], Donghyen Lee[1], Seung-Bo Park[1], Kyung-Jin Lee[1], Hwan Myung Lee[2], Junghwan Kim[3], Bokyung Kim[1] and Kyung Jong Won[1]

[1] *Department of Physiology, School of Medicine, Konkuk University, Gwangjin-gu, Seoul, Korea*
[2] *Department of Cosmetic Science, College of Life and Health Sciences, Hoseo University, Asan, Korea*
[3] *Department of Physical Therapy, College of Public Health and Welfare, Yongin University, Yongin, Korea*

Abstract. In a previous study, we produced antibodies from rats immunized with human umbilical vein endothelial cells (HUVECs) and determined the vascular function of the monoclonal antibodies. However, unanswered question remains still about their role in vascular function. The current study explored vasoreactivity, in particular, focusing on the vascular contractility of a functional antibody against proteins expressed on the plasma membrane of HUVECs developed in a previous study. Among the antibodies developed, A-7 significantly attenuated endothelium-dependent vasorelaxation in response to acetylcholine (ACh) but not to sodium nitroprusside or histamine. In addition, the A-7 antibody did not affect norepinephrine-stimulated contraction in both endothelium-intact and -denuded aorta. Immunocytochemical and immunoblotting analyses showed that A-7 attenuated ACh-increased expression of ACh receptor on the plasma membrane of HUVECs. These findings suggest that the monoclonal A-7 antibody may act as an inhibitor of endothelium-dependent vasorelaxation, probably in part *via* downregulation of ACh receptor expression.

Key words: Antibody — HUVEC — Membrane protein — Acetylcholine receptor — Vasorelaxation

Introduction

The normal function of vascular endothelial cells (ECs) is essential for the regulation of vascular contractility and is closely linked to maintenance of blood pressure and blood flow in the body. Vascular ECs along with smooth muscle cells (SMCs) contribute to vascular contractility regulated *via* vascular contraction and relaxation (Rodrigo and Herbert 2018). ECs produce various vasoactive factors such as vasoconstrictors and vasodilators. Physiological stimuli such as physical forces, circulating hormones, platelet products, and prostaglandins can regulate endothelium-dependent vascular contractility (Khaddaj Mallat et al. 2017). Acetylcholine (ACh) is known to induce endothelium-dependent vasorelaxation (Furchgott 1999). The ACh-induced vasorelaxation is mediated by the activation of muscarinic ACh receptors (mAChRs) (Boulanger et al. 1994). The mAChRs comprise the five subtypes (m1AChR through m5AChR) in the vascular system (Wessler et al. 2003; Zarghooni et al. 2007; Wessler and Kirkpatrick 2008). Among the different subtypes, the m3AChR is the main receptor mediating ACh-stimulated endothelium-dependent vasorelaxation (Boulanger et al. 1994; Gericke et al. 2011). Nitric oxide (NO) is one of the main EC-derived vasodilators and is induced in response to ACh (Köhler and Milstein 1975; Furchgott 1983; Versari et al. 2009). NO production in ECs is triggered by the activation of NO synthase that converts l-arginine to l-citrulline. The NO diffuses into SMC layers and activates guanylate cyclase, which induces the elevation of cyclic guanosine monophosphate to elicit vascular SMC relaxation through multiple mechanisms (Omori and Kotera 2007). These responses suggest that NO plays an essential

* These two authors contributed equally to the manuscript.

Correspondence to: Kyung Jong Won, Department of Physiology, Konkuk University School of Medicine, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Korea
E-mail: kjwon@kku.ac.kr

role in the regulation of vascular contractility (Pintérová et al. 2011). Human body is exposed to various external stimuli that change blood flow and blood pressure, which are closely associated with NO (Furchgott 1983). Therefore, identification of new functional proteins linked to NO role in ECs may facilitate the study of vascular dilation, although the interaction between NO function and ECs is reported in vascular cells under physiological and pathophysiological conditions.

Plasma membrane in various cells separates the cell from the external environments. It expresses various functional proteins that respond to extracellular stimuli and play important roles in maintaining normal biological responses (Lee et al. 2009; Volonté and D'Ambrosi 2009). Plasma membrane proteins or membrane-associated proteins act as potential targets for biomarkers, drugs and antibodies (Rucevic 2011). Previous studies reported that a third of all known biomarker candidates are membrane proteins (Polanski and Anderson 2007; Josic et al. 2008). Many investigators have demonstrated that a monoclonal antibody can be strategically used against cell membrane antigens to elucidate the function of isolated proteins (Miraglia 1997; Yin et al. 1997). Moreover, the application of antibodies alone or in combination with other drugs has been of clinical and therapeutic interest (Lee et al. 2018). Antibodies display high specificity toward a defined target antigen, and are therefore, considered as unique therapeutics (Hicklin et al. 2001). Therefore, the identification of new functional molecules may provide basic insight into and understanding of their physiological mechanisms in targeted cells including vascular cells. Our laboratory has developed 22 monoclonal antibodies from rats inoculated with HUVECs (Won et al. 2013). Although these antibodies display functional vascular responses, they may be additional antibodies possessing potential vascular reactivity. In the current study, we sought to identify novel functional molecules expressed on the membranes of HUVECs based on their vascular reactivity, especially, vascular contractility.

Materials and Methods

Materials

ACh, norepinephrine (NE), histamine, bovine serum albumin (BSA), and sodium nitroprusside (SNP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was obtained from HyClone Laboratories Inc (Logan, UT, USA). The anti-mAChR and IgG2α antibodies were purchased from Santa Cruz (Santa Cruz, CA, USA). Alexa 488-conjugated anti-rabbit IgG antibody was ordered from Molecular Probes (Eugene, OR, USA).

Tissue preparation and measurement of isometric contraction

Animal care and all the experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Instituetes of Health (NIH publication NO. 85-23, revised 1996) and approved by the Animal Subjects Committee and institutional guidelines of Konkuk University, Korea. Male Sprague-Dawley (SD) rats (7-weeks-old, 180–190 g, $n = 10$) were purchased from Orient Bio (Korea). The animals were euthanized using CO_2 gas inhalation and bled rapidly by severing the carotid arteries (Won et al. 2013; Raffetto et al. 2019). The thoracic aorta was rapidly and carefully removed and placed in a physiological salt solution (PSS) containing the following composition (in mM): NaCl 136.9; KCl 5.4; $CaCl_2$ 1.5; $MgCl_2$ 1.0; $NaHCO_3$ 23.8; EDTA 0.01. The aorta was cleaned by removing fat and connective tissue, and cut into 2-mm ring segments. In some experiments, the endothelium was removed by gently rubbing the inner surface of the vessel with cotton balls soaked in PSS.

The prepared aortic ring segments were mounted on two L-shape holders, of which one end was attached to a stainless-steel rod, and the other to a force transducer (FT03; Grass-Telefactor Instruments, West Warwick, RI, USA), in 3 ml organ baths containing PSS. Changes in muscle force were isometrically recorded on Grass 79E polygraphs (Grass-Telefactor Instruments). After the segments were mounted under resting tension of 10 mN, they were equilibrated for 30 min in an organ bath filled with PSS and sequentially exposed to 70 mM K^+ and PSS. The high K^+ solution was prepared by replacing NaCl with an equimolar amount of KCl. All bath solutions were saturated with a mixture of 95% O_2 and 5% CO_2 at pH 7.4 and 37°C.

HUVEC culture

HUVECs were enzymatically isolated from human umbilical cord veins as described previously (Jaffe et al. 1973). The endothelial cells were cultured in gelatin (0.1%)-coated culture flasks filled with M199 medium (Welgene, Korea) containing 20% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 3 ng/ml bFGF, 5 units/ml heparin and 250 μg/ml fungizone. The cells were used in passages two to six for each experiment.

A-7 antibody production

The A-7 monoclonal antibody against HUVECs was produced by injecting HUVECs into 6-week-old SD rats (Orient Bio) as described previously (Won et al. 2013). Briefly, rats were immunized by injecting 3×10^5 HUVECs on days 0 and 7 in both hand footpads. After a second injection, lymphocytes from lymph node were prepared and fused to SP2/0

myeloma cells. Fused cells were cultured on 96-well plates in Dulbecco's Modified Eagle's medium (DMEM; Welgene, Daegu, Republic of Korea) supplemented with 20% FBS and incubated at 37°C in 5% CO_2. For screening hybridoma cells, culture supernatants were screened on HUVECs using a fluorescence-activated cell analyzer (FACS) Calibur (BD Biosciences, San Jose, CA, USA). Monoclonal antibodies were traced by phycoerythrin (PE)-conjugated mouse anti-rat immunoglobulin G antibody (IgG) (BD Pharmingen, San Diego, CA, USA). The experimental monoclonal A-7 antibody was prepared from ascites fluid by purifying protein-A column.

To determine the specific binding ability of A-7 antibody, HUVECs were stained with anti-A-7 antibody and PE-conjugated anti-rat IgG as the secondary antibody. The stained cells were analyzed with a FACS Calibur (BD Biosciences) and the data were collected using only live cells labeled with propidium iodide (PI). The frequencies in quadrant corners are given as percentages of gated cells. The collected data were analyzed using the CELLQUEST software (BD Biosciences).

Immunofluorescence staining

Expression of AChR in the cultured HUVECs was determined using a standard immunostaining method. Briefly, the cells were cultured on a cover glass coated with poly-d-lysine, and fixed with 4% formaldehyde at 37°C for 30 min. After washing in PBS, cells were blocked by incubation with 1% BSA for 1 hour followed by treatment with primary antibodies and rabbit anti-mAChR (1:100) antibodies at room temperature (RT) for 2 hours. After washing, the secondary Alexa 488-conjugated anti-rabbit IgG antibody was used at a dilution of 1:400 for 1 hour at RT. The immunostained cells were observed by fluorescence microscopy (Axio Observer A1; Carl Zeiss, Jena, Germany). Immunohistochemical staining of images was quantified using MetaMorph imaging software (ver 7.1; Molecular Devices, Downingtown, PA, USA).

Immunoblotting

The cell lysates were centrifuged at 17,000 × *g* for 15 min at 4°C and the supernatants were collected as protein samples. Proteins (35 μg/lane) were separated on 8% SDS-polyacrylamide gel and then transferred electrophoretically to a polyvinylidene fluoride membrane (Millipore, Bedford, MA, USA). The membranes were blocked with PBS containing 5% BSA, incubated overnight at 4°C in the primary antibodies (1:1000 dilution), and subsequently incubated in the peroxidase-conjugated secondary (1:3000 dilution) for 1 hour at RT. The blots were incubated in enhanced chemiluminescence solution (Amersham-Pharmacia, Piscataway, NJ, USA) and the bands were visualized using a Luminescent Image Analyzer LAS-4000 (Fujifilm, Tokyo, Japan). Band intensity was quantified using ImageJ software (NIH, Bethesda, MD, USA).

Statistical analysis

Data are expressed as the mean ± SE. All data were analyzed using GraphPad Prism (GraphPad Software, San Diego,

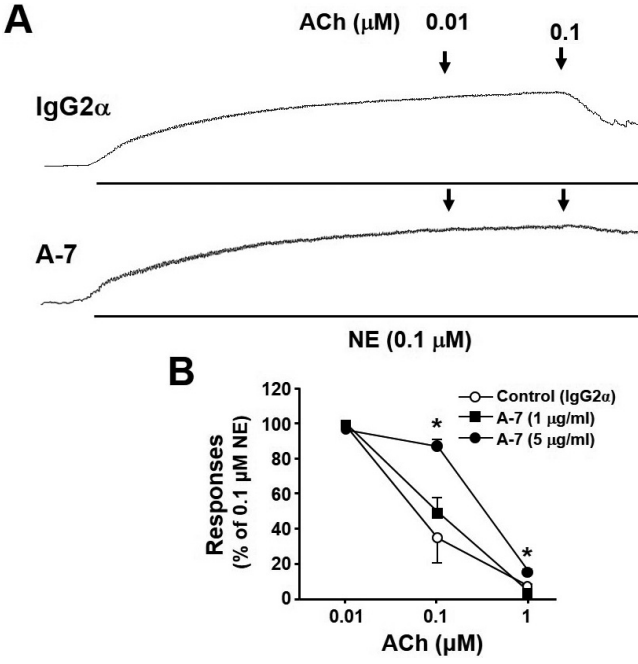

Figure 1. Effects of A-7 antibody on endothelium-dependent vasorelaxation in response to acetylcholine (Ach). Endothelium-intact aortic rings were treated with A-7 antibody (1 and 5 μg/ml) or IgG2α (5 μg/ml) for 60 min followed by stimulation of norepinephrine (NE; 0.1 μM) and cumulative treatment with ACh at the indicated concentrations. **A.** Representative recording chart images show changes in ACh-elicited relaxation induced by treatment with A-7 antibody. **B.** Statistical graph obtained from panel A; the magnitude of NE (0.1 μM)-induced contractile response before treatment with ACh was expressed as 100% ($n = 4$). Data was statistically analyzed by two-way repeated-measures ANOVA with Tukey's *post-hoc* test ($F(4,18) = 9.171$, $p = 0.0003$, interaction; $F(2,9) = 10.51$, $p = 0.0044$, group; $F(2,18) = 243.9$, $p < 0.0001$, concentration (ACh)). * significant difference compared to ACh-stimulated states in the presence of IgG2α ($p < 0.05$).

CA, USA). Statistical evaluation of data was performed with a Student's *t*-test (Figs. 2C and 3B) for comparisons between pairs of groups and by a one-way ANOVA (Figs. 4B and 5B) or a two-way repeated-measures ANOVA (Figs. 1C, 2A,B and 3A) followed by a Tukey's *post-hoc* test for multiple group comparison. The value $p < 0.05$ was considered statistically significant.

Results

Effect of A-7 antibody on endothelium-dependent vasorelaxation

In a previous study, we generated 22 monoclonal antibodies that regulated the activities of vascular system and identified a vasoactive antibody (Won et al. 2013). To further investigate another monoclonal antibody associated with vascular function, we tested the effects of antibodies with specific reactivity to HUVECs other than the antibody (C7) with vascular reactivity, which is one of the seven antibodies exhibiting more than 80% specific reactivity to HUVECs in a previous study. Among the different antibodies, A-7 displayed 45.9% specific reactivity to HUVEC as reported previously (Won et al. 2013) and affected vascular reactivity as shown in Figure 1. Treatment of ACh (0.01–1 μM) elicited concentration-dependent inhibition of NE (0.1 μM)-induced contraction in endothelium-intact aortic ring isolated from rats, with a maximum inhibition at 1 μM of ACh (Figure 1; $n = 4$). Treatment with A-7 antibody at a concentration of 5 μg/ml, but not at a concentration of 1 μg/ml, inhibited the ACh-induced suppression of NE (0.1 μM)-stimulated contractile response in the aortic ring. On the other hand, the ACh-induced inhibitory response of NE-stimulated contraction did not differ between groups treated with and without IgG2α (Figure 1).

Effect of A-7 antibody on vasorelaxant- or vasoconstrictor--induced reactivity in endothelium-intact aorta

To confirm whether A-7 antibody inhibits only the ACh-induced response, the effect of A-7 antibody was also tested against histamine-induced relaxation in endothelium-intact aortic rings. Treatment with histamine at a concentration range of 1 to100 μM attenuated the contractile response to NE (0.1 μM) in the endothelium-intact aortic rings in a concentration-dependent manner. However, this histamine-inhibited response was not affected by treatment with 5 μg/ml of A-7 antibody (Figure 2A; $n = 5$).

To determine the effect of A-7 antibody on vasoreactivity by endothelium-independent vasodilator, we induced endothelium-independent vasodilator SNP-dependent effect against NE-induced precontraction in aortic ring.

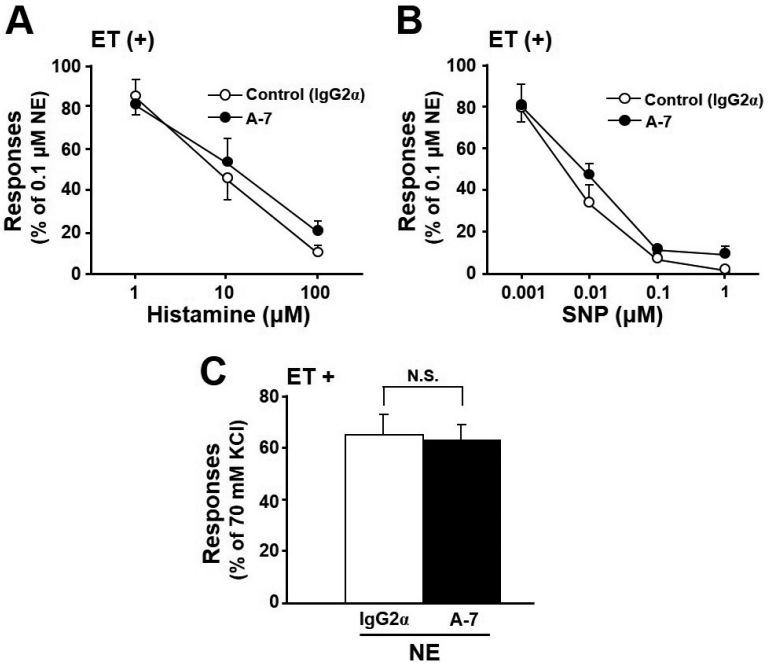

Figure 2. Effects of A-7 antibody on vascular reactivity in endothelium-intact aorta. **A** and **B.** Effects of A-7 antibody on vascular relaxation in endothelium-intact aorta. After treatment with A-7 antibody (5 μg/ml) or IgG2α (5 μg/ml) for 60 min, endothelium-intact ET (+) aortic rings were stimulated with norepinephrine (NE; 0.1 μM) and cumulatively treated with histamine (A; $n = 5$) or sodium nitroprusside (SNP) (B; $n = 4$). The magnitude of NE (0.1 μM)-induced contraction before treatment with histamine (A) or SNP (B) was considered as 100%. Data was statistically analyzed by two-way repeated-measures ANOVA [(A: $F(2,14) = 0.9341$, $p = 0.4161$, interaction; $F(1,6) = 0.2114$, $p = 0.6596$, group; $F(3,18) = 67.61$, $p < 0.0001$, concentration) (B: $F(3,18) = 0.3744$, $p = 0.7725$, interaction; $F(1,6) = 1.360$, $p = 0.2879$, group; $F(3,18) = 72.48$, $p < 0.0001$, concentration)]. **C.** Effect of A-7 antibody on NE-stimulated contraction of aortic rings. Endothelium-intact (ET (+)) aortic ring was stimulated with NE (0.1 μM) in the presence or absence of A-7 antibody (5 μg/ml). The magnitude of 70 mM high K^+-induced aortic contraction just before initiation of the experiments is expressed as 100% ($n = 7$). N.S., not significant ($p > 0.05$, Student's *t*-test).

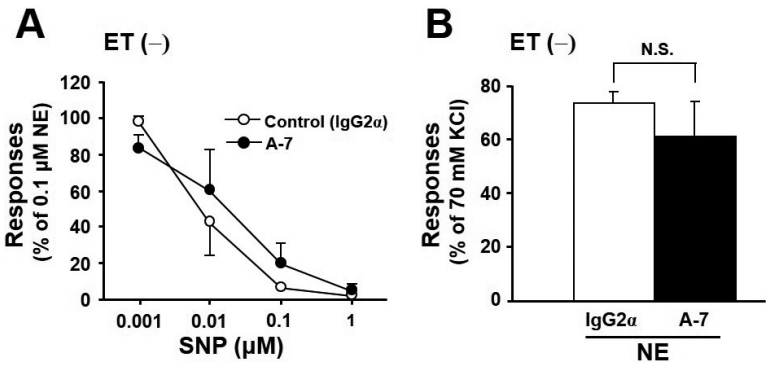

Figure 3. Effects of A-7 antibody on vascular reactivity in aortic smooth muscle. **A.** Effect of A-7 antibody on sodium nitroprusside (SNP)-induced response of aortic smooth muscle. After treatment with A-7 antibody (5 μg/ml) or IgG2α (5 μg/ml) for 60 min, endothelium-denuded ET (-) aortic rings were stimulated with norepinephrine (NE; 0.1 μM) and cumulatively treated with SNP. The magnitude of NE (0.1 μM)-induced contraction before treatment with SNP was defined as 100% (n = 4). Data was statistically analyzed by two-way repeated-measures ANOVA ($F(3,18) = 1.028$, $p = 0.4037$, interaction; $F(1,6) = 0.1725$, $p = 0.6923$, group; $F(3,18) = 34.86$, $p < 0.0001$, concentration (SNP)). **B**. Effect of A-7 antibody on NE-induced contraction of aortic smooth muscle. Endothelium-denuded (ET(-)) aortic rings were stimulated with NE (0.1 μM) with or without A-7 antibody (5 μg/ml). The magnitude of 70 mM high K^+-induced aortic contraction immediately before NE application is expressed as 100% (n = 4). N.S., not significant ($p > 0.05$, Student's t-test).

Treatment with SNP at a concentration range of 0.001 to 1 μM showed concentration-dependent inhibition of the NE-induced contraction in endothelium-intact aortic ring. The SNP-inhibited response of NE-induced contraction did not vary between A-7 antibody (5 μg/ml)-treated group and gG2α-treated control (Figure 2B; n = 4). Moreover, treatment with A-7 antibody (5 μg/ml) did not alter NE (0.1 μM)-induced contraction in endothelium-intact aortic ring (Figure 2C; n = 7). The A-7 antibody and IgG2α control antibodies did not show any effect on the resting tension of aortic ring (data not shown; n = 7). The IgG2α control antibody also had no effect on the NE-stimulated contractile response (Figure 2).

Effect of A-7 antibody on SNP- or NE-induced vascular smooth muscle reactivity

To determine the effect of A-7 antibody on endothelium-independent vascular reactivity in aorta, we also examined the SNP-induced response to NE-triggered contraction in endothelium-denuded aortic ring. As shown in Figure 3A, NE (0.1 μM)-stimulated contraction showed concentration-dependent relaxation in the endothelium-denuded aortic ring following treatment with SNP (0.001–1 μM). Pretreatment with A-7 antibody (5 μg/ml) did not significantly inhibit the SNP-induced vasorelaxation of NE (0.1 μM)-stimulated aortic contraction (Figure 3A; n = 4). In addition,

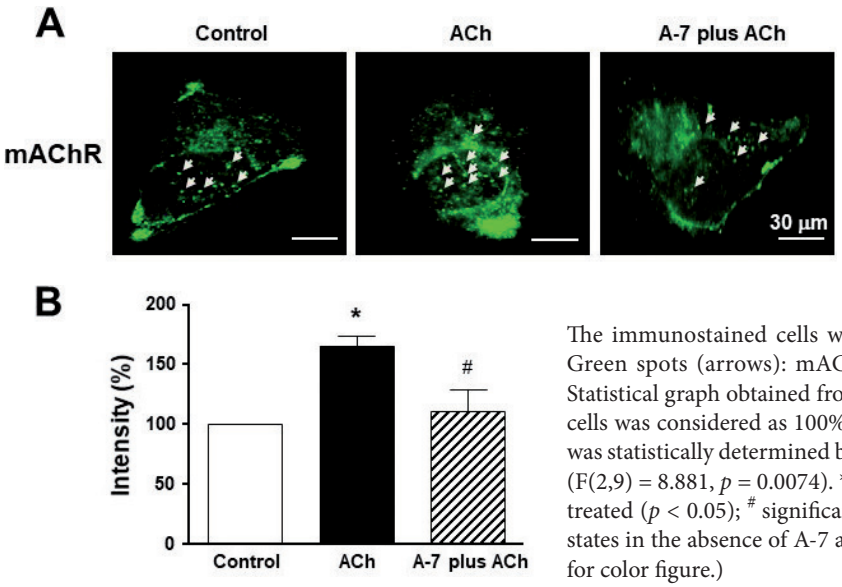

Figure 4. Effect of A-7 antibody on AChR expression on membranes of HUVEC. **A**. HUVECs were incubated with IgG2α (control) or A-7 antibody (5 μg/ml) for 60 min and were treated with or without acetylcholine (ACh; 1 μM) for 10 min. HUVECs were stained with anti-muscarinic M3 AChR (mAChR) antibody and visualized using Alexa Fluor 488-conjugated secondary antibody (n = 4). The immunostained cells were observed using fluorescence microscopy. Green spots (arrows): mAChR positive responses. Scale bar: 30 μm. **B**. Statistical graph obtained from panel A. AChR expression in IgG2α-treated cells was considered as 100%. The difference between treatment conditions was statistically determined by a one-way ANOVA with Tukey's *post-hoc* test ($F(2,9) = 8.881$, $p = 0.0074$). * significant difference compared to the IgG2α-treated ($p < 0.05$); # significant difference compared to the ACh-stimulated states in the absence of A-7 antibody control ($p < 0.05$). (See online version for color figure.)

treatment with A-7 antibody (5 μg/ml) did not alter NE (0.1 μM)-induced contractions in endothelium-denuded aorta (Figure 3B; $n = 4$). A-7 and IgG2α antibodies showed no effect on the resting tension in aortic ring (data not shown; $n = 4$) and IgG2α also did not affect the NE-induced contraction (Figure 3).

Effect of A-7 treatment on AChR expression on HUVEC membrane

Based on the results of ACh-induced vasorelaxation of A-7 mentioned above, we determined the correlation between A-7 and AChR expression on the endothelial cellular membrane to elucidate the role of A-7 in ACh-induced endothelial cell activity. As shown in Figure 4, an immunofluorescence staining showed that treatment of HUVECs with ACh (1 μM) significantly increased the level of AChR expression and this increase was inhibited by pretreatment with A-7 antibody (5 μg/ml) ($n = 4$).

In addition, the effect of A-7 antibody on AChR expression in HUVECs was confirmed using immunoblotting technique. Similar to the results of immunofluorescence staining, treatment of HUVECs with A-7 antibody (5 μg/ml) significantly reduced the AChR expression level that was increased in HUVECs by treatment of ACh (1 μM) (Figure 5; $n = 4$).

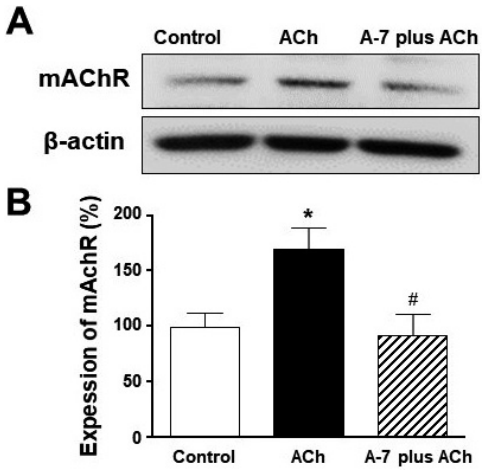

Figure 5. Effect of A-7 antibody on AChR expression in HUVEC. **A**. HUVECs were incubated with IgG2α (control) or A-7 antibody (5 μg/ml) for 60 min and were treated with or without acetylcholine (ACh; 1 μM) for 10 min. The expression level of AChR in HUVECs was examined by immunoblotting using anti-muscarinic M3 AChR (mAChR) antibody. **B**. Statistical results obtained from panel A. AChR expression in IgG2α-treated cells was expressed as 100% ($n = 4$). The difference between treatment conditions was statistically determined by one-way ANOVA analysis with Tukey's post-hoc test ($F(2,9) = 6.544$, $p = 0.0176$). * significant difference compared to the IgG2α-treated control ($p < 0.05$); # significant difference compared to the ACh-stimulated states in the absence of A-7 antibody ($p < 0.05$).

Discussion

In the present study, we found that A-7, one of the monoclonal antibodies induced by injection of HUVECs, statistically significantly inhibited ACh-induced vasorelaxation in endothelium-intact aorta from rats, indicating that A-7 antibody may have an inhibitory activity on ACh-induced vasorelaxation. However, the vascular response induced by A-7 at concentrations higher than 5 μg/ml may need to be investigated in order to more clarify correlation between A-7 and ACh-induced response. Furthermore, in our previous study, a functional monoclonal antibody was detected among antibodies directed against HUVECs (Won et al. 2013). ACh evokes vascular SMC relaxation mediated by signals triggered by the AChR activation in the membrane of ECs (Moncada et al. 1991). Signals induced by AChR activation participate in NO generation contributing to guanylate cyclase activation and cyclic guanosine monophosphate elevation, resulting in vascular SMC relaxation (Jaffe 1985). Therefore, these findings demonstrate that A-7 antibody may be a potential functional protein expressed in ECs associated with EC-dependent relaxation in response to ACh.

Vascular ECs that serve as a barrier between blood and tissue contribute to the maintenance of vascular tone by various substances regulating vascular reactivity (Moncada et al. 1991). NO is induced in ECs by stimuli including histamine as well as ACh in vascular system in a receptor-dependent manner and is diffused into SMCs (Köhler and Milstein 1975). In the present study, the A-7 antibody showed a very weak inhibitory effect on histamine-induced endothelium-dependent vasorelaxation, which was not statistically significant, implying that it may not significantly affect endothelium-dependent vasorelaxation *via* histamine-linked signaling. Moreover, the A-7 antibody controlled ACh-induced endothelium-dependent vasorelaxation and attenuated ACh-increased mAChR induction as shown in immunocytochemistry and immunoblot results. Therefore, it is assumed that the A-7 antibody may participate in mAChR-, but not in histamine receptor-, mediated responses. NE stimulates the activation of adrenoceptors on the plasma membranes of both ECs and SMCs (Endemann and Schiffrin 2004). The activation of adrenoceptors in ECs promotes NO production, leading to smooth muscle relaxation, and induces phospholipase C activation and Ca^{2+} influx in SMCs, resulting in SMC contraction (Endemann and Schiffrin 2004). The present study demonstrated that the A-7 antibody did not significantly affect the NE-stimulated contractile response in endothelium-intact or -denuded

aorta, indicating that A-7 antibody was not associated with adrenoceptor-associated vascular relaxation and contraction. Moreover, A-7 antibody had a statistically non-significant slight inhibitory effect on the vascular response induced by the endothelium-independent NO donor SNP in endothelium-intact and -denuded condition, implying that A-7 antibody may not exert a significant effect on the direct NO generation-induced vascular response. Therefore, these results suggest that A-7 may play a role in signal transduction responses, especially NO generation, triggered by the activation of mAChR in vascular system.

It is well known that mAChRs distributed throughout the human body are classified into five subtypes m1, m2, m3, m4, and m5AchR (Eglen 2012; Kruse et al. 2014). Activated mAChRs control physiological responses such as heart rate, smooth muscle contraction, glandular secretion and multiple activities in the central nervous system (Eglen 2012). Recently, it was reported that mAChRs play an important role in a variety of cellular functions including brain development (Lebois et al. 2018). Moreover, abnormal mAChR receptors have been implicated in diseases such as Alzheimer, Parkinson's, and schizophrenia (Wess et al. 2007; Kruse et al. 2014). The m3AchR is located in vascular and gastric SMCs, and in salivary glands (Wess et al. 2007; Tobin et al. 2009). Therefore, the monoclonal antibodies generated by the injection of cells can be considered as a unique therapeutic response clinically.

In summary, we demonstrated that monoclonal A-7 antibody generated by the injection of HUVECs inhibited endothelium-dependent vasorelaxation in response to ACh but not to histamine. The A-7 antibody did not affect NE-induced contraction in endothelium-intact and -denuded aorta. Moreover, the immunocytochemical and immunoblotting analysis revealed that ACh-increased mAChR expression was attenuated by treatment with A-7 antibody in HUVECs. These findings indicate that the A-7 antibody has an inhibitory effect on endothelium-dependent vasorelaxation in response to ACh, probably at least partially resulting from the downregulation of mACh receptor expression. Therefore, monoclonal A-7 antibody may be a valuable molecule to elucidate or better understand the potential functional mechanism linked to ACh-receptor-mediated pathway in the vascular system.

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Conflicts of interest. The authors declare that they have no conflict of interest.

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