Biochemical and Biophysical Research Communications 401 (2010) 7–12

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Inhibition of dipeptidyl peptidase 4 regulates microvascular endothelial growth induced by inflammatory cytokines

Wataru Takasawa [a], Kei Ohnuma [b], Ryo Hatano [a], Yuko Endo [a], Nam H. Dang [c], Chikao Morimoto [a,b,*]

[a] Division of Clinical Immunology, The Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan
[b] Department of Rheumatology and Allergy, Research Hospital, The Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan
[c] Division of Hematology/Oncology, University of Florida, 1600 SW Archer Road, Box 100278, Room MSB M410A, Gainesville, FL 32610, USA

ARTICLE INFO

Article history:
Received 13 August 2010
Available online 7 September 2010

Keywords:
CD26/dipeptidyl peptidase 4
TNF-α
IL-1β
Endothelial cell
Vascularization

ABSTRACT

CD26/DPP-4 is abundantly expressed on capillary of inflamed lesion as well as effector T cells. Recently, CD26/dipeptidyl peptidase 4 (DPP-4) inhibition has been used as a novel oral therapeutic approach for patients with type 2 diabetes. While accumulating data indicate that vascular inflammation is a key feature of both micro- and macro-vascular complications in diabetes, the direct role of CD26/DPP-4 in endothelial biology is to be elucidated. We herein showed that proinflammatory cytokines such as tumor necrosis factor or interleukin-1 reduce expression of CD26 on microvascular endothelial cells, and that genetical or pharmacological inhibition of CD26/DPP-4 enhances endothelial growth both *in vitro* and *in vivo*. With DPP-4 inhibitors being used widely in the treatment of type 2 diabetes, our data strongly suggest that DPP-4 inhibition plays a pivotal role in endothelial growth and may have a potential role in the recovery of local circulation following diabetic vascular complications.

1. Introduction

In addition to being a marker of T cell activation, CD26/DPP-4 is also associated with T cell signal transduction processes as a costimulatory molecule, and the enzymatic activity of CD26/DPP-4 appears to play an important role in enhancing cellular responses to external stimuli [1,2]. While CD26 expression is increased following activation of resting T cells, $CD4^{+}CD26^{high}$ T cells respond maximally to recall antigens such as tetanus toxoid. Moreover, crosslinking of CD26 and CD3 with solid-phase immobilized monoclonal antibodies can induce T-cell costimulation and IL-2 production by $CD26^{+}$ T-cells [1,3]. Furthermore, high CD26 cell surface expression is correlated with the production of TH1-type cytokines, while $CD26^{+}$ T-helper cells stimulate antibody synthesis in B-cells [4]. Recently, we have demonstrated that caveolin-1 is a costimulatory ligand for CD26, and that CD26 on activated memory T-cells interacts with caveolin-1 on tetanus toxoid (TT)-loaded monocytes [5]. Moreover, following CD26–caveolin-1 interaction on TT-loaded monocytes, caveolin-1 is phosphorylated, with linkage to NF–κB activation, followed by upregulation of CD86, a ligand for CD28 [6]. Taken together with the data that effector T cells in inflamed lesions express high levels of CD26/DPP-4 [7–9], it is conceivable that CD26 plays an important role in the inflammatory process.

Inhibition of DPP-4 represents a novel oral treatment approach for type 2 diabetes, since it increases insulin secretion and reduces glucagon secretion by preventing the inactivation of glucagon-like peptide-1, thereby lowering glucose levels [10]. While cardiovascular disease accounts for approximately 70% of all deaths in diabetic patients [11], it remains to be clarified whether diabetic vascular complications can be more effectively treated by targeting disease-specific mechanisms rather than by focusing only on achieving optimal metabolic control [12]. The role of inflammation and immunity in the development of diabetic vascular complications including atherosclerosis is receiving increased attention, leading potentially to the identification of novel targets for prevention and treatment [13]. Activation of T cells and proinflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β) play an important role in endothelial homeostasis of metabolic vascular disease [13]. Moreover, we have previously shown that exogenous recombinant DPP-4 strongly regulates the migratory ability of T cells through endothelial cells (ECs) [14]. In this regard, while CD26/DPP-4 is a novel target of diabetes treatment [10], and is recognized as an activation antigen on T cells localized to inflammatory lesions [9], it is unclear whether CD26/DPP-4 plays a role in the regulatory mechanism of the inflammatory process of ECs. To examine the potential role of DPP-4 activity in inflammatory endothelial responses, we evaluated in the present study CD26/DPP-4-dependent microvascular EC proliferation,

* Corresponding author at: Division of Clinical Immunology, The Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan. Fax: +81 354 495 448.
E-mail address: morimoto@ims.u-tokyo.ac.jp (C. Morimoto).

0006-291X/$ - see front matter © 2010 Published by Elsevier Inc.
doi:10.1016/j.bbrc.2010.08.112

migration, and neovascularization in inflammatory settings, through the use of small interfering RNA (siRNA) targeting CD26/DPP-4 mRNA, DPP-4 enzyme inhibitors or CD26-knockout (CD26-KO) mice.

2. Materials and methods

2.1. Cell culture, siRNA transfection and animals

Human dermal microvascular endothelial cells (HMVEC) were purchased from Sanko Junyaku Co., Ltd. and cultured in EGM2-GV medium at 37 °C, 5% CO_2, and 100% humidifier, according to the manufacturer's instructions. Human CD26/DPP-4 siRNAs (Hs_DPPIV_1 HP (S1) and Hs_DPPIV_2 HP (S2)) were purchased from Qiagen. Negative control siRNA (mock-siRNA) was purchased from Qiagen (All Star Negative Control siRNA). Transfection of siRNA into HMVEC was performed with TransPass HUVEC Transfection Reagent according to the manufacturer's instructions (New England BioLabs). In *in vivo* experiments, 8-to-9-week-old male wild-type C57BL/6c ($CD26^{+/+}$) mice from CLEA Japan Inc. were used. CD26-KO ($CD26^{-/-}$) mice developed from C57BL/6c mice were gifted from the laboratory of Takeshi Watanabe at Kyusyu University (Fukuoka) [15]. All animal experiments were approved by the institutional animal care and ethics committee.

2.2. Cell proliferation assay

HMVEC seeded (5×10^4/well) in 96-well titer plates were incubated for 48 h in medium in the presence or absence of human TNF-α (PeproTech EC), IL-1β (PeproTech EC) or DPP-4 inhibitors (diprotinin-A or P32/98 from Enzo Life) at indicated concentrations. For CD26 knockdown experiments, 20 nM of siRNAs (mock, S1 or S2) were transfected into HMVEC before incubation. Cell proliferation was assayed using TetraColor ONE according to the manufacturer's instructions (Seikagaku Biobuisiness Corp.). Living cells were measured by the absorbance value at 450 nm. Data represent mean values ± standard errors calculated from at least three independent experiments in triplicate.

2.3. Western blot analysis and flow cytometry

After HMVEC was cultured in the presence or absence of TNF-α or IL-1β (0, 5, or 20 ng/ml) for 24 h, cells were lysed with HBST lysis buffer (0.5% Triton X-100, 150 mM NaCl, 1 mM PMSF, 10 mM HEPES pH 7.4, Protease inhibitor cocktail). Cell lysates were subjected to SDS–PAGE and Western blotting as described previously [16]. For flow cytometry (FCM) analysis, HMVEC were cultured for various hours as above, removed using 0.05% trypsin–MEM (Invitrogen), and subjected to FCM as described previously [17].

2.4. Reverse transcription–polymerase chain reaction (RT–PCR) assay

In experiments assessing expression of CD26 mRNA, HMVEC were cultured in the presence or absence of TNF-α or IL-1β (0, 5, or 20 ng/ml) for 24 h, and total RNA was then extracted using RNeasy Micro Kit (Qiagen). cDNA was produced by using Thermo-Script III reverse transcriptase (Life Technologies Inc.) with oligo $(dT)_{12-18}$ primers. The quantities of mRNA were adjusted equally through PCR analysis of glyceraldehyde-3-phosphate dehydrogenase (GADPH) (forward primer, 5′-ACCACAGTCCATGCCATCAC-3′; reverse primer, 5′-TCCACCACCCTGTTGCTGTA-3′) as the internal control. CD26 mRNA was amplified with primers designed to amplify the partial coding sequence of CD26 (forward primer, 5′-CGGTCCTGGTCTGCCCCTCTA-3′; reverse primer, 5′-CGCCACGGCTATTCCACACTT-3′). The PCR reactions were performed using TaKaRaEx Taq (Takara Bio Inc.) as follows: 94 °C for 4 min, then denaturing at 98 °C for 10 s, annealing at 55 °C for 30 s, and extending at 72 °C for 1 min at 35 cycles. Amplified DNA was then electrophoresed in 2% agarose gel, and stained with ethidium bromide.

2.5. HMVEC scratch-wound assay

HMVEC were seeded (3×10^4/well) into 8-well chambers (Nalgen Nunc Int.) and grown to 70–90% confluency. Cells were transfected with siRNAs or incubated in medium containing 10 μM of DPP-4 inhibitor (diprotinin-A or P32/98), and cultured in the presence or absence of 20 ng/ml of TNF-α or IL-1β. The monolayer was then scraped using P200 micropipette tip. Forty-eight hours after wounding, cells were washed in phosphate buffered saline, and fixed in 10% paraformaldehyde. Cells in the denuded area (each three random fields) were quantified by light microscopy under 100 × magnification.

2.6. Aortic ring assay

Aortic rings were prepared from the thoracic and abdominal aorta of $CD26^{+/+}$ or $CD26^{-/-}$ mice and processed as described previously [18]. The rings were then embedded in Matrigel (BD Bioscience) in 8-well chamber slides and incubated in medium containing 300 ng/ml of Endothelial Cell Growth Supplement (Sigma–Aldrich). After 48-h incubation, the medium was changed to Medium199 (Invitrogen) containing 20 ng/ml of TNF-α or IL-1β, or 10 μM of DPP-4 inhibitor P32/98. Three days later, cells sprouted from the rings were quantified by light microscopy under 100 × magnification (each three random fields) in the same manner as described in the literature [19].

The *in vivo* Matrigel plug assay $CD26^{+/+}$ or $CD26^{-/-}$ mice were injected subcutaneously (bilateral flanks) with 500 μl of Matrigel containing heparin (64U/ml, Mochida Pharmaceutical Co. Ltd.), bFGF (300 ng/ml) and TNF-α or IL-1β (each 100 ng/ml) in the presence or absence of DPP-4 inhibitor P32/98 (10 μM). Four days later, plugs were resected and fixed in 10% paraformaldehyde, and 4 μm cross-sections were stained with Hematoxylin–Eosin, visualized using light microscopy. Vessels were confirmed by immunostaining with anti-mouse CD31 (Immunotech), and CD26 expression was evaluated by immunostaining with anti-mouse CD26 (R&D Systems).

2.7. Photo image analysis and statistics

ImageJ software (the National Institutes of Health) was used for image analysis of band intensity, cell count or cell intensity. Student's *t* test was used to determine whether the difference between control and sample was significant ($p < 0.05$ being significant).

3. Results

3.1. TNF-α or IL-1β induces HMVEC proliferation with associated reduction of CD26 expression

Since proinflammatory cytokines have been reported to induce activation of ECs [20], we first tested the effect of IL-2, IL-6, interferon-γ (IFN-γ), TNF-α or IL-1β on EC proliferation. As shown in Fig. 1A, we also observed that TNF-α or IL-1β enhanced proliferation of HMVEC (* or **). While CD26 expression in T cells is increased by IL-2 stimulation [21], it remains to be elucidated whether proinflammatory cytokines other than IL-2 regulate expression of CD26, especially in EC. Therefore, we next evaluated the level of CD26 expression on HMVEC in the presence of TNF-α or IL-1β. As shown in Fig. 1B, cell surface expression of CD26 on HMVEC decreased in the presence of TNF-α or IL-1β (panels a, c

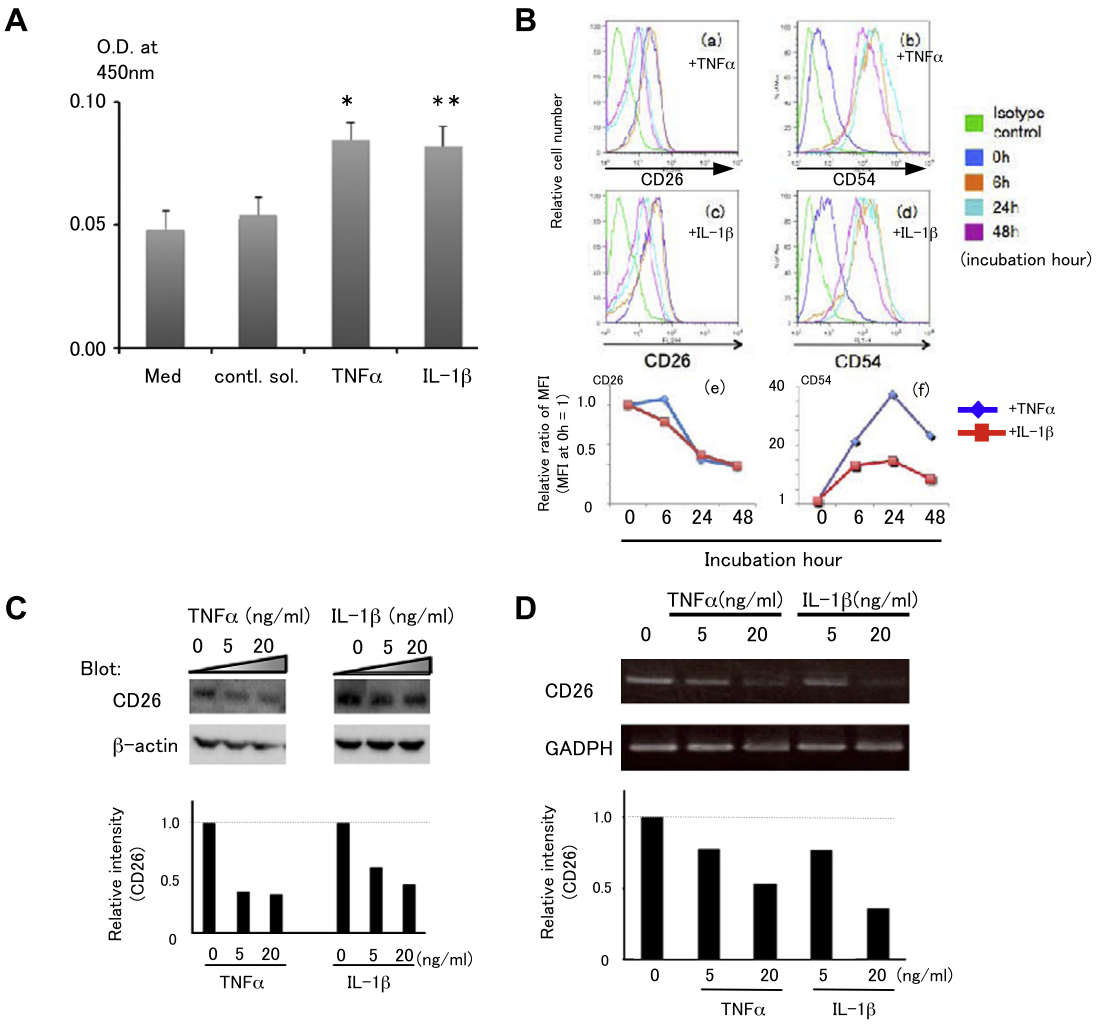

Fig. 1. Expression of CD26 is decreased on human dermal microvascular endothelial cells (HMVEC) with stimulation of TNF-α or IL-1β. (A) Proliferation of HMVEC was assayed by MTT as described in Section 2. TNF-α (*) or IL-1β (**) significantly enhanced cellular proliferation. Med or contl.sol. denotes medium alone or control solvent, respectively. (B) TNF-α or IL-1β changed CD26 (panels a or c) or CD54 (ICAM-1) (panels b or d) expression on HMVEC, assayed by flow cytometry. Panels e (CD26) and f (CD54) show relative changes of mean fluorescence intensity (MFI), compared to MFI value at 0 h. Data are representative of three independent experiments, all of which showed similar results.(C) HMVEC were cultured in the presence of various concentrations of TNF-α or IL-1β (0, 5, 20 ng/ml), in a similar manner as in A. CD26 expression was assayed by Western blot, and membranes were subsequently reprobed with anti-β-actin to determine equality of mounted proteins. Band intensity of CD26 expression (the intensity of sample at 0 ng/ml = 1.0) is indicated in the bottom graphs. (D) Following culturing of HMVEC as described in C, CD26 mRNA levels were assayed by RT–PCR, and band intensity of CD26 (the intensity of sample at 0 ng/ml = 1.0) is indicated in the bottom graphs.

and e), while the EC activation molecule CD54 (ICAM-1) was increased maximally at 24 h after TNF-α or IL-1β treatment (panels b, d and f). Decreased levels of CD26 protein and mRNA were also observed in the presence of TNF-α or IL-1β at a dose-dependent manner (Fig. 1C and D). On the other hand, IL-2, IL-6 or IFN-γ had no effect on EC proliferation nor its CD26 expression (data not shown). These data suggest that downregulation of CD26 may be associated with EC proliferation in the presence of TNF-α or IL-1β.

3.2. CD26 siRNA or DPP-4 inhibition enhances TNF-α or IL-1β-induced EC proliferation

Since our data above suggest that CD26 may negatively regulate EC proliferation induced by proinflammatory cytokines, we next conducted loss-of-function experiments using CD26 siRNA. Genetic suppression of CD26 by siRNA (inbox panels of Fig. 2A) enhanced TNF-α- or IL-1β-induced proliferation of HMVEC (* or ** in Fig. 2A). Since we have shown previously that DPP-4 enzymatic activity has an important role in T-cell costimulation via CD26 [6,22], we next examined whether chemical inhibition of DPP-4 would have an effect on EC proliferation. As shown in Fig. 2B, inhibition of DPP-4 by either diprotinin-A or P32/98 enhanced TNF-α- or IL-1β-induced proliferation of HMVEC (* or **). To confirm these effects of CD26 siRNA or DPP-4 inhibitors on cytokine-induced EC proliferation, we performed scratch-wound assay, with results being assessed by quantifying cell numbers in the denuded area. This reparative process, which involves both migration and proliferation induced by TNF-α or IL-1β, was enhanced by CD26 siRNA or DPP-4 inhibitors (Fig. 2C). These data strongly suggest that inhibition of the DPP-4 enzymatic activity of CD26 as well as suppression of CD26 expression augments EC proliferation induced by TNF-α or IL-1β.

3.3. Loss of CD26/DPP-4 enhances aortic sprouting induced by TNF-α or IL-1β

To examine in more details the effect of CD26/DPP-4 inhibition on proinflammatory cytokine-induced EC proliferation and neovascularization, we performed *ex vivo* and *in vivo* experiments using *CD26*$^{-/-}$ mice. For this purpose, we first conducted *ex vivo* aortic sprouting assay. We confirmed by Western blotting that

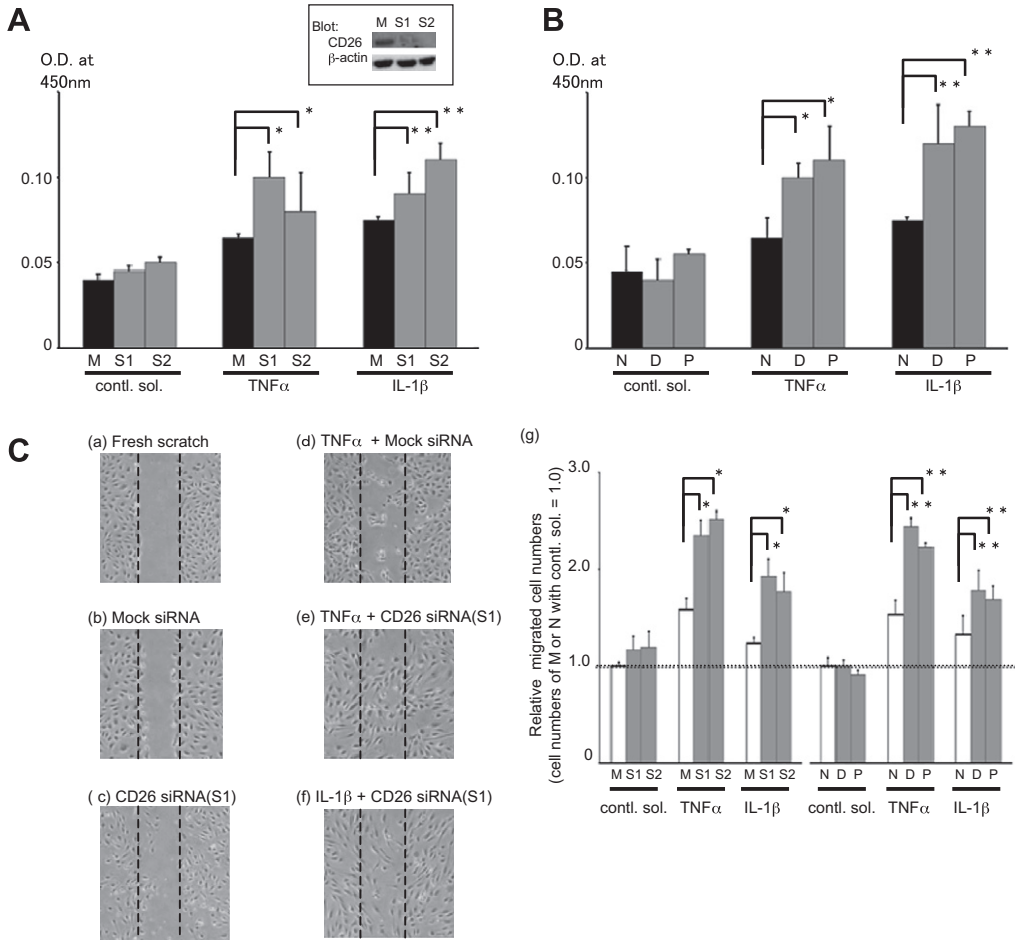

Fig. 2. CD26 siRNA or DPP-4 inhibition enhances HMVEC proliferation stimulated by TNF-α or IL-1β. (A) Proliferation of HMVEC transfected with 20 nM of mock or CD26 siRNAs was assayed by MTT. CD26 expression was clearly decreased following transfection of two independent siRNAs against human CD26 (S1 or S2) (inbox panels of upper right. M denotes mock-siRNA). TNF-α- or IL-1β-induced HMVEC proliferation was significantly increased in the presence of CD26 siRNAs (* or **). (B) HMVEC were cultured in the same manner as in Fig. 1A, in the presence or absence of 10 μM of DPP-4 inhibitors D, diprotinin-A or P, P32/98). TNF-α- or IL-1β-induced HMVEC proliferation was significantly increased in the presence of DPP-4 inhibitors (* or **). N denotes control solvent. (C) Scratch-wound assay using HMVEC was performed in the presence of CD26 siRNA (S1 or S2) or DPP-4 inhibitors (D, diprotinin-A or P, P32/98) as described in Section 2. Injury was performed by scraping the monolayer (denuded area is between dotted lines of panels a–f). After 48-h incubation in the presence or absence of cytokines (20 ng/ml of TNF-α or IL-1β) or control solvent (contl.sol.), cell regrowth from the wound edge was observed by light microscopy. Quantification of cell numbers in the denuded area is demonstrated in the graph in panel g, in which the data are shown as the relative ratio of regrown cell numbers in the denuded area, compared to the number of control culture (M or N in contl.sol.) (*, ** $p < 0.05$). The photographs shown here are representative of three separate experiments for each CD26-targeted agent (siRNAs or DPP-4 inhibitors), all of which had similar results.

the expression of murine CD26 protein was detected in *CD26*$^{+/+}$ EC, and that CD26 was not detected in *CD26*$^{-/-}$ EC (data not shown). TNF-α or IL-1β slightly induced sprouting of *CD26*$^{+/+}$ aortic rings (panels a, d and g of Fig. 3A and white bars in Fig. 3B). In this condition, TNF-α or IL-1β-induced sprouting of *CD26*$^{-/-}$ aortic rings was enhanced with significantly high level, compared to that of *CD26*$^{+/+}$ aortic rings (panels b, e and h of Fig. 3A and * in Fig. 3B). Moreover, addition of DPP-4 inhibitor P32/98 in culture medium for *CD26*$^{+/+}$ aortic sprouting (*CD26*$^{+/+}$ + P32/98) resulted in significantly greater level of TNF-α- or IL-1β-induced sprouting (panels c, f and i of Fig. 3A and ** in Fig. 3B).

3.4. Loss of CD26/DPP-4 enhances capillary formation induced by TNF-α or IL-1β in the in vivo Matrigel model

Finally, we conducted *in vivo* murine Matrigel capillary formation assay to verify the *in vitro* or *ex vivo* results shown above. Subcutaneous Matrigel implantation containing TNF-α or IL-1β in *CD26*$^{+/+}$ mice triggers a neovascularizing response from the host vasculature and the eventual colonization of the plugs (panels a, d and g of Fig. 4A). Blood vessels or ECs were confirmed by immunostaining with anti-CD31 antibody (data not shown). In this system, capillary formation in *CD26*$^{-/-}$ mice was observed to occur at significantly higher levels in the plugs with TNF-α or IL-1β (panels b, e and h of Fig. 4A and * of Fig. 4B). Furthermore, adding DPP-4 inhibitor P32/98 in the Matrigel for *CD26*$^{+/+}$ mice (*CD26*$^{+/+}$ + P32/98) resulted in significantly greater level of TNF-α- or IL-1β-induced capillary formation in the plugs (panels c, f and i of Fig. 4A and ** in Fig. 4B) These results confirm the notion that suppression of CD26/DPP-4 enhances EC proliferation and neovascularization induced by TNF-α or IL-1β.

4. Discussion

ECs are major target of cytokine signals from various immune cells and vascular cells, which can mediate EC dysfunction and vascular inflammation [20]. While we and other investigators have reported that CD26^{+} T lymphocytes play an important role as effector cells in inflammatory lesions [9,23,24], and that expression of CD26 in ECs is changed in hypoxic conditions [25], the association between CD26 expression and EC function in inflammatory conditions remains to be clarified. In this study, we demonstrate that

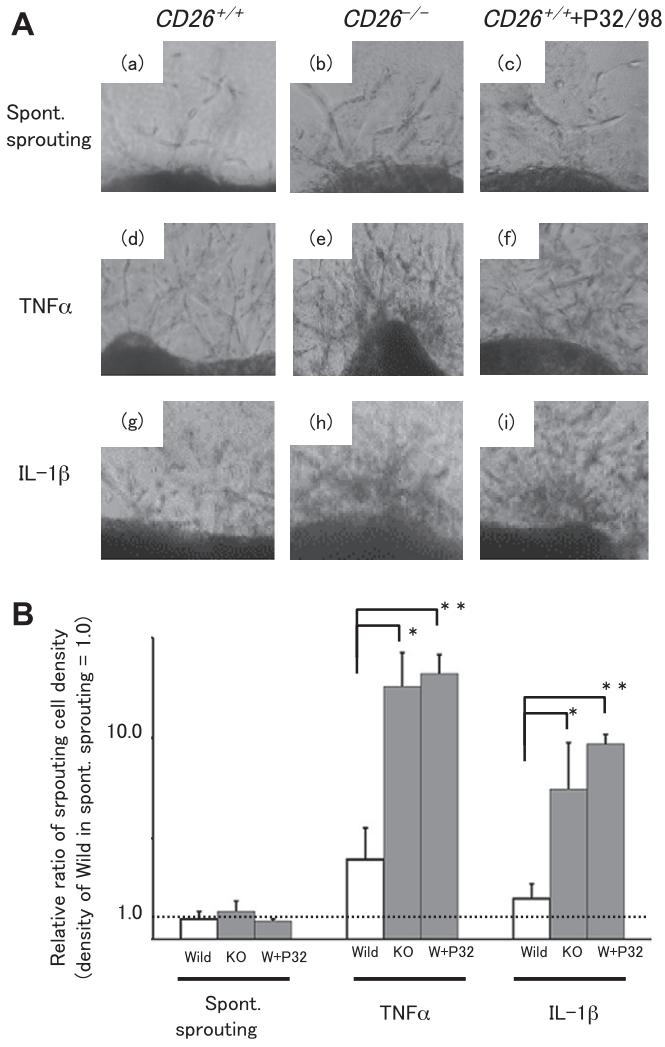

Fig. 3. Genetical elimination of CD26 or pharmacological inhibition of DPP-4 enhances TNF-α- or IL-1β-induced sprouting of aortic rings. (A). Aortic rings were obtained from *CD26+/+* or *CD26*$^{-/-}$ mice, and embedded and cultured in medium containing 20 ng/ml of cytokines (TNF-α or IL-1β) as described in Section 2. For DPP-4 inhibitor experiments, aortic rings were incubated in medium containing 10 μM of P32/98 (*CD26*$^{+/+}$+P32/98). Photographs shown from each mouse are representative of results from three independent experiments for each mouse strain. (B). Quantification of sprouting cell density in graph form was determined as described in Section 2. Data are shown as relative ratio of sprouting cell density, compared to that of spontaneous sprouting in *CD26*$^{+/+}$mice (*, ** $p < 0.05$). Wild and W denote *CD26*$^{+/+}$ aortic sprouting, and KO denotes *CD26*$^{-/-}$ aortic sprouting.

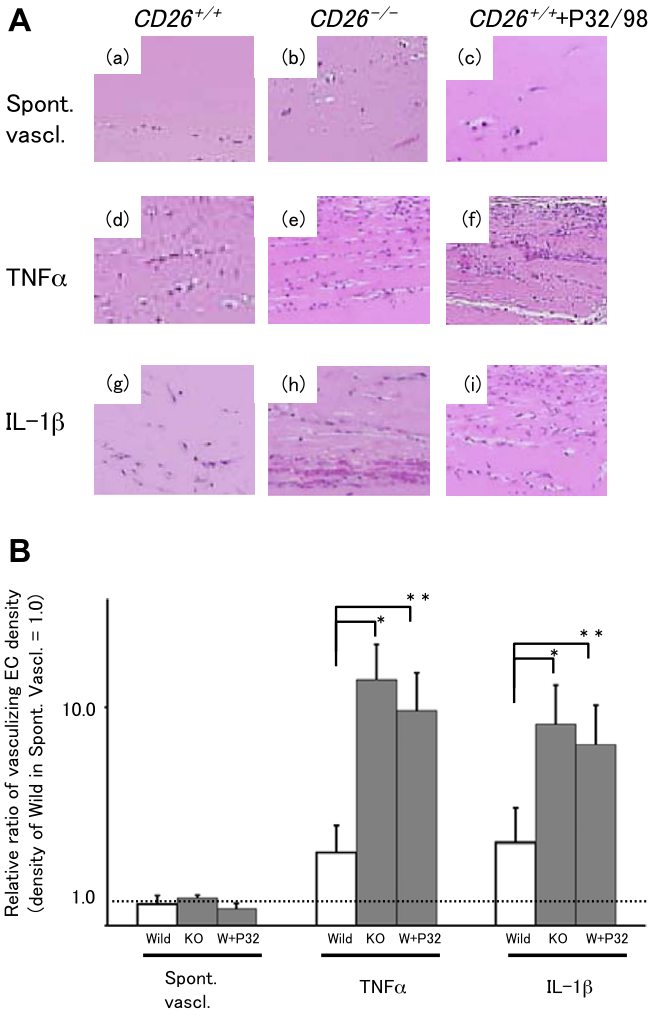

Fig. 4. TNF-α- or IL-1β-induced capillary formation is enhanced by loss of CD26/DPP-4. (A) Spontaneous, TNF-α- or IL-1β-induced capillary in-growth (vascl., vascularization) into subcutaneous Matrigel plugs from *CD26*$^{+/+}$ (panels a, d and g), *CD26*$^{-/-}$ mice (panels b, e and h) was shown by microscopy. Capillary formation from *CD26*$^{+/+}$ mice was performed in the presence of the DPP-4 inhibitor P32/98 in the Matrigel (panels c, f and i). Photographs shown from each mouse are representative of results from three independent experiments for each mouse strain. (B) Quantification of EC in graph form was determined as described in Section 2. Data are shown as relative ratio of EC density, compared to that of spontaneous vascularization in *CD26*$^{+/+}$ mice (*, ** $p < 0.05$). Wild or W denotes *CD26*$^{+/+}$ mice, and KO denotes *CD26*$^{-/-}$ mice.

CD26 expression in HMVEC is genetically suppressed during proliferation induced by TNF-α or IL-1β (Fig. 1), and that siRNAs targeting CD26 or DPP-4 chemical inhibitors enhance TNF-α- or IL-1β-induced regrowth in an *in vitro* model of wounding (Fig. 2). In addition, the effect of CD26 targeting on TNF-α- or IL-1β-induced EC proliferation and neovascularization is further confirmed by aortic sprouting assay (Fig. 3) and Matrigel capillary formation assay (Fig. 4), using *CD26*$^{-/-}$ mice or DPP-4 chemical inhibitors. These data strongly suggest that inhibition of enzymatic activity of CD26/DPP-4 is associated with neovascularization induced by TNF-α or IL-1β stimulation.

Recently, DPP-4 inhibitors have been used worldwide in the clinical setting for the treatment of patients with type 2 diabetes [10]. While well-controlled blood glucose level has been achieved with administration of DPP-4 inhibitors, it needs to be clarified as to whether diabetic vascular complications can be more effectively prevented by targeting disease-specific mechanisms rather than by focusing only on achieving optimal metabolic control and traditional risk factor intervention [12]. The role of inflammation and immunity in the development of diabetic vascular complications is receiving increasing attention, raising hopes that novel targets for prevention and treatment can be identified in the future [13]. Studies by other investigators have suggested the use of pharmacological inhibition of DPP-4 and G-CSF-based stem cell mobilization as a therapeutic concept for treatment following myocardial infarction [26]. They indicated that CD26 depletion promotes post-translational stabilization of active SDF-1α, which is cleaved and inactivated by CD26/DPP-4, and that inhibition of CD26/DPP-4 preserves the cardiac SDF-1-CXCR4 homing axis of endothelial progenitor cells. However, the direct effect of DPP-4 inhibitors on EC has not been demonstrated previously. Our data shown in the present study strongly suggest that inhibition of DPP-4 is directly associated with EC proliferation and migration and neovascularization in inflammatory settings. Therefore, these results suggest that

DPPIV inhibition may be a useful approach for recovery of EC proliferation and local circulation after diabetic vascular damage.

We cannot exclude the possibility that DPP-4-mediated processing of other angiogenic peptides released from ECs in the presence of TNF-α or IL-1β is responsible for the results seen in our studies. For example, some proliferation factors, which are cleaved and inactivated by CD26/DPP-4, are produced from ECs by TNF-α or IL-1β stimulation, and that cleaving the proliferation factors by CD26/DPP-4 results in inactivation, leading to increasing EC proliferation. Recently, other investigators have shown that the proteolytic activity of DPP-4 is essential for the ability of neuropeptide Y (NPY) to promote cell migration and wound closure [27], concluding that DPP-4 enzyme activity is required for NPY-mediated chemotaxis. If NPY is shown to be associated with inflammatory vascularization in diabetes, the effect of DPP-4 inhibition may be tested in such conditions.

In conclusion, we have shown in the present study that proinflammatory cytokines such as TNF-α or IL-1β reduce expression of CD26 in microvascular endothelial cells, and that genetical or pharmacological inhibition of CD26/DPP-4 enhances endothelial growth both *in vitro* and *in vivo*. Since DPP-4 inhibitors are widely used in the treatment of diabetes, our data strongly suggest that this effect of DPP-4 inhibition on endothelial growth may be of potential use in treating diabetic vascular complications, as well as diabetes itself.

Acknowledgments

This work was supported by Grant-in-Aid of The Ministry of Education, Science, Sports (K.O. and C.M.) and Culture, Ministry of Health, Labour, and Welfare, Japan (C.M.), and by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (C.M.).

References

[1] C. Morimoto, S.F. Schlossman, The structure and function of CD26 in the T-cell immune response, Immunol. Rev. 161 (1998) 55–70.
[2] K. Ohnuma, N. Takahashi, T. Yamochi, et al., Role of CD26/dipeptidyl peptidase IV in human T cell activation and function, Front Biosci. 13 (2008) 2299–2310.
[3] N.H. Dang, Y. Torimoto, K. Shimamura, et al., 1F7 (CD26): A marker of thymic maturation involved in the differential regulation of the CD3 and CD2 pathways of human thymocyte activation, J. Immunol. 147 (1991) 2825–2832.
[4] C. Morimoto, Y. Torimoto, G. Levinson, et al., 1F7, a novel cell surface molecule, involved in helper function of CD4 cells, J. Immunol. 143 (1989) 3430–3439.
[5] K. Ohnuma, T. Yamochi, M. Uchiyama, et al., CD26 up-regulates expression of CD86 on antigen-presenting cells by means of caveolin-1, Proc. Natl. Acad. Sci. USA 101 (2004) 14186–14191.
[6] K. Ohnuma, M. Uchiyama, T. Yamochi, et al., Caveolin-1 triggers T-cell activation via CD26 in association with CARMA1, J. Biol. Chem. 282 (2007) 10117–10131.
[7] K. Eguchi, Y. Ueki, C. Shimomura, et al., Increment in the Ta1+ cells in the peripheral blood and thyroid tissue of patients with Graves' disease, J. Immunol. 142 (1989) 4233–4240.
[8] A. Mizokami, K. Eguci, A. Kawakami, et al., Increased population of high fluorescence 1F7 (CD26) antigen on T cells in synovial fluid of patients with rheumatoid arthritis, J. Rheumatol. 23 (1996) 2022–2026.
[9] K. Ohnuma, H. Inoue, M. Uchiyama, T. Yamochi, O. Hosono, N.H. Dang, C. Morimoto, T-cell activation via CD26 and caveolin-1 in rheumatoid synovium, Mod. Rheumatol. 16 (2006) 3–13.
[10] A. Karasik, P. Aschner, H. Katzeff, M.J. Davies, P.P. Stein, Sitagliptin, a DPP-4 inhibitor for the treatment of patients with type 2 diabetes: A review of recent clinical trials, Curr. Med. Res. Opin. 24 (2008) 489–496.
[11] J.A. Beckman, M.A. Creager, P. Libby, Diabetes and atherosclerosis: Epidemiology pathophysiology, and management, JAMA 287 (2002) 2570–2581.
[12] C.M. Clark Jr., D.A. Lee, Prevention and treatment of the complications of diabetes mellitus, N. Engl. J. Med. 332 (1995) 1210–1217.
[13] J. Nilsson, E. Bengtsson, G.N. Fredrikson, H. Bjorkbacka, Inflammation and immunity in diabetic vascular complications, Curr. Opin. Lipidol. 19 (2008) 519–524.
[14] H. Ikushima, Y. Munakata, S. Iwata, K. Ohnuma, S. Kobayashi, N.H. Dang, C. Morimoto, Soluble CD26/dipeptidyl peptidase IV enhances transendothelial migration via its interaction with mannose 6-phosphate/insulin-like growth factor II receptor, Cell Immunol. 215 (2002) 106–110.
[15] B. Jones, S. Adams, G.T. Miller, M.I. Jesson, T. Watanabe, B.P. Wallner, Hematopoietic stimulation by a dipeptidyl peptidase inhibitor reveals a novel regulatory mechanism and therapeutic treatment for blood cell deficiencies, Blood 102 (2003) 1641–1648.
[16] T. Ishii, K. Ohnuma, A. Murakami, et al., CD26-mediated signaling for T cell activation occurs in lipid rafts through its association with CD45RO, Proc. Natl. Acad. Sci. USA 98 (2001) 12138–12143.
[17] T. Inamoto, T. Yamochi, K. Ohnuma, et al., Anti-CD26 monoclonal antibody-mediated G1-S arrest of human renal clear cell carcinoma Caki-2 is associated with retinoblastoma substrate dephosphorylation, cyclin-dependent kinase 2 reduction, $p27^{kip1}$ enhancement, and disruption of binding to the extracellular matrix, Clin. Cancer Res. 12 (2006) 3470–3477.
[18] R.F. Nicosia, A. Ottinetti, Growth of microvessels in serum-free matrix culture of rat aorta. A quantitative assay of angiogenesis in vitro, Lab Invest. 63 (1990) 115–122.
[19] E.W. Lee, D.S. Grant, S. Movafagh, Z. Zukowska, Impaired angiogenesis in neuropeptide Y-Y2 receptor knockout mice, Peptides 24 (2003) 99–106.
[20] A.H. Sprague, R.A. Khalil, Inflammatory cytokines in vascular dysfunction and vascular disease, Biochem. Pharmacol. 78 (2009) 539–552.
[21] N.H. Dang, D.A. Hafler, S.F. Schlossman, J.B. Breitmeyer, FcR-mediated crosslinking of Ta1 (CDw26) induces human T lymphocyte activation, Cell Immunol. 125 (1990) 42–57.
[22] T. Tanaka, J. Kameoka, A. Yaron, S.F. Schlossman, C. Morimoto, The costimulatory activity of the CD26 antigen requires dipeptidyl peptidase IV enzymatic activity, Proc. Natl. Acad. Sci. USA 90 (1993) 4586–4590.
[23] I. De Meester, S. Korom, J. Van Damme, S. Scharpe, CD26, let it cut or cut it down, Immunol. Today 20 (1999) 367–375.
[24] B. Fleischer, CD26: A surface protease involved in T-cell activation, Immunol. Today 15 (1994) 180–184.
[25] D.T. Dang, S.Y. Chun, K. Burkitt, et al., Hypoxia-inducible factor-1 target genes as indicators of tumor vessel response to vascular endothelial growth factor inhibition, Cancer Res. 68 (2008) 1872–1880.
[26] M.M. Zaruba, H.D. Theiss, M. Vallaster, et al., Synergy between CD26/DPP-IV inhibition and G-CSF improves cardiac function after acute myocardial infarction, Cell Stem Cell 4 (2009) 313–323.
[27] G. Ghersi, W. Chen, E.W. Lee, Z. Zukowska, Critical role of dipeptidyl peptidase IV in neuropeptide Y-mediated endothelial cell migration in response to wounding, Peptides 22 (2001) 453–458.