Biochemical and Biophysical Research Communications 421 (2012) 305–311

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Nuclear IL-33 is a transcriptional regulator of NF-κB p65 and induces endothelial cell activation

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ARTICLE INFO

Article history:
Received 23 March 2012
Available online 7 April 2012

Keywords:
Cell adhesion molecules
High endothelial venules
Interleukin-33
NF-HEV
Inflammation
NF-κB p65

ABSTRACT

Interleukin (IL)-33, an IL-1 family member, acts as an extracellular cytokine by binding its cognate receptor, ST2. IL-33 is also a chromatin-binding transcriptional regulator highly expressed in the nuclei of endothelial cells. However, the function of IL-33 as a nuclear factor is poorly defined. Here, we show that IL-33 is a novel transcriptional regulator of the p65 subunit of the NF-κB complex and is involved in endothelial cell activation. Quantitative reverse transcriptase PCR and Western blot analyses indicated that IL-33 mediates the expression of intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 in endothelial cells basally and in response to tumor necrosis factor-α-treatment. IL-33-induced ICAM-1/VCAM-1 expression was dependent on the regulatory effect of IL-33 on the nuclear factor (NF)-κB pathway; NF-κB p65 expression was enhanced by IL-33 overexpression and, conversely, reduced by IL-33 knockdown. Moreover, NF-κB p65 promoter activity and chromatin immunoprecipitation analysis revealed that IL-33 binds to the p65 promoter region in the nucleus. Our data provide the first evidence that IL-33 in the nucleus of endothelial cells participates in inflammatory reactions as a transcriptional regulator of NF-κB p65.

1. Introduction

Inflammation is a typical response to infectious agents or tissue injury that involves activation of vascular endothelial cells and local recruitment of circulating leukocytes from the blood to the inflamed site [1]. Endothelial cell activation induces the expression of cell adhesion molecules, such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, that assist in the recruitment of leukocytes during the inflammatory process. Binding of interleukin-1 (IL-1) family members to their receptors leads to the induction of inflammatory genes, such as cytokines, chemokines, and cell adhesion molecules, via nuclear factor-κB (NF-κB) and the modulation of inflammatory responses [2,3].

IL-33, also known as the nuclear factor from high endothelial venules (NF-HEVs), is a member of the IL-1 cytokine family that includes IL-1 and IL-18. IL-33 is expressed in fibroblasts, epithelium, and endothelium [4,5]. IL-33 is synthesized as an approximately 30-kDa precursor and in response to tissue injury, the full length form of IL-33 is thought to be released as a cytokine [6]. Cytokine IL-33 binds to a heterodimer of ST2 receptor and IL-1R accessory protein (IL-1RAcP) on inflammatory cells, such as mast cells, T lymphocytes, eosinophils, basophils, and neutrophils and modulates inflammatory diseases like sepsis, asthma, anaphylaxis, and arthritis [7].

The 30-kDa precursor form of IL-33, like IL-1α and high motility group box 1 (HMGB1), likely acts as an intracellular protein that exerts biological activities in the nucleus as IL-33 is constitutively expressed in the nucleus even in the absence of proinflammatory stimuli [8]. IL-33 is expressed in the nuclei of MECA 79-positive HEVs in tonsils, Peyer's patches, and lymph nodes as well as in inflamed tissues in inflammatory conditions, such as Crohn's disease, rheumatoid arthritis, and atherosclerosis [4,9]. The IL-33N-terminal homeodomain-like helix-turn-helix motif is responsible for nuclear targeting and chromatin binding. This short chromatin-binding motif (CBM) of IL-33 associates with the acidic pocket formed by H2A and H2B [10]. Despite the fact that IL-33 acts as a nuclear factor and a cytokine, neither the transcriptional targets nor the pathophysiological role of nuclear IL-33 in endothelial cells have been examined. Our results demonstrate a nuclear role for IL-33 in the transcriptional regulation of NF-κB p65 through which

Abbreviations: CBM, chromatin-binding motif; ChIP, chromatin immunoprecipitation; HEV, high endothelial venule ; HMGB1, high-mobility group protein B1; HUVECs, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; NF-κB, nuclear factor-κB; qRT-PCR, quantitative reverse transcription-PCR; siRNA, small interfering RNA; VCAM-1, vascular cell adhesion molecule.

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0006-291X/
http://dx.doi.org/10.1016/j.bbrc.2012.04.005

IL-33 induces the expression of cell adhesion molecules (ICAM-1 and VCAM-1). Nuclear IL-33 exerts its biological effect in a receptor-independent manner.

2. Materials and methods

2.1. Cell culture

Human umbilical vein endothelial cells (HUVECs) were isolated from human umbilical cord veins by collagenase treatment and used at passages 2–7. Cells were grown in M199 medium (Invitrogen, Carlsbad, CA) supplemented with 20% fetal bovine serum, 1% penicillin/streptomycin, 3 ng/ml basic fibroblast growth factor (R&D Systems Minneapolis, MN), and 5 U/ml heparin (Sigma, St. Louis, MO) at 37 °C in a humidified 95–5% (v/v) mixture of air and CO_2.

2.2. Quantitative real-time reverse transcription PCR (qRT-PCR)

Real-time PCR was performed with SYBR Green (Invitrogen) in a Bio-Rad real-time PCR detection system. The primers used for qRT-PCR were as follows: IL-33, 5′-CCATTACTTTTGCTTTGGAGGA-3′ and 5′-CCATTACTTTTGCTTTGGAGGA-3′; ICAM-1, 5′-GAAGTGGTGGGGGAGACATA-3′ and 5′-CAAGGGTTGGGGTCAGTAGA-3′; VCAM-1, 5′-AAAAGCGGAGACAGGAGACA-3′ and 5′-GCAAAATAGAGCACGAGAAGC-3′; p65, 5′-ACTGTTCCCCCTCATCTTCC-3′ and 5′-TGGTCCTGTGTAGCCATTGA-3′; ST2, 5′-AAGGAGTTTGCCTACGAGCA-3′ and 5′-CCACTTGATGGTCCCCTGTA-3′; GAPDH, 5′-ACCCAGAAGACTGTGGATGG-3′ and 5′-TCTAGACGGCAGGTCAGGTC-3′.

2.3. Transient transfection and luciferase assays

Total RNA was isolated from HUVECs and cDNA was synthesized using M-MLV reverse transcriptase (Promega, Madison, WI). IL-33 cDNA (817 bp) was amplified from HUVEC cDNA and subcloned into pFLAG-CMV2. ICAM-1 (−1350 bp), VCAM-1 (−1716 bp), and 4x-κB luciferase reporter constructs were used as previously described [11]. The NF-κB p65 promoter was amplified from a BAC clone (RP11–856B14) and subcloned into pGL3-Basic (Promega). HUVECs were transfected with expression plasmid, reporter construct, and pRL-CMV for normalization using Lipofectamine as per the manufacturer's instructions (Invitrogen). After 24 h, HUVECs were lysed with passive lysis buffer and luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega).

2.4. Transfection with small interfering (si)RNA

HUVECs were transfected with control siRNA and IL-33 siRNA (Dharmacon, Lafayette, CO) using Lipofectamine for 3 h. Cells were assayed 48 h after transfection. For IL-33, siRNAs were used: 5′-GCACAUACAAUGAUCAAUC-3′. To silence ST2 expression, a ST2 siRNA sequence (Dharmacon; 5′-CGAAAGAGCAGGCGGCACAUU-3′) was used.

2.5. Monocyte adhesion assays

HUVECs were transfected with siRNA in 60-mm plates. After 24 h, HUVECs were re-plated in 96-well plates at 2×10^4 cells/well overnight. THP-1 cells were labeled with 5 μM calcein-AM (Sigma) and incubated at 37 °C for 30 min. Calcein-AM-labeled cells were co-cultured with confluent endothelial cells for 1 h. The cells were washed with PBS to remove unbound cells. Fluorescence intensities were measured with a FLUOstar Omega (BGM LABTECH, Offenburg, Germany) with excitation and emission wavelengths set to 494 and 517 nm, respectively.

2.6. Western blot analysis

HUVECs were washed with cold PBS, harvested in buffer (0.1% NP-40, 10 nM NaCl, 5 mM $MgCl_2$, 10 nM NaH_2PO_4 [pH 7.4], 65 mM sodium orthovanadate), and centrifuged at 1200*g* for 15 min. After centrifugation, nuclei were pelleted and suspended in nuclear buffer (1 mM EDTA, 3.5% SDS, 10% glycerol, and 70 mM Tris–Cl), as described previously [8]. The proteins were separated by SDS–PAGE. Immunoblotting was performed with antibodies to IL-33 (R&D Systems), ICAM-1, VCAM-1, p65, β-actin (Santa Cruz Biotechnology, Santa Cruz, CA), and histone H3 (Millipore, Billerica, MA).

2.7. Chromatin immunoprecipitation (ChIP) assay

ChIP was performed using a ChIP assay kit (Millipore) according to the manufacturer's instructions. HUVECs were transfected with a FLAG-tagged IL-33 expression vector for 48 h. FLAG-specific monoclonal antibodies (Sigma) or mouse control IgG (Santa Cruz Biotechnology) were added to precleared chromatin and incubated overnight at 4 °C. The DNA was amplified with primers specific to the p65 promoter (5′-GCACTGTGGGGTCACATGACAGAA-3′ and 5′-AGGGCTCGTCCCTCTCCAGCTAAA-3′) or with negative control primers (5′-GATTGAAGCCCTCCAAAAGC-3′ and 5′-ACCATCAGGACAGGGGAA AA-3′).

2.8. Immunocytochemistry

HUVECs were fixed in 3.7% formaldehyde for 10 min and permeabilized with 0.1% Triton X-100 in PBS. Cells were blocked with 1% BSA (Millipore) for 1 h at room temperature. The cells were washed with PBS, labeled with anti-p65 antibody (Santa Cruz Biotechnology) for 2 h at room temperature, rinsed in PBS, and incubated with AlexaFluor-488 for 60 min at room temperature. Samples were examined with a fluorescence microscope (Zeiss, 400×).

2.9. Statistical analysis

Data were analyzed with Student's *t*-tests and expressed as means ± standard deviation (SD). All experiments were performed at least three times and representative results are shown.

3. Results

3.1. IL-33 regulates ICAM-1 and VCAM-1 expression in resting endothelial cells

Cell adhesion molecules, including ICAM-1 and VCAM-1, control cell integrity under physiological condition and regulate inflammation under pathological conditions [12]. Endothelial cells of HEVs in healthy and inflamed tissues express cell adhesion molecules, such as ICAM-1 and VCAM-1, as well as IL-33 [13]. We examined the interplay between IL-33 and specific cell adhesion molecules. Exposure of HUVECs to IL-33-targeting siRNA decreased the mRNA and protein levels of ICAM-1 and VCAM-1 according to qRT-PCR (Fig. 1A) and Western blot analyses (Fig. 1B), respectively. Conversely, ectopic expression of IL-33 in HUVECs increased ICAM-1 and VCAM-1 at both the mRNA and protein levels (Fig. 1C and D). In addition, ICAM-1 and VCAM-1 promoter activities were reduced by exposure of HUVECs to IL-33 siRNA and elevated in response to IL-33 (Fig. 1E and F).

IL-33 as a cytokine upregulates ICAM-1 and VCAM-1 via interactions with ST2 receptor in ST2-positive cells [14]. To exclude the possibility that the upregulation of ICAM-1/VCAM-1 in our

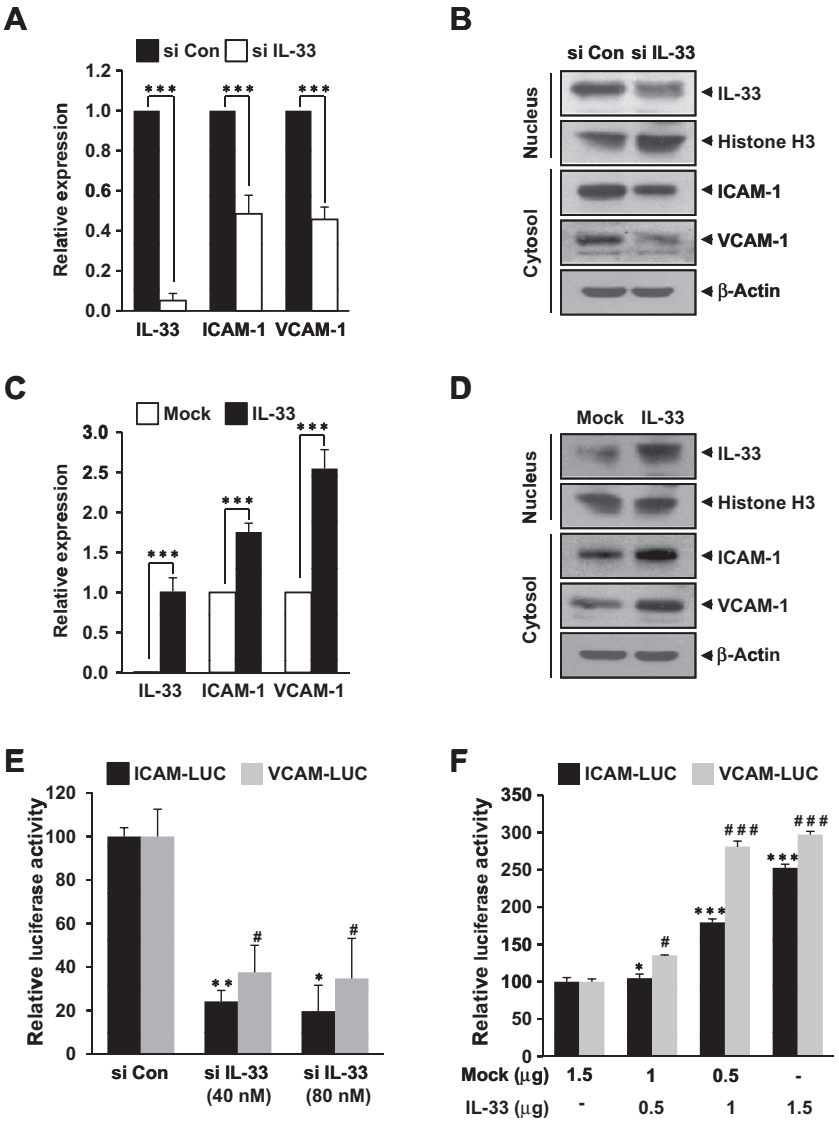

Fig. 1. IL-33 regulates ICAM-1 and VCAM-1 expression in HUVECs. (A and B) HUVECs were transfected with control siRNA or IL-33 siRNA for 48 h. (C and D) HUVECs were transfected with mock or IL-33 constructs for 48 h. (A and C) RNA levels of ICAM-1 and VCAM-1 were quantified by qRT-PCR and normalized to GAPDH levels. (B and D) Nuclear and cytoplasmic extracts were prepared and protein expression was assessed by Western blot analysis with the indicated antibodies. $^{***}P < 0.001$ versus control cells. (E) HUVECs were transfected with control siRNA or IL-33 siRNA for 24 h, followed by transfection with ICAM-1 or VCAM-1 reporter constructs for 24 h. (F) HUVECs were co-transfected with two reporter constructs and either mock or IL-33 expression plasmids for 24 h. Firefly luciferase activity from ICAM-1 or VCAM-1 reporter constructs was measured. Transfection efficiency was normalized to *Renilla* luciferase activity from co-transfected pRL-CMV. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus control vector for ICAM-1 promoter activity. $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ versus control vector for VCAM-1 promoter activity.

experiments was due to extracellular rather than intracellular IL-33, we knocked down ST2 receptor expression using siRNA prior to IL-33 overexpression (Fig. 2A). ICAM-1 and VCAM-1 were still upregulated in ST2-silenced HUVECs (Fig. 2B and C). Thus, regulation of ICAM-1 and VCAM-1 by IL-33 is due to its intracellular functions rather than a receptor-mediated effect. These data demonstrate that nuclear-localized IL-33 mediated ICAM-1 and VCAM-1 expression in resting endothelial cells.

3.2. IL-33 regulates ICAM-1 and VCAM-1 expression in TNF-α-activated endothelial cells

Inflammatory mediators, including TNF-α and LPS, induce expression of cell adhesion molecules and modulate inflammatory reactions [2]. Given that IL-33 acts in a first wave of the inflammatory response, nuclear IL-33 in endothelial cells may respond to inflammatory mediators to increase endothelial VCAM-1/ICAM-1 expression. Knockdown of IL-33 by siRNA attenuated TNF-α-induced ICAM-1 and VCAM-1 expression at both the mRNA and protein levels (Fig. 3A and B). Moreover, the VCAM-1/ICAM-1 promoter activity induced by TNF-α was attenuated in IL-33 siRNA-treated HUVECs compared with control cells (Fig. 3C and D). These data demonstrate that, in HUVECs, nuclear IL-33 can regulate inflammatory mediator-induced ICAM-1 and VCAM-1 expression as well as their basal expression levels.

3.3. Silencing IL-33 in endothelial cells inhibits monocyte adhesion

During inflammation, LFA1/VLA1-expressing leukocytes are recruited to the inflamed tissue through interactions with ICAM-1/VCAM-1-expressing endothelial cells [15,16]. To confirm the biological function of IL-33-mediated ICAM-1/VCAM-1 expression in HUVECs, we investigated the extent of THP-1 monocyte adhesion on IL-33-silenced HUVECs. Knocking down IL-33 in both resting

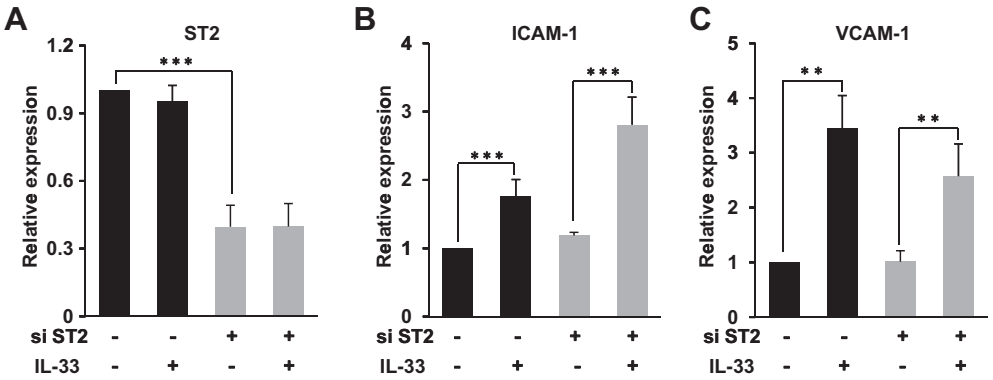

Fig. 2. IL-33 increases ICAM-1 and VCAM-1 expression in an ST2 receptor-independent manner. (A–C) HUVECs were transfected with control siRNA or ST2 siRNA for 24 h and then endothelial cells were transfected with IL-33 expression vector or a control vector. After an additional 40 h, mRNA levels were quantified by qRT-PCR. $^{**}P < 0.01$ and $^{***}P < 0.001$ versus control cells.

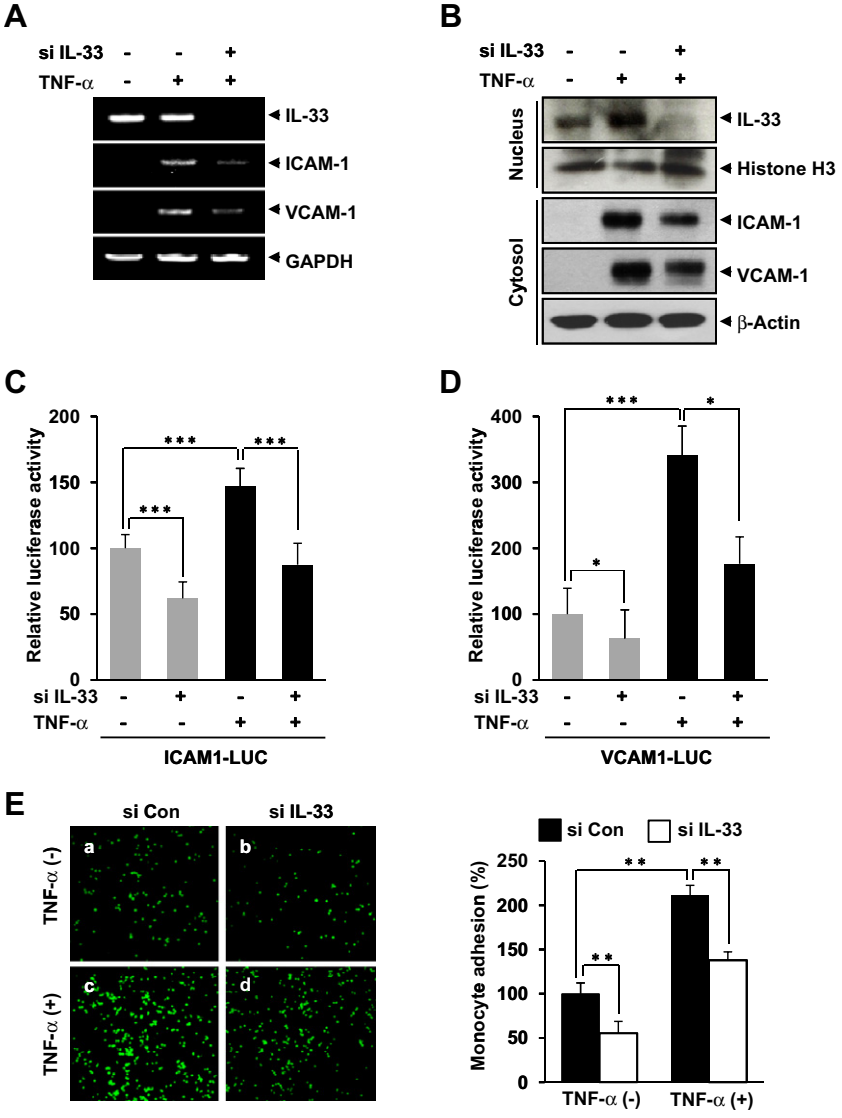

Fig. 3. Silencing of IL-33 decreases ICAM-1 and VCAM-1 expression in TNF-α-activated endothelial cells. (A and B) Endothelial cells were transfected with siRNA for 48 h and then treated with TNF-α (10 ng/ml) for 4 h. The mRNA levels (A) and protein expression levels (B) were measured. (C and D) HUVECs were transfected with control or IL-33 siRNAs for 24 h, followed by transfection with ICAM-1 or VCAM-1 reporter constructs for 24 h. (E) HUVECs were transfected with control or 80 nM IL-33 siRNAs for 24 h. Monolayer endothelial cells were treated with TNF-α (10 ng/ml) for 4 h and co-cultured with calcein AM-labeled monocytes for 1 h. The fluorescence intensities for attached monocytes were quantified with a fluorometer. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus control siRNA untreated and TNF-α-treated cells.

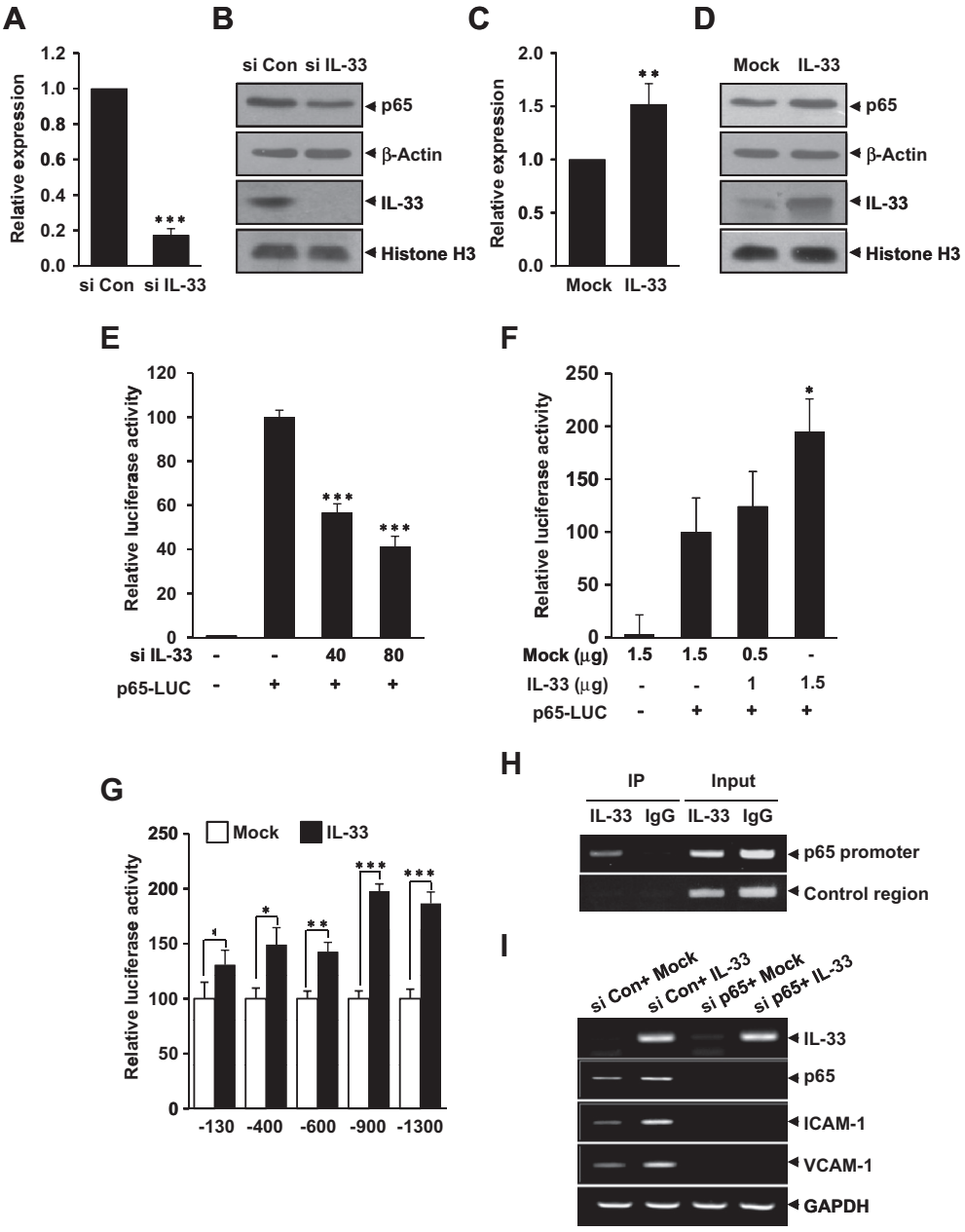

Fig. 4. IL-33 upregulates NF-κB p65 expression in endothelial cells. (A and B) HUVECs were transfected with control or IL-33 siRNA for 48 h. (C and D) HUVECs were transfected with mock or IL-33 constructs for 48 h. The mRNA levels (A and C) and total protein levels (B and D) of p65 were measured. (E and F) Luciferase activity regulated by the full p65 reporter construct was measured. (E) Deletion reporter constructs of the p65 promoter were co-transfected with or without the IL-33 expression vector. Relative normalized luciferase activities are presented. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus control cells. (D) After expression of the FLAG-IL-33 vector, a ChIP assay was performed using anti-FLAG antibodies or normal mouse IgG. Genomic DNA was amplified by PCR using primers spanning the p65 promoter region from −900 to −600 bp. For the negative control region, genomic DNA was amplified using primers specific to the p65 3′ coding sequence region. (E) HUVECs were transfected with control siRNA or p65 siRNA for 24 h, followed by transfection with either mock or IL-33 constructs for 24 h. The mRNA levels were measured.

and TNF-α-stimulated HUVECs resulted in an approximately 40% decrease in the ability of THP-1 monocytes to adhere compared with control siRNA-treated endothelial cells (Fig. 3E). These data suggest that cell adhesion molecule expression induced by IL-33 controls monocyte adhesion in resting and TNF-α-activated HUVECs.

3.4. IL-33 modulates the NF-κB pathway in endothelial cells

NF-κB-dependent pathways contribute to cell adhesion molecule expression to regulate inflammatory responses [2]. The response elements of NF-κB are localized in the proximal promoter regions of cell adhesion molecule genes, including those of ICAM-1 and VCAM-1 [11]. Therefore, to explore whether IL-33-mediated ICAM-1/VCAM-1 promoter activity is dependent on the NF-κB pathway, we examined the effect of IL-33 on the NF-κB response elements by measuring luciferase activity of a 4x-κB reporter gene. Knocking down IL-33 attenuated (Supplementary Fig. 1A), and overexpressing IL-33 potentiated (Supplementary Fig. 1B), the luciferase activity of the 4x-κB reporter gene in HUVECs.

NF-κB is an inducible factor that responds to inflammatory mediators. The NF-κB pathway is regulated by several mechanisms in both the cytoplasm and the nucleus, such as nuclear translocation, DNA binding activity, post-translational modification, and

proteasomal degradation [17]. Therefore, to determine the key steps in the regulation of NF-κB activity, we investigated changes in the expression of the p65 subunit following IL-33 knockdown in HUVECs. Interestingly, endogenous p65 expression was significantly attenuated in IL-33-silenced HUVECs (Supplementary Fig. 1C). These results suggest that IL-33 may modulate the NF-κB pathway to induce ICAM-1 and VCAM-1 expression.

3.5. IL-33 upregulates NF-κB p65 expression in endothelial cells

NF-κB p65 levels were reduced following IL-33 knockdown (Fig. 4A and B) and elevated following IL-33 overexpression (Fig. 4C and D) as determined by qRT-PCR and Western blot analyses. These findings indicated that IL-33 upregulates NF-κB p65 expression to potentiate the expression of cell adhesion molecules, such as ICAM-1 and VCAM-1.

To determine whether IL-33 transcriptionally regulates p65, we performed a p65 promoter reporter assay. The promoter activity of p65 was downregulated by IL-33 siRNA treatment (Fig. 4E) and dose-dependently upregulated by IL-33 overexpression in HUVECs (Fig. 4F). To identify more specifically the region of the p65 promoter that is responsible for the IL-33 effect, we constructed several mutant forms of the p65 promoter. The region between −600 and −900 bp is important for IL-33-mediated induction of p65 (Fig. 4G). Therefore, we performed a ChIP assay to determine whether IL-33 interacts with the p65 promoter. DNA was amplified with primers spanning from −900 to −600 bp of the p65 promoter, but no PCR products were produced with primers corresponding to the 3′ region of the p65 locus (Fig. 4H), suggesting that nuclear IL-33 most likely induces p65 transcription by binding to the p65 promoter. Overexpression of IL-33 in p65-depleted HUVECs did not increase ICAM-1/VCAM-1 mRNA levels as measured by RT-PCR (Fig. 4I). Overall, IL-33 acts as a p65 transcriptional regulator to control ICAM-1/VCAM-1 expression.

4. Discussion

Here, we describe the role of IL-33 as a nuclear factor in regulating tissue inflammatory properties. Nuclear IL-33 increases NF-κB p65 mRNA expression by binding to the NF-κB p65 promoter in endothelial cells. This leads to the expression of ICAM-1 and VCAM-1 that increase monocyte adhesion in basal and TNF-α-stimulated conditions.

Under normal conditions, IL-33 is expressed in the nucleus of HEV endothelial cells [4] that have morphologies distinct from those cells of normal vessels and express specific cell adhesion molecules (ICAM, VCAM) that promote the recruitment of lymphocytes [18]. IL-33 and cell adhesion molecules are also found in the vessel of inflammatory diseases, such as atherosclerosis and rheumatoid arthritis [9,19]. Thus, we expected a relationship between the expression of IL-33 and that of cell adhesion molecules. Interestingly, we found that knockdown or ectopic expression of IL-33 in endothelial cells regulated ICAM-1 and VCAM-1 expression. In addition, TNF-α-activated endothelial cells showed IL-33-dependent ICAM-1 and VCAM-1 induction and enhanced monocyte adherence. According to numerous reports, IL-33 functions as a cytokine released from damaged cells under necrotic conditions rather than secreted normally through a ER-Golgi-dependent pathway [6]. The released IL-33 binds to ST2 receptor and is involved in activating inflammatory reactions. For example, IL-33, as a cytokine, induces the expression of ICAM-1, VCAM-1, E-selectin, and MCP-1 through the NF-κB pathway in human carotid atherosclerotic plaques [14]. Contrary to this, our data revealed that ectopic IL-33 expression increased ICAM-1 and VCAM-1 expression even after ST2 had been silenced by siRNA treatment, implying IL-33 as an intracellular factor under these conditions, not as a cytokine.

Previous reports suggested that nuclear IL-33 has properties of transcription repressor and the N-terminal region of the IL-33 protein has a chromatin binding motif (CBM) structure, resembling the chromatin binding motif in Kaposi sarcoma herpesvirus (KSHV) LANA (latency-associated nuclear antigen). LANA and IL-33 attach to heterochromatin and mitotic chromatin through the CBM [10]. Because KSHV LANA attenuates p53 promoter activity and promotes CDK2 promoter activity [20], IL-33 may also directly bind to target chromatin via its N-terminal chromatin binding motif or through a protein–protein interaction with other transcription factors through its C-terminal region. Accordingly, depending on the gene and cell type-specific promoters, IL-33 could act as a transcriptional activator or repressor.

Because the NF-κB transcription factor binding site is highly conserved in the promoter regions of ICAM-1 and VCAM-1, we hypothesized that IL-33 acts as a nuclear regulator of the NF-κB pathway, [11]. In support of this idea, IL-33 shares very similar biological characteristics with IL-1α and HMGB1 that possess dual functions as cytokines and nuclear factors. Full-length IL-1α in the absence of IL-1 receptor binding activates luciferase activity of NF-κB and AP1 and expression of the NF-κB-dependent cytokines IL-6 and IL-8 [21]. HMGB1 also enhances the DNA binding efficiency of p65/p50 to increases expression of the NF-κB target gene, VCAM-1 [22]. As with those factors, IL-33 as a nuclear factor may be involved in the modulation of the NF-κB pathway. NF-κB/Rel family (RelA, RelB, c-Rel, p100/p52, and p105/p50) are constitutively expressed in endothelial cells [23]. Their expression is autoregulated by the NF-κB pathway, except for p65 (RelA) as the promoter region of p65 contains no NF-κB response element [24]. Currently, transcriptional regulation of p65 has not been well studied. The Sp1 transcription factor is solely known as a regulator of p65 expression. For instance, MDM2 directly binds to the Sp1 site which is in the proximal region of the p65 coding sequence to induce p65 transcription [25]. In addition, human cytomegalovirus infection leads to induction of Sp1 both at the mRNA and protein levels and Sp1 protein induces p65 transcription [26]. However, nuclear IL-33 was unlikely to affect mRNA expression (data not shown). We found that IL-33 markedly upregulated p65 mRNA, we further examined p65 promoter activity and IL-33 promoter binding. We concluded that IL-33 binds to the p65 promoter to ultimately upregulate cell adhesion molecules in endothelial cells, even though it remains to be determined whether IL-33 is a transcription factor or a cofactor.

Overall, the data suggest that endogenous IL-33 as a nuclear factor regulates not only basal levels of cell adhesion molecules in normal endothelial cells, but also promotes cell adhesion molecule expression to recruit leukocytes in cytokine-exposed endothelial cells. We provide evidence that nuclear IL-33 upregulates NF-κB p65 mRNA and subsequently promotes the expression of cell adhesion molecules. Therefore, we demonstrate that nuclear IL-33 is likely to contribute to endothelial cell activation and is required for maintaining endothelial homeostasis through regulating the inflammatory response.

Acknowledgment

This study was supported by Grants from the Korea Health 21 R&D Project, Ministry of Health Welfare & Family Affairs, Republic of Korea (A085136), the National Research Foundation of Korea (NRF) funded by the Korean government (MEST, 2011-0020403), and the Biomedical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST, 2011-0019267).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.04.005.

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