藥學博士學位論文

免役細胞에서 AIMP1에 의해 誘導되는 TNF 生産 關聯 受容體 糾明 및 信號傳達經路에 관한 硏究

Studies on the identification of a functional receptor and its signal transduction mechanism involving AIMP1-induced TNF production in immune cells

2012年 8月

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ABSTRACT

ARS-interacting multifunctional protein 1 (AIMP1/p43) can be secreted to trigger proinflammatory molecules while it is predominantly bound to a cytoplasmic macromolecular protein complex that contains several different aminoacyl-tRNA synthetases. Although its activities as a secreted signaling factor have been well-characterized, the functional receptor for its proinflammatory activity has not yet identified. In this study, I have identified the receptor molecule for AIMP1 that mediates the secretion of TNF-α from THP-1 monocytic cells and primary human peripheral blood mononuclear cells (PBMCs). In a screen of 499 soluble receptors, I identified CD23, a known low-affinity receptor for IgE, as a high affinity binding partner of AIMP1. I found that down-regulation of CD23 attenuated AIMP1-induced TNF-α secretion and AIMP1 binding to THP-1 and PBMCs. I also observed that in THP-1 and PBMCs, AIMP1-induced TNF-α secretion mediated by CD23 involved activation of ERK1/2. Interestingly, endothelial monocyte activating polypeptide II (EMAP II), the C-terminal fragment of AIMP1 that is also known to work as a proinflammatory cytokine, was incapable of binding to CD23 and of activating

ERK1/2. Therefore, identification of CD23 not only explains the inflammatory function of AIMP1 but also provides the first evidence by which the mode of action of AIMP1 can be distinguished from that of its C-terminal domain, EMAP II.

Keywords: AIMP1, TNF-α, CD23, EMAP II, monocyte, cytokine

Student ID: 2007-30468

CONTENTS

LIST OF ABBREVIATIONS

ADAM10, a disintegrin and metalloproteinase domain-containing protein 10

AIMP, aminoacyl-tRNA synthetase-interating multi-functional protein

ARS, arginyl-tRNA synthetase

CLEC10A, C-type lectin domain family 10 member A

CXCR3, chemokine (C-X-C motif) receptor 3

EMAP, endothelial monocyte activating polypeptide

ELISA, enzyme-linked immunosorbant assay

ERK, extracellular signal-regulating kinase

FACS, fluorescence-activated cell sorter

HSP, heat-shock protein

IL-4, interleukin-4

IL20Rb, interleukin 20 receptor beta

JNK, jun-N-terminal kinase

MAPK, mitogen-activated protein kinase

NF-κB, nuclear factor kappa B

PBMC, peripheral blood mononuclear cell

PKB, protein kinase B, AKT

PKCγ, protein kinase C gamma

PLAC9, placenta specific protein 9

RT-PCR, reverse transcription-polymerase chain reaction

sFRP1, secreted frizzled-related protein 1

TGF-β, transforming growth factor

TNF-α, tumor necrosis factor alpha

TNFSF13, tumor necrosis factor ligand superfamily member 13

VEGFR1/2, vascular endothelial growth factor receptor 1/2

LIST OF FIGURES

INTRODUCTION

AIMP1 (also known as p43), was identified as one of three auxiliary factors of the mammalian aminoacyl tRNA synthetase (ARS) complex [1]. AIMP1 binds and facilitates the catalytic reaction of arginyl-tRNA synthetase [2]. AIMP1 is also involved in diverse physiological processes [3], including extracellular cytokine activities involving monocytes [4-6], endothelial cells [7], and fibroblasts [8], and glucagon-like hormonal activity [9]. It is recently reported that the intracellular physical interaction between AIMP1 and gp96 controls the ER retention of gp96, thereby preventing its extracellular presentation [10].

AIMP1's functional involvement in the immune response was initially discovered through the finding that a polypeptide with cytokine activity called endothelial monocyte activating polypeptide II (EMAP II) [1, 11], comprises the C-terminal portion of AIMP1. This finding suggested that AIMP1 is an inactive precursor of EMAP II. However, subsequent investigations demonstrated that intact AIMP1 itself is secreted from intact mammalian cells and actively works as a cytokine to trigger the proinflammatory response through monocytes and macrophages [4]. The secretion of intact AIMP1 is found in different types of cells including adenoma, immune cells and

transfected cells [12-15]. It was recently shown that the cleavage of AIMP1 to EMAP II and its secretion are modulated by proteasome and arginyl-tRNA synthetase [16]. Together, these data indicate that AIMP1 functions as a bona fide cytokine under physiological conditions.

Amongst the AIMP1-induced genes, a robust increase in the expression of TNF-α was observed [5]. This AIMP1-induced TNF-α production is mediated mainly through activation of mitogen-activated protein kinases (MAPKs) relayed by phospholipase Cγ (PLCγ), protein kinase C (PKC), and nuclear factor-kappa B (NF-kB) [4-6]. AIMP1 also increases the expression of inflammatory molecules including interleukin-8 (IL-8), monocyte chemotacticprotein-1 (MCP-1), macrophage inflammatory protein-1 (MIP-1), and IL1-β, as well as IL-12 production through the activation of NF-kB in macrophages [17] and bone marrow-derived dendritic cells [18]. Elucidating the signaling mechanism through which AIMP1 induces TNF-α production is essential for understanding the physiological and pathophysiological role of AIMP1 in inflammation. Although several reports have described functional receptor candidates for AIMP1 or EMAP II [19-21], the receptor mediating AIMP1-induced TNF-α production has not yet been identified.

Macrophages and monocytes play important roles in allergic inflammation [22] and

the macrophages and monocytes of individuals with allergic diseases express high levels of CD23 on their cell surfaces. Cells expressing CD23 can be occupied by IgE, which equips these cells for effector functions in IgE-dependent inflammation [23]. The cross-linking of CD23-bound IgE by allergen activates cells to release inflammatory cytokines such as TNF-α, IL-6, and IL-1β [24]. The role of CD23 in inflammatory diseases was suggested by studies which showed an anti-CD23 antibody can decrease both cellular infiltration of the synovial sublining layer and destruction of cartilage in collagen-induced arthritis models [25]. Accordingly, CD23-deficient mice showed delayed onset and reduced severity of collagen-induced arthritis [26]. Thus, CD23 might be a target in the treatment of inflammatory diseases.

In this study, I show that CD23 is an AIMP1 receptor in human monocytic THP-1 cells and primary human peripheral blood mononuclear cells (PBMCs) and plays an essential role in the AIMP1-induced immune response. I identified CD23 as an AIMP1 binding protein by screening a soluble receptor library, and showed it binds to AIMP1 with high affinity. Knockdown of CD23 suppressed cell surface binding of AIMP1, as well as AIMP1-induced ERK phosphorylation and TNF-α production. However, EMAP II, the portion of AIMP1 that mediates its association with the ARS complex, did not bind to CD23 and knockdown of CD23 had no effect on EMAP II-induced

TNF-α production. These results suggest that CD23 is a specific receptor for AIMP1 and may mediate the pathophysiological activity of AIMP1 in inflammation, independent of the EMAP II.

MATERIALS AND METHODS

Cell culture and materials

THP-1cells were obtained from American Type Culture Collection (ATCC) and grown in RPMI medium containing 10% fetal bovine serum and 50μg/ml streptomycin and penicillin. Transwell chambers for the THP-1 cell migration assay were purchased from Corning.Anti-AIMP1 polyclonal antibody (Abcam), CD23 (Abcam), tubulin (Abcam), integrin (Santa Cruz), and HSP (Santa Cruz) antibodies were used for western blot analysis. FITC-conjugated anti-CD23 monoclonal antibody, clone MHM6 (Dako), was used for the neutralizing test and Fluorescence-activated cell sorter **(**FACS) analysis. IL-4, which was used as an inducer for CD23 expression, was purchased from R&D systems. Mouse IgG1/FITC (Dako) was used as a negative control. All siRNAs used in this study were obtained from Invitrogen. Stealth universal RNAi (Invitrogen) was used as a negative control. siRNAs were transfected by electroporation using a Microporator-mini (Digital Bio Technology).

Isolation of human peripheral blood mononuclear cells (PBMCs)

All blood samples and procedures in this study were approved by the Seoul National University Institutional Review Board, approval number 1205/001-002, in accordance to the guidelines of National Bioethics Committee and were conducted in accordance to the Declaration of Helsinki. PBMCs were obtained from blood of healthy donors using BD Vacutainer CPT (BD Bioscience) ficoll gradient centrifugation at 1,800*g* for 30 minutes at room temperature. After the separation, a thin layer of PBMCs was isolated and washed twice with RPMI 1640. The pellet was resuspended in RPMI 1640 with streptomycin. Isolated PBMCs were cultured in RPMI 1640 supplemented with 10% fetal bovine serum.

Preparation of recombinant human AIMP1or EMAP II

Human AIMP1 and EMAP II cDNAs, encoding 312 and 166 amino acids respectively, were cloned into pET-28a (Novagen) and overexpressed in *Escherichia coli* BL21 (DE3) (Invitrogen) by induction with 0.5 mM IPTG. His-tagged AIMP1 (amino acid 1-312) and EMAP II (amino acid 148-312) were purified using nickel affinity chromatography (Invitrogen), following the manufacturer's instructions. Briefly, cells were resuspended in lysis buffer (50 mM KH_2PO_4, 500 mM NaCl, 0.2 mM EDTA, and

10% glycerol, pH 7.8) and lysed by sonication. After centrifugation at 10,000*g* for 30 minutes, the lysate was loaded on a nickel affinity column. The proteins bound to the column were eluted by 300 mM imidazole buffer (300 mM imidazole, 50 mM KH_2PO_4, 500 mM NaCl, 0.2 mM EDTA, and 10% glycerol, pH 6.0). To remove the lipopolysaccharide (LPS), each protein-containing solution was loaded to polymyxin resin (Bio-Rad), incubated for 2 hours, and eluted. To further remove the residual LPS, the solution was filtered through an Acrodisc unit with a Mustang E membrane (Pall Gelman Laboratory).

Preparation of recombinant human AIMP1 deletions

The constructs of whole AIMP1, AIMP1-(1–312), and AIMP1 deletions (namely AIMP1-(1–192), AIMP1-(193–312), AIMP1-(1–47), AIMP1-(47–192), AIMP1-(101–192), AIMP1-(114–192)) were described previously [27]. Each of the whole AIMP1 and AIMP1-deleted constructs was expressed as GST-tag fusion protein in *Escherichia coli* BL21 (DE3) and purified by glutathione S bead as described previously [4]. To remove lipopolysaccharide, the protein solution was dialyzed in pyrogen-free buffer (10 mM PBS, pH 6.0, 100 mM NaCl). After dialysis, the protein was loaded to polymyxin resin (Bio-Rad) pre-equilibrated with the same buffer, incubated for 20 minutes, and

eluted. The concentration of the residual LPS was below 20 pg/ml when determined using the Limulus Amebocyte Lysate QCL-1000 kit (BioWhittacker).

Soluble receptor binding assay

To identify the binding partner of AIMP1, soluble Fc-fused receptor proteins were used in an ELISA-based binding assay. Human cDNAs that encode extracellular region of membrane receptor except seven transmembrane proteins were subcloned into pYK602 vector, which were constructed to facilitate Fc-fused protein purification in mammalian cell, at sites for *Sfi I* restriction enzymes. These clones were transfected into HEK293 cells (ATCC). After 24 hours, transfected cells were incubated with serum free DMEM for 3 days. Cultured media were harvested and incubated with protein Aagarose bead. Bead-bound Fc-fusion proteins were eluted and dialyzed [28]. The MaxiSorp plates (Nunc) were coated with recombinant AIMP1 (1 μg/ml) in PBS for 12 hours at 4℃ and then blocked with 4% non-fat milk in PBS. After the Fc-fused extracellular domains of receptor proteins (1 μg/ml) were added to the plate and washed, the plates were incubated with anti-human IgG1 Fc-HRP (Thermo). The plates were washed 3 times, TMB solution was added, and the plates were read at 490 nm using a microplate reader. The equilibrium dissociation constants were calculated using ProteOn Manager^{TM}

software (version 2.1) and data were evaluated using a Langmuir 1:1 binding model.

Quantitative RT-PCR analysis

Cells were seeded and incubated for 12 hours prior to transfection of siRNAs targeting receptor candidates by a microporator. To confirm siRNAs inhibitory activities, total RNAs were extracted using the RNeasy Mini Kit (QIAGEN), and quantitative RT-PCR was performed with primers specific to the receptor candidates. The following primers were used: VEGFR1, forward 5′-ATGGTCTTTGCCTGAAATGGTGAG-3′, reverse 5′-CTGTGAAGCCAGTGTGGTTTGC-3’; VEGFR2, forward 5′-GGAAATGACACT-GGAGCCTACAAG-3′, reverse 5′-GGACCCGAGACATGGAATCACC-3′; sFRP1, forward 5′-GGCGGAGGTGAAGCAGCAG-3′, reverse 5’-CGAAGAGCGAGCAGA-GGAAGAC-3′; TNFSF13, forward 5′-CCTGGAAGCCTGGGAGAATGG-3′, reverse 5′-A-TGTCACATCGGAGTCATCCTTGG-3′; PLAC9, forward 5’-GCCGCTGCCGA-ACCCTTC-3′, reverse 5′-CCACGGTCTTCTCTACCATCTCC-3’; CLEC10A, forward 5′-GCTGGTCATCATCTGTGTGGTTG-3′, reverse 5′-TGCCTGCCGTTCCT-GCTTG-3′; CD23, forward 5′-TGCTGACTCTGCTTCTCCTGTG-3′, reverse 5′-TCT-GCGTGGACTGGGATTTCTG-3′; IL20Rb, forward 5′-TCTTGATGTGGAGCCCA-GTGATC-3′, reverse 5′-TCAGGACCTTCAGTGAGTGAGC-3′; CXCR3, forward 5′-

CCGACACCTTCCTGCTCCAC-3′, reverse 5′-GCTCCTGCGTAGAAGTTGATGTT-G-3′; GAPDH, forward 5′-CGCTCTCTGCTCCTCCTGTTC-3′, reverse 5′-TTGACT-CCGACCTTCACCTTCC-3′ and TNF-α, forward 5′-GGCGTGGAGCTGAGAGAT-AAC-3′, reverse 5′-GGTGTGGGTGAGGAGCACAT-3′. Glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA levels were used as internal controls. Human TNF-α mRNA levels were measured to verify TNF-α production by induction of AIMP1 or EMAP II.

TNF-α enzyme-linked immunosorbant (ELISA) analysis

Cells were cultured on 12-well plates in RPMI medium with 10% FBS and 1% antibiotics for 12 hours and starved in serum-free RPMI medium for 3 hours. The indicated concentrations of AIMP1 (100 nM) were added to the serum-free medium for the indicated times, and the medium was harvested by centrifugation at 3,000*g* for 10 minutes. The secreted TNF-α was detected using a TNF-α ELISA Kit, according to the manufacturer's instructions (R&D systems).

Transwell migration assay

THP-1 migration assays were performed using Transwell chambers (24-well) with

polycarbonate membranes (8.0 μm pore size, Costar) as described, with slight modifications [29]. Briefly, the wells were coated with 0.5 mg/ml gelatin (Sigma) in PBS and allowed to air-dry. THP-1 cells were suspended in serum-free RPMI and added to the upper chamber at 5×10^5 cells per well. 50 nM of AIMP1 was placed in the lower chamber, and the cells were allowed to migrate for 10 hours at 37°C in a 5% CO_2 incubator. After incubation, non-migrant cells were removed from the upper face of the membrane with a cotton swab. The migrant cells, which attached to the lower face, were fixed in 100% methanol and visualized by hematoxylin (Sigma) staining. The migrant cells were counted in high power fields.

Cell binding assay

To obtain biotin-labeled AIMP1, purified His-AIMP1 (1 mg) was incubated with 0.25 mg Sulfo-NHS-SS-biotin (Pierce) in PBS for 4 hours followed by 100 mM Tris-HCl (pH 7.4) for quenching. Cells were seeded and incubated for 12 hours. After preserved cells were incubated in serum-free RPMI medium for 30 minutes, biotinylated AIMP1 was added to the culture medium and further incubated for the indicated times. The cells were washed 3 times with cold PBS, lysed in 50 mM Tris-HCl (pH 7.4) lysis buffer containing 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.2% sodium

deoxycholate, 10 mM NaF, 1 mM sodium orthovanadate, 10% glycerol, and protease inhibitors, and centrifuged at 18,000*g* for 15 minutes. The extracted proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and detected by streptavidin-coupled HRP (Pierce).

Fluorescence-activated cell sorter (FACS) analysis

To monitor the level of CD23 surface expression by flow cytometry, cells were transfected with specific siRNAs (50 nM) for 48 hours and treated with IL-4 (10 ng/ml) for 72 hours. Cells were resuspended, incubated with the anti-CD23 antibody, and stained with Alexa488-conjugated secondary antibody (Invitrogen) in FACS buffer (PBS containing 1% BSA) for 1 hour. Cells were then washed 3 times with PBS and analyzed by flow cytometry using Cell Quest software (BD Biosciences)

Mitogen-activated protein kinase (MAPK) analysis

THP-1 and PBMCs were cultured on 6-well plates for 12 hours, washed twice, and starved in serum-free medium for 3 hours. Cells were incubated with the indicated concentrations of AIMP1 or EMAP II (100 nM) for the indicated times or with the indicated dose for 1 hour, and washed. The proteins were extracted with 25 mM Tris-

HCl (pH 7.4) lysis buffer containing 150 mM NaCl, 1 mM EDTA, 1mM sodium orthovanadate, 20 mM sodium fluoride, 12 mM β-glycerophosphate, 10% glycerol, 1% Triton X-100, and protease inhibitors and resolved by SDS-PAGE. Total and phosphorylated MAPKs were detected by their specific antibodies (Cell Signaling).

Pull-down assay

To confirm interactions by the *in vitro* pull-down assay, purified Fc-CD23 and control protein (2 μg/ml) were incubated with His-AIMP1 or EMAP II (2 μg/ml) for 1 hour. Immune-precipitation was performed using the Fc-fused sCD23 (the soluble form of CD23 encoding the COOH-terminal 172 amino acids) or control protein using protein A/G agarose and analyzed by immunoblotting with anti-AIMP1 to detect the interaction. Thy-1 (RLE): Fc (Enzo Life Sciences) was used as a negative control.

Statistical analysis

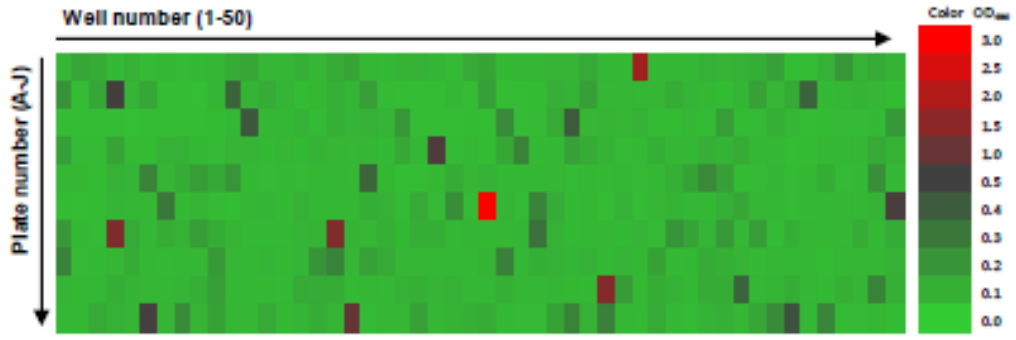
A paired t-test was used to establish statistically significant differences between treatment groups. P values < 0.05 were considered to represent statistically significant differences. Where applicable, the mean ± SEM of multiple measurements is reported, as indicated.

RESULTS

Screening to identify AIMP1-binding receptors

Potential binding partners of AIMP1 were identified by an ELISA-based binding assay, whereby recombinant AIMP1 was coated on a plate and reacted with 499 different soluble receptor proteins [28]. Among them, 6 soluble receptor proteins were selected as having high binding affinity for AIMP1, including CD23, CLEC10A, FRP1, IL20Rb, TNFSF13, and PLAC9 and designated as candidates AIMP1 receptors (**Fig. 1A**). Previously, vascular endothelial growth factor receptor 1 (VEGFR1) was identified as a receptor for EMAP II, the C-terminal fragment of AIMP1 [21], but AIMP1 did not interact with VEGFR1 in this screening system. To investigate the specificities of candidate receptor binding to AIMP1, I analyzed the binding ratio using recombinant AIMP1 and BSA. Among 6 soluble receptor proteins, only CD23 and CLEC10A showed high AIMP1/BSA binding ratios (2.2631 and 2.6641, respectively), suggesting their binding is specific for AIMP1 (**Fig. 1B**).

A



B

Clone (plate-well)	Protein	BSA	AIMP1	Ratio (AIMP1/BSA)
A-35	CD23	0.1395	0.3157	2.2631
F-26	PLAC9	0.3864	0.4857	1.2570
G-04	CLEC10A	0.1024	0.2728	2.6641
G-17	IL20RB	0.3112	0.3192	1.0257
I-33	sFRP1	0.2558	0.3219	1.2584
J-18	TNFSF13	0.2849	0.2845	0.9986
J-50	Mock	0.0768	0.0680	0.8854

Fig. 1. Screening to detect potential AIMP1-binding receptors. (A) Heat map of primary screening data. Binding of soluble receptors to AIMP1 was monitored as described in Methods. The signal is indicated as the OD_{490} value and the degree of the signal is represented in the heat map from 0 (green) to 3 (red). The soluble receptor binding assay was performed using 1 μg of recombinant AIMP1 for pre-coating of the

wells, along with cell culture supernatant containing each Fc-fused soluble receptor expression construct. Binding was detected using HRP conjugated anti-hFc antibody and measuring absorbance at 490nm. Mock represents the internal control (no soluble receptor protein added). (B) The assay was performed using 100 ng of recombinant AIMP1 or BSA as a control, in each well for pre-coating. BSA was used as the negative control and mock represents the internal control (no soluble receptor protein added). Then aliquots of cell culture supernatant containing 6 candidate soluble receptors were applied to wells. Absorbance values for BSA and AIMP1 are indicated in each column. The ratio was calculated as the absorbance value of AIMP1-receptor binding divided by that of BSA-receptor binding.

AIMP1 binds to CD23

I next determined the effects of receptor candidate knockdown on TNF-α secretion in THP-1 human monocyte cells. I first tested the effects of nine siRNAs, each targeting different receptor candidates for AIMP1. All siRNAs reduced target mRNA expression by at least 50%, as determined by quantitative RT-PCR (**Fig. 2A**). Among them, CD23 siRNA specifically suppressed AIMP1-induced TNF-α secretion in THP-1 cells (**Fig. 2B**). Interestingly, knockdown of VEGFR1, which is a known receptor for EMAP II, and CLEC10A, significantly increased AIMP1-induced TNF-α secretion, suggesting that cross-talk exists between CD23 and VEGFR1 and/or CLEC10A.

To investigate whether AIMP1 specifically binds to CD23, I tested whether the binding affinity between AIMP1 and CD23 is saturable. Data were subjected to Scatchard analysis to determine maximum binding (B_{max}) and the equilibrium constant (K_D). The B_{max} and K_D were determined as 0.35 and 4.3, respectively (**Fig. 2C**). In contrast, CLEC10A and sFRP1 showed relatively low affinity binding for AIMP1 (K_D = 159.1 nM and K_D = 325.2 nM, respectively). These results suggest that CD23 is a candidate for a functional receptor for AIMP1.

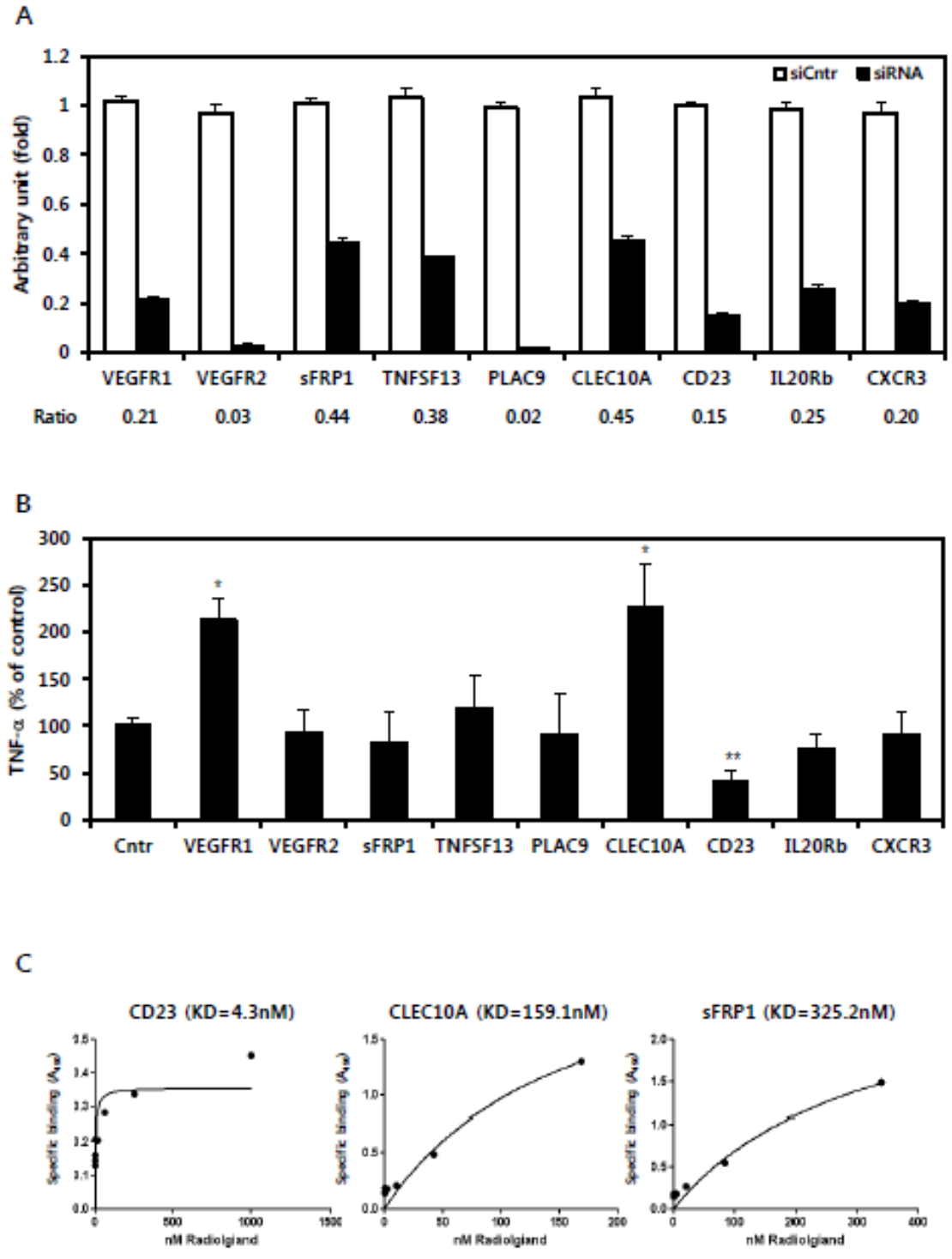


Fig. 2. AIMP1 binding to CD23. (A) THP-1 cells were transfected with each siRNA

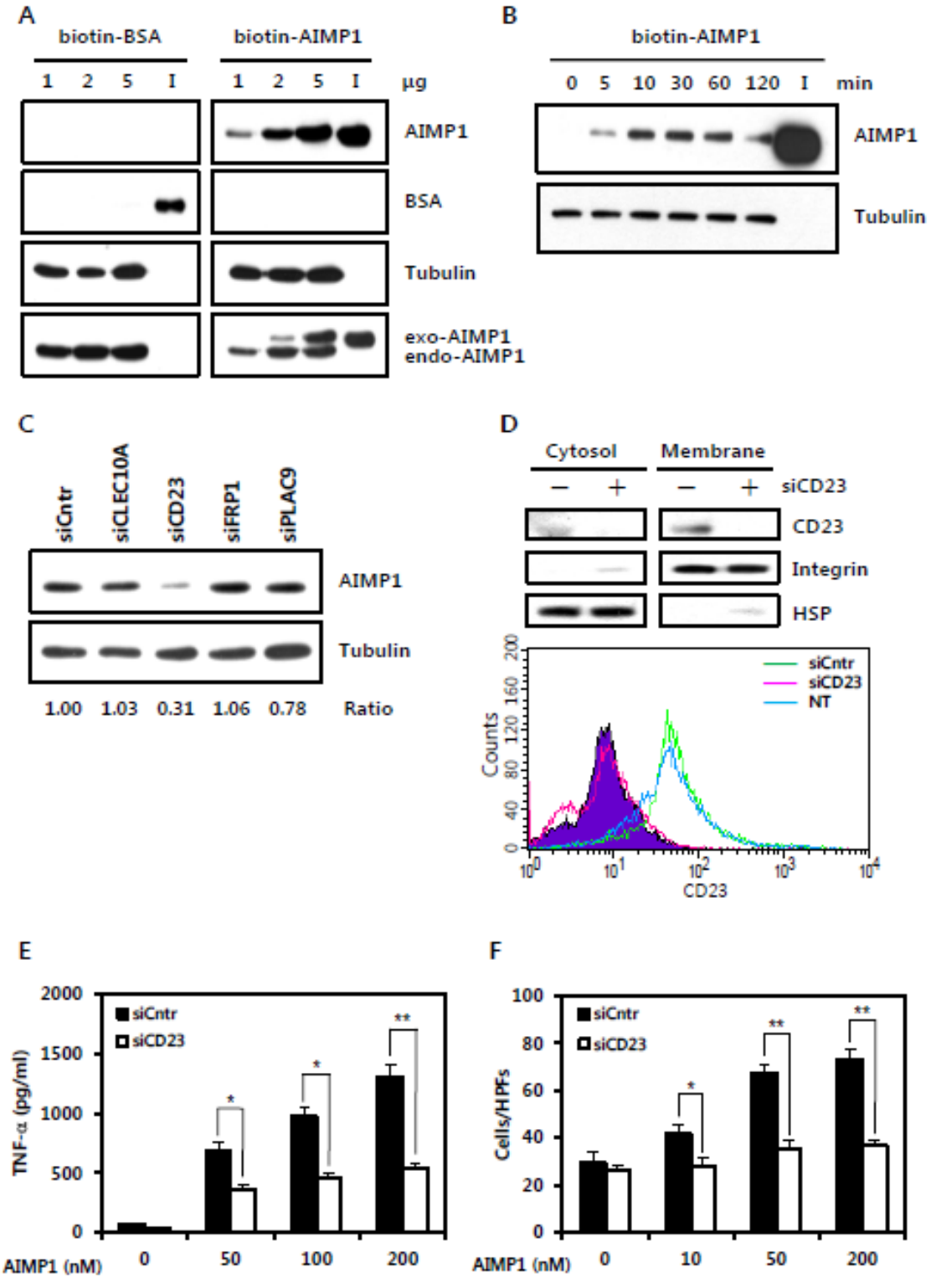
for 48 hours and the effect of siRNA treatment on receptor candidate gene expression was analyzed by quantitative RT-PCR. The ratio was calculated as the level in candidate receptor siRNA-transfected cells divided by the level in control siRNA-transfected cells. GAPDH was used as the internal control. (B) The effects of candidate receptor knockdown on AIMP1-induced TNF-α secretion. Data are expressed as the mean ± SEM (n=6). * and ** denote significant differences from negative control siRNA ($p < 0.05$ and $p < 0.01$ respectively). (C) Dose-dependent interaction of AIMP1 with Fc-fused candidate receptors. The indicated concentrations of candidate receptors (CD23, CLEC10A, or sFRP1 proteins) were added to AIMP1 coated on the surface of microtiter wells, and Fc- receptor bound to AIMP1 was detected with anti-Fc-conjugated peroxidase. The equilibrium dissociation constant (K_D) was determined for the interactions of AIMP1 with each candidate receptor.

CD23 mediates AIMP1 cell surface binding and AIMP1-induced TNF-α secretion

If AIMP1 binds to CD23 on the surface of THP-1 cells, the cell surface binding of AIMP1 should be closely related to the level of CD23 expression. I tested this possibility by monitoring the effect of CD23 siRNA on AIMP1 cell surface binding. To determine the optimal condition for this assay, THP-1 cells were treated with biotinylated AIMP1 at various concentrations and incubation times, and bound AIMP1 was detected with streptavidin-conjugated horseradish peroxidase or anti-AIMP1 antibody. I found that biotinylated AIMP1, but not biotinylated BSA, bound to THP-1 in a dose- and time-dependent manner (**Fig. 3A, B**). In addition, CD23 knockdown specifically reduced cell surface binding of AIMP1 (**Fig. 3C**). PLAC9 knockdown showed a slight decrease of AIMP1 binding to THP-1 cells, but not involved in the reduction of TNF-α secretion (**Fig. 2B**), indicating that PLAC9 is not an AIMP1 receptor that mediate TNF-α secretion in THP-1 cells despite its ability to bind to AIMP1.

To confirm that CD23 siRNA efficiently reduced its cell surface expression, CD23 levels were quantified by immunoblotting and FACS analysis (**Fig. 3D**). To investigate whether AIMP1-induced TNF-α secretion is mediated by CD23, THP-1 cells were

treated with different doses of recombinant biotinylated AIMP1 (50, 100, and 200 nM). I found that CD23 siRNA, but not by control siRNA, suppressed AIMP1-induced TNF-α secretion (**Fig. 3E**). In addition, CD23 siRNA suppressed AIMP1-induced migration of THP-1 cells in a dose-dependent manner (**Fig. 3F**). Together, these results suggest that CD23 specifically mediates the cell surface binding of AIMP1 and AIMP1-induced TNF-α secretion and cell migration.


A
biotin-BSA
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siCLEC10A
siCD23
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siPLAC9
AIMP1
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CD23
Integrin
HSP
siCntr
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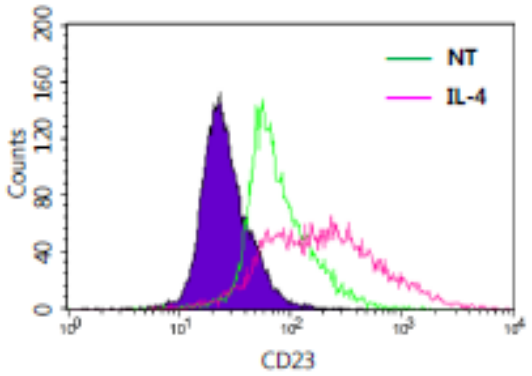
siCD23
TNF-α (pg/ml)
AIMP1 (nM)
0 50 100 200
F
siCntr
siCD23
Cells/HPFs
AIMP1 (nM)
0 10 50 200

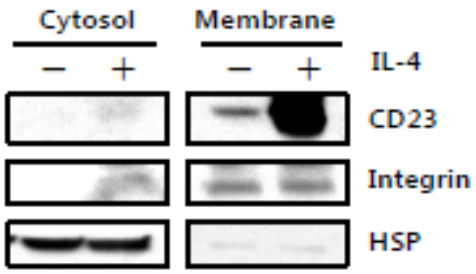
Fig. 3. The effect of CD23 knockdown on AIMP1-induced TNF-α secretion and migration in THP-1 cells. THP-1 cells were treated with different concentration of biotinylated BSA and -AIMP1 for 1 hour (A) or with 1 μg of biotinylated AIMP1 for the indicated time (B). Exo-AIMP1 and endo-AIMP1 represent the level of externally applied biotinylated AIMP1 and endogenous AIMP1, respectively. Input (I) means the biotinylated BSA or -AIMP1 that were used as the size controls. THP-1 cells treated with biotinylated BSA or -AIMP1 were bound to streptavidin-bound sepharose beads and harvested. The cells were lysed and the extracted proteins were subject to western blotting with horseradish peroxidase-conjugated anti-streptavidin (the 1st and 2nd), anti-tubulin (the 3rd) and anti-AIMP1 antibody (the 4th panel). (C) The effect of candidate receptor knockdown on biotinylated AIMP1 binding to THP-1. Bound AIMP1 was analyzed by western blotting with streptavidin-conjugated peroxidase. The ratio represents the value of the level of AIMP1 in control siRNA-transfected cells divided by that in candidate receptor siRNA-transfected cells. Tubulin was used as the internal control. (D) The effect of CD23 knockdown on surface CD23 expression on THP-1 cells. The filled area indicates mock used as the negative control (no anti-CD23 antibody added). Integrin and HSP (heat-shock protein) were used as markers of the membrane and the cytosol, respectively. Cell surface CD23 expression was analyzed by

western blotting and FACS analysis with the anti-CD23 antibody. Non-siRNA-transfected cells (NT) and control siRNA-transfected cells (siCntr) were used as negative controls. (E) The effect of CD23 knockdown on AIMP1-induced TNF-α secretion. THP-1 cells were transfected with control or CD23 siRNA, treated with AIMP1 at the indicated concentrations for 3 hours, and TNF-α secretion was monitored. Data are expressed as the mean ± SEM (n = 3). * and ** denote significant differences from negative control siRNA ($p < 0.05$ and $p < 0.01$ respectively). (F) The effect of CD23 knockdown on AIMP1-induced migration in THP-1 cells. The cell migration assay was performed using a transwell-chamber with a gelatin-coated polycarbonate membrane. THP-1 cells were suspended in the upper chamber and AIMP1 (50 nM) was added to the lower chamber. Migrated cells were counted by light microscopy using high power field (HPF). Data are expressed as the mean ± SEM (n= 3). * and ** denote significant differences from negative control siRNA ($p < 0.05$ and $p < 0.01$ respectively).

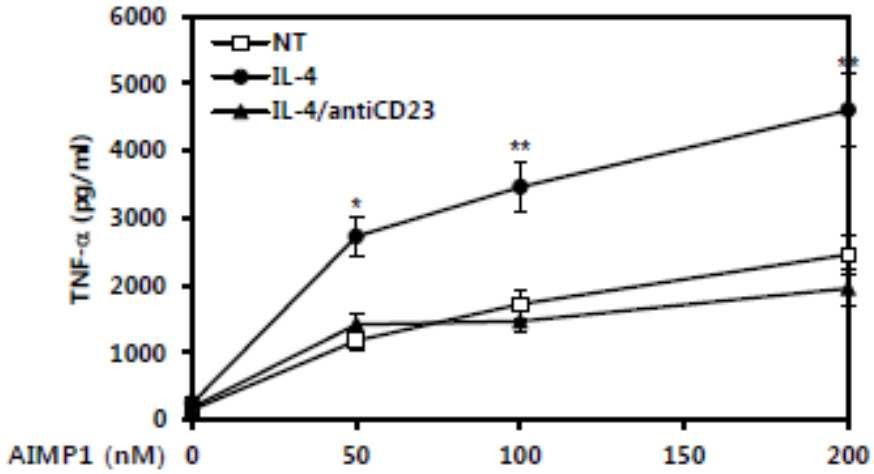
Next, I confirmed whether CD23 mediates AIMP1-induced TNF-α secretion through a set of experiments where I increased or decreased the cellular levels of CD23. Since IL-4 has known as an inducer for CD23 expression [30], CD23 levels on THP-1 cells treated with IL-4 were quantified by immunoblotting and FACS analysis (**Fig. 4A**). AIMP1 enhanced TNF-α secretion in the IL-4-treated cells than in non-treated cells (**Fig. 4B**). In addition, treatment with a neutralizing anti-CD23 antibody suppressed AIMP1-enhanced TNF-α secretion in IL-4-treated cells. To test whether soluble CD23 protein competed with AIMP1 for TNF-α secretion, AIMP1 was pre-incubated with CD23-Fc soluble receptor prior to addition of THP-1 cells. I found that AIMP1 induced TNF-α secretion in control Fc-treated cells, whereas CD23-Fc significantly suppressed AIMP1-induced TNF-α secretion (**Fig. 4C**). Although previous studies indicated that soluble CD23 activates monocytes and triggers cytokine release [31], the duration of treatment with AIMP1 and soluble CD23 was relatively short in my study, and I found that soluble CD23 itself had no effect on TNF-α secretion in THP-1 cells. Together, these results suggest that CD23 is a functional receptor for proinflammatory AIMP1 in THP-1 cells and that the AIMP1 binding site of CD23 overlaps with the epitope of the neutralizing anti-CD23 antibody.

A


NT
IL-4
Counts
0
40
80
120
160
200
CD23


Cytosol
Membrane
IL-4
CD23
Integrin
HSP

B


NT
IL-4
IL-4/antiCD23
TNF-α (pg/ml)
AIMP1 (nM)
0
50
100
150
200
1000
2000
3000
4000
5000
6000

C

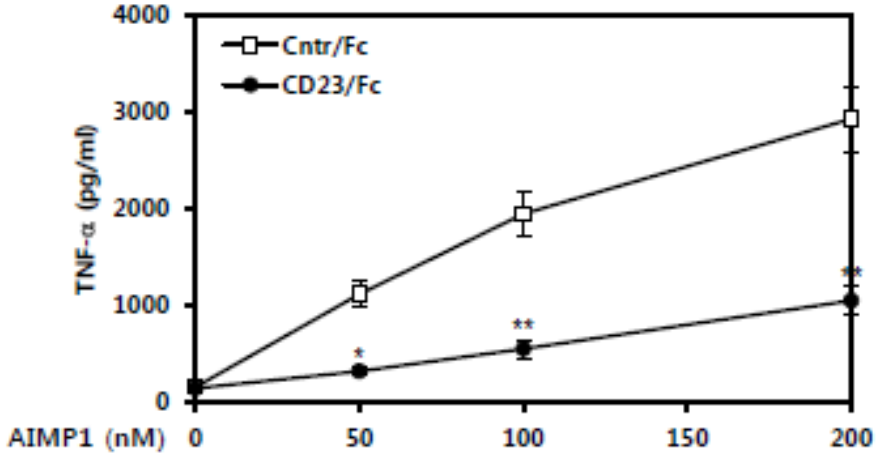

Cntr/Fc
CD23/Fc
TNF-α (pg/ml)
AIMP1 (nM)
0
50
100
150
200
1000
2000
3000
4000

Fig. 4. The effect of CD23 expression on AIMP1-induced TNF-α secretion. (A) The effect of IL-4 treatment on surface CD23 expression on THP-1 cells. The filled area indicates mock used as the negative control (no anti-CD23 antibody added). Integrin and HSP (heat-shock protein) were used as markers of the membrane and the cytosol, respectively. Cell surface CD23 expression was analyzed by western blotting and FACS analysis with the anti-CD23 antibody. Non-siRNA-transfected cells (NT) and control siRNA-transfected cells (siCntr) were used as negative controls. (B) The effect of CD23 overexpression on AIMP1-induced TNF-α secretion on THP-1 cells. THP-1 cells were pretreated with IL-4 (20 μg/ml) for 72 hours and/or followed treatment with anti-CD23 antibody (10 μg/ml). Cells were treated with indicated concentrations of AIMP1 for 3 hours and TNF-α secretion was monitored. Data are expressed as the mean ± SEM (n = 3). * and ** denote significant differences from negative control not treated with IL-4 and/or anti-CD23 antibody ($p < 0.05$ and $p<0.01$ respectively). (C) The effect of the Fc-fused sCD23 protein on AIMP1-induced TNF-α secretion. AIMP1 (2 μg) was preincubated with Fc-fused sCD23 or control protein (2 μg each) and added to THP-1 cells for 3 hours. Data are expressed as the mean ± SEM (n = 3). * and ** denote significant differences from negative control treated with control protein($p < 0.05$ and $p < 0.01$ respectively).

ERK pathway is functionally linked to CD23 for AIMP1-induced TNF-α secretion

In THP-1 cells, AIMP1 activates MAPK family members and NF-κB [5]. ERK1/2 is rapidly activated in 5–10 minutes in THP-1 cells through cross-linking with an anti-CD23 antibody [32]. Furthermore, JNK is associated with CD23-triggered TNF-α production in human intestinal epithelial cells [33]. Because MAPK family members mediate TNF-α production upon cellular exposure to LPS and other cytokines, I investigated whether AIMP1-induced CD23-mediated TNF-α secretion is mediated by MAPKs signaling pathways. I found that treating THP-1 cells with recombinant AIMP1 increased phosphorylation of ERK1/2 but not phosphorylation of JNK and AKT/protein kinase B (PKB) **(Fig. 5A, B**). CD23 knockdown also decreased AIMP1-induced phosphorylation of ERK1/2 (**Fig. 5C**). Furthermore, pre-treatment with the MEK inhibitor U0126 suppressed AIMP1-induced TNF-α secretion and ERK1/2 phosphorylation, whereas the JNK inhibitor SP600125 had no effect (**Fig. 5D**). Therefore, these results suggest that within the MAPK pathway, ERK family members are functionally linked to the CD23 downstream pathway for AIMP1-induced TNF-α secretion.

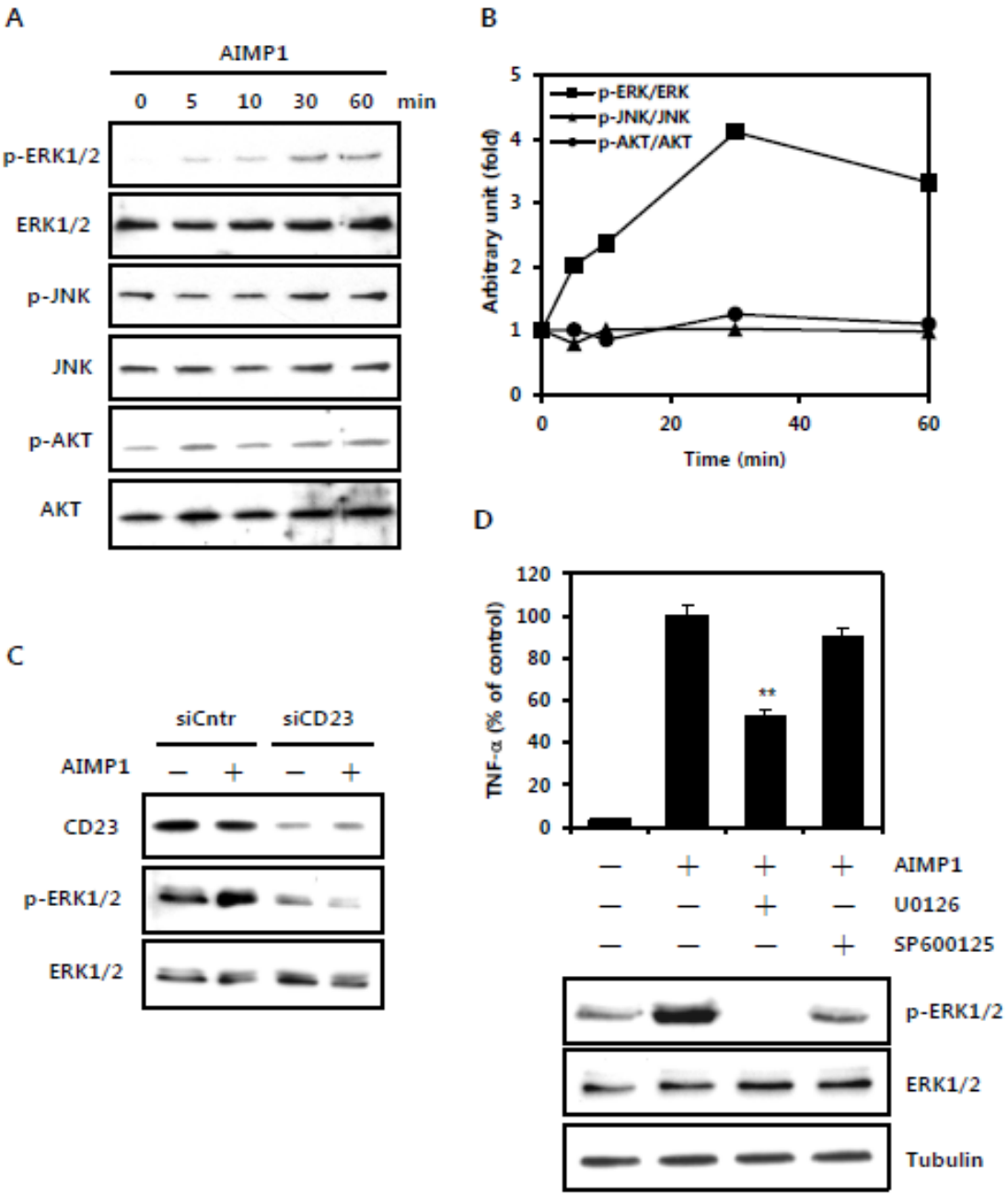


Fig. 5. The effect of AIMP1 on ERK1/2, JNK, and AKT phosphorylation in THP-1 cells. (A) THP-1 cells were treated with AIMP1 (100 nM) and changes in ERK1/2, JNK, and AKT phosphorylation status during the time of AIMP1 treatment was

determined. (B) Quantitative analysis of the fold ratio of phospho/total protein from (A) was performed by using densitometer. The changes in ERK1/2, JNK, and AKT phosphorylation status were calculated as the phosphorylation level of each MAP kinase for indicated time (5, 10, 30, 60 minutes) by the level in start time (0 minute). (C) The effect of CD23 knockdown on AIMP1-induced ERK1/2 phosphorylation in THP-1 cells. THP-1 cells were transfected with CD23 siRNA for 48 hours followed by treatment with AIMP1 for 30 minutes. Cell lysates were analyzed using anti-CD23, p-ERK1/2, and ERK1/2 antibodies. (D) THP-1 cells were pre-incubated with U0126 or SP600125, inhibitors of ERK or JNK, respectively, for 30 minutes before treatment with AIMP1. The effects of these inhibitors on AIMP1-induced TNF-α secretion were analyzed by the TNF-α ELISA kit. Data are expressed as the mean ± SEM ($n = 3$). ** denotes a significant difference from negative control not treated with either inhibitor ($p < 0.01$).

The central region of AIMP1 (amino acid 101–192) mediates CD23 binding and TNF-α secretion

To determine which regions of AIMP1 are involved in binding with CD23 and TNF-α secretion, several deletion derivatives of AIMP1 were used [27]. The deletion derivatives were purified as GST-tagged fusion proteins using a bacterial expression system, and analyzed by SDS-polyacrylamide gel electrophoresis. I compared the binding affinities of deletion AIMP1 constructs for CD23 by *in vitro* pull-down assay (**Fig. 6A**). I determined that AIMP1-(1–312), AIMP1-(1–192), AIMP1-(47–192), and AIMP1-(101–192) bound to the CD23-Fc fusion protein. In contrast, AIMP1-(1–46) and AIMP1-(193–312) did not bind the CD23-Fc fusion protein. Together, these data revealed that the CD23-Fc protein bound to the central region of AIMP1 (amino acid 101–192). To investigate TNF-α secretion by the AIMP1 deletion derivatives, the purified recombinant proteins were added to THP-1 cells and the levels of secreted TNF-α were determined by ELISA (**Fig. 6B**). I found that AIMP1-(1–312), AIMP1-(1–192), AIMP1-(47–192), and AIMP1-(101–192) showed cytokine activity. In contrast, AIMP1-(1–46) and AIMP1-(193–312) did not activate TNF-α secretion. The results suggest that AIMP1-(101–192) binds to CD23 and this region is closely linked to cytokine effect of AIMP1.To confirm that TNF-α secretion induced by the central

region of AIMP1 is CD23 dependent, AIMP1-(1–312) and AIMP1-(101–192) were added to CD23 knockdown THP-1 cells and the levels of secreted TNF-α were determined (**Fig. 6C**). I found that CD23 knockdown suppressed AIMP1-(1–312) and AIMP1-(101–192)-induced TNF-α secretion. These results suggested that in THP-1 cells, the central region of AIMP1 (amino acid 101–192) is closely linked to the cytokine effect of AIMP1 by its binding to CD23.

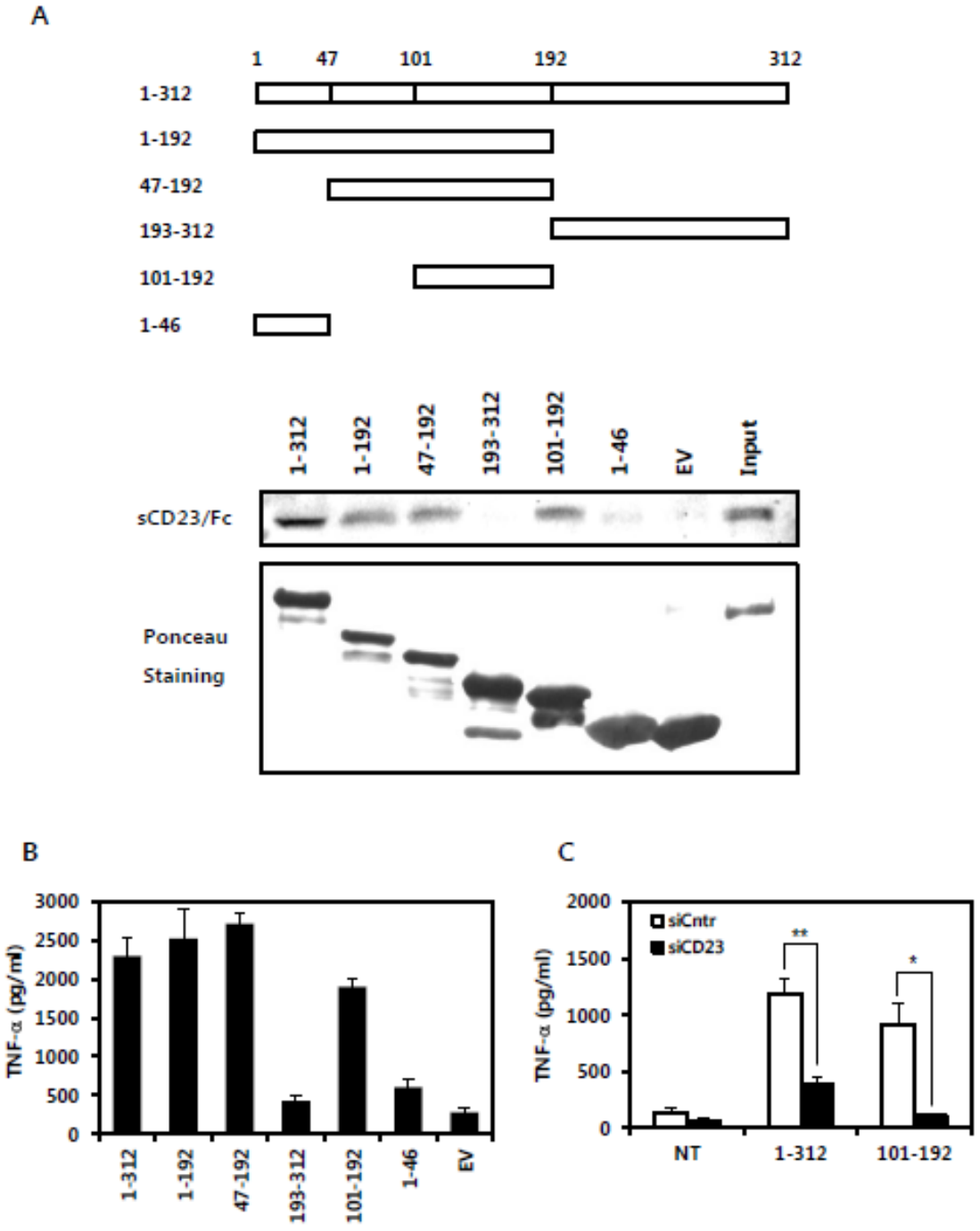


Fig. 6. CD23 binding to the central domain of AIMP1. (A) A schematic drawing of the AIMP1 deletion mutant proteins used in this study and the binding affinities of the

constructs for CD23.AIMP1 and its deletion mutant protein were mixed with Fc-fused sCD23 protein prior to the addition of protein A/G agarose to the mixture. Bound AIMP1 and its deletion mutant protein were investigated using the human IgG coupled HRP. Empty vector (EV) was used as the control. Addition of Fc-fused CD23 soluble receptor was used as loading controls. (B) The effects of AIMP1 and its deletion mutant proteins on TNF-α induction. THP-1 cells were treated with AIMP1 and its deletion constructs, and TNF-α secretion was monitored. Empty vector (EV) was used as the control. Data are expressed as the mean ± SEM (n = 3). (C) The effects of AIMP1 and its deletion mutant, AIMP1-(101–192), on TNF-α induction in CD23 knockdown THP-1 cells. THP-1 cells were transfected with control or CD23 siRNA for 48 hours followed by treatment with 100 nM of AIMP1-(1–312) and AIMP1-(101–192) respectively, and TNF-α secretion was monitored. Data are expressed as the mean ± SEM (n = 3). * and ** denote significant differences from negative control siRNA (p < 0.05 and p < 0.01 respectively).

AIMP1, but not EMAP II, induces TNF-α secretion via CD23

EMAP II, which is the truncated C-terminal portion of AIMP1, was first discovered as a secreted peptide in the culture medium. It was later found to possess cytokine activity including angiostatic or pro-apoptotic functions [34, 35]. In this study, my data shows that AIMP1-(101–192) is a binding site for CD23 and induces TNF-α secretion. To determine whether CD23 is a specific receptor for AIMP1, even in the absence of the EMAP II portion of the protein, I directly compared the binding affinities of AIMP1 and EMAP II for CD23. *In vitro* pull-down assays revealed that the CD23-Fc protein bound to AIMP1 but not to EMAP II (**Fig. 7A**). Thus, these results indicate that the central region of AIMP1 not containing EMAP II (amino acid 101–146) mediates CD23 binding. Consistent with the previous report [4], EMAP II induced TNF-α secretion, but the potency of EMAP II was lower than that of AIMP1. CD23 knockdown suppressed AIMP1-induced TNF-α secretion, but had no effect on EMAP II-induced TNF-α secretion (**Fig. 7B**). EMAP II did not activate the ERK pathway in a manner different from AIMP1 (**Fig. 7C**). Furthermore, pre-treatment with the MEK inhibitor U0126 did not suppress EMAP II-induced TNF-α secretion (**Fig. 7D**). These data suggest that in monocytic cells, AIMP1, but not EMAP II, binds to CD23 to induce TNF-α secretion, and this effect is mediated by the ERK pathway.

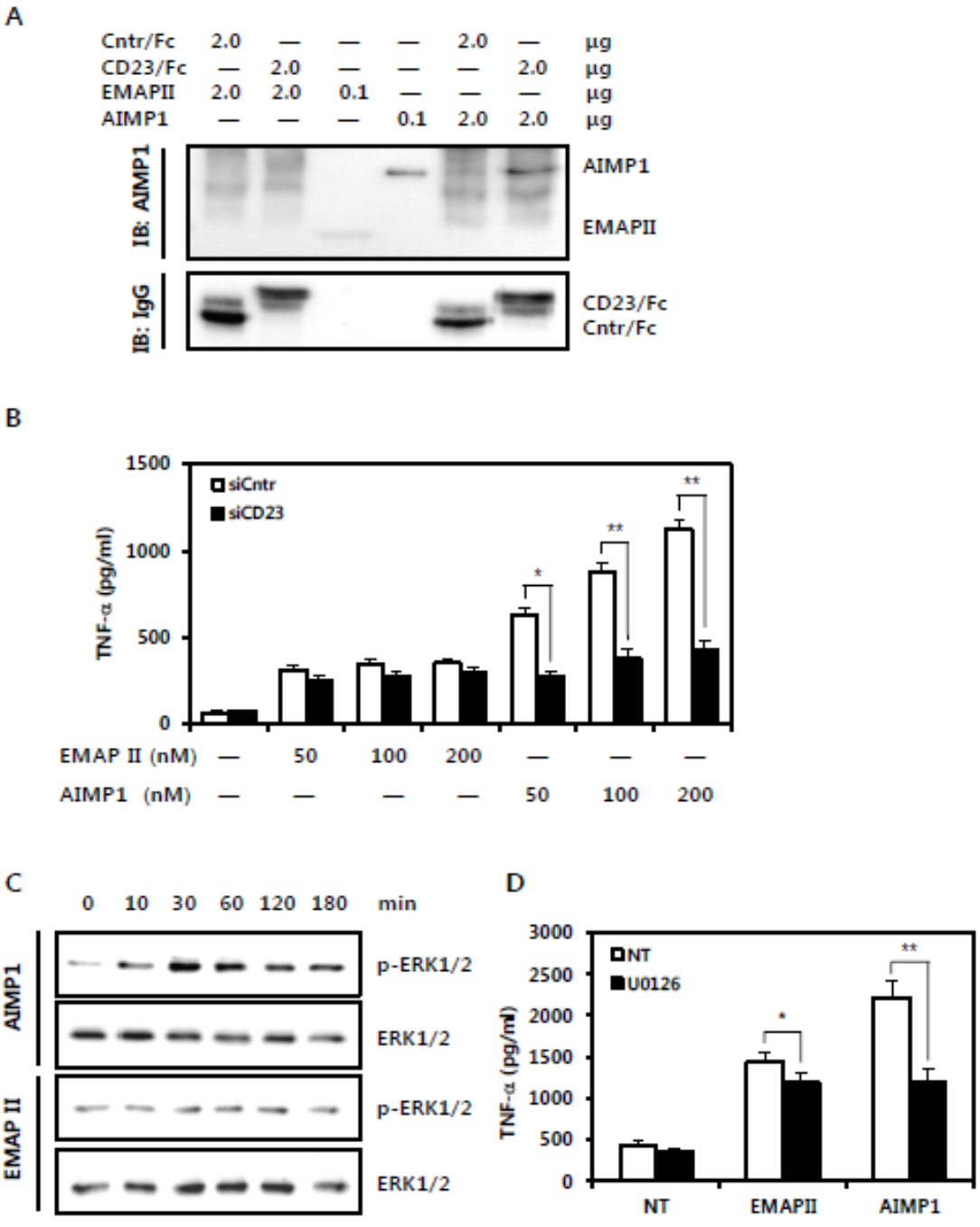


Fig. 7. The effects of AIMP1 and EMAP II on CD23-mediated TNF-α secretion.

(A) AIMP1 or EMAP II protein was mixed with CD23 soluble receptor for 1 hour

prior to the addition of protein A/G agarose to the mixture. Levels of bound AIMP1 or EMAP II were tested using the anti-AIMP1 antibody and human IgG coupled HRP. AIMP1 and EMAP II were used as loading controls. (B) The effect of CD23 knockdown on AIMP1 or EMAP II-induced TNF-α secretion. Data are expressed as the mean ± SEM (n = 3). * and ** denote significant differences from negative control siRNA ($p < 0.05$ and $p < 0.01$ respectively).(C) The effect of EMAP II on ERK1/2 phosphorylation was investigated in THP-1 cells. THP-1 cells were treated with AIMP1 or EMAP II for the indicated times and the change in ERK phosphorylation was determined. (D) THP-1 cells were pre-incubated with U0126, which is an inhibitor of ERK, before treatment with EMAP II (100 nM) or AIMP1 (100 nM). The effects of these inhibitors on EMAP II- or AIMP1- induced TNF-α secretion were analyzed by TNF-α ELISA. Data are expressed as the mean ± SEM (n = 3). * and ** denote differences from negative control (p = 0.237 and p = 0.005, respectively).

CD23 is a functional receptor for AIMP1 in primary immune cells

In this study, I identified CD23 as a functional receptor for AIMP1 in monocytic cells such as THP-1 cells. To confirm that AIMP1 induces TNF-α production through its binding to CD23 on primary immune cells, I used primary human PBMCs to verify CD23-mediated AIMP1 binding and TNF-α production, and compared this effect with that of EMAP II. I determined the effects of CD23 knockdown on TNF-α production in PBMCs and compared it with knockdown of other known receptors of EMAP II such as VEGFR1 and CXCR3. All siRNAs reduced target mRNA expression by at least 50%, as determined by quantitative RT-PCR (**Fig. 8A**). Among them, CD23 siRNA specifically suppressed AIMP1-induced TNF-α production in PBMCs (**Fig. 8B**). In addition, CD23 knockdown suppressed the cell surface binding of AIMP1 (**Fig. 8C**). CXCR3 knockdown showed a slight decrease of AIMP1 binding to PBMCs, but not involved in the reduction of TNF-α secretion (**Fig. 8B**), indicating that CXCR3 is not an AIMP1 receptor that mediate TNF-α secretion in PBMCs despite its ability to bind to AIMP1. Consistent with the result using THP-1 cells, CD23 knockdown suppressed AIMP1-induced TNF-α secretion, but had no effect on EMAP II-induced TNF-α secretion (**Fig. 8D**). CD23 knockdown also decreased AIMP1-induced phosphorylation of ERK1/2, but EMAP II did not activate the ERK pathway in a

manner different from AIMP1 in PBMCs (**Fig. 8E**).

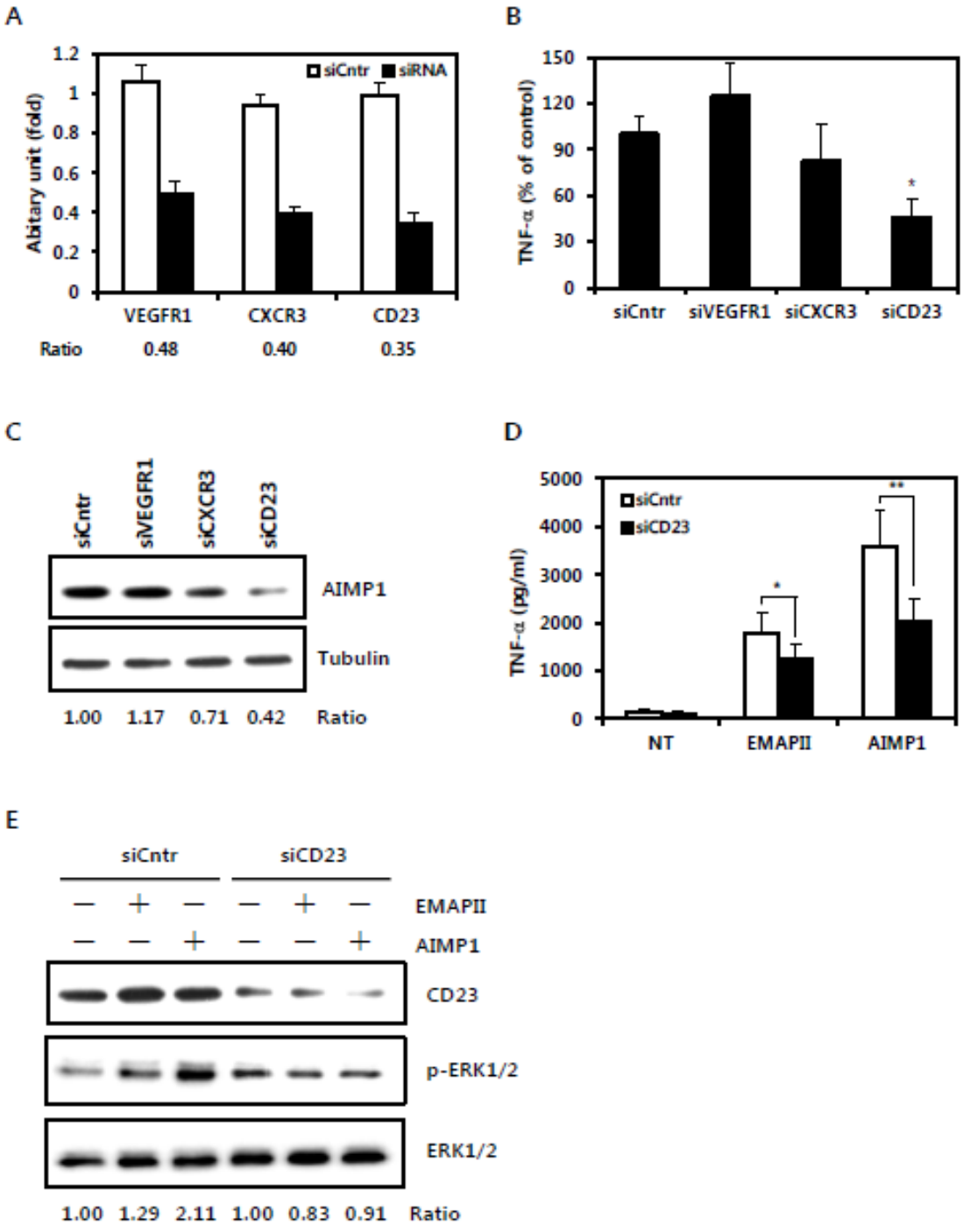


Fig. 8. The effects of CD23onAIMP1-induced TNF-α secretion in primary human PBMCs. (A) PBMCs were transfected with each siRNA for 48 hours and the effect on

receptor candidate gene expression was analyzed by quantitative RT-PCR. The ratio was calculated as the level in the receptor siRNA-transfected cells divided by the level in control siRNA-transfected cells. GAPDH was used as the internal control. (B) The effects of knockdown of CD23 and other known receptors of EMAP II on AIMP1-induced TNF-α production. PBMCs transfected with each siRNA were treated 100 nM of AIMP1 for 3 hours and the mRNA level of TNF-α were analyzed by quantitative RT-PCR. Data are expressed as the mean ± SEM (n = 3). * denotes a significant difference from negative control siRNA ($p < 0.05$). (C) The effect of receptor knockdown on biotinylated AIMP1 binding to PBMCs. Bound AIMP1 was analyzed by western blotting with streptavidin-conjugated peroxidase. The ratio represents the value of the level of AIMP1 in control siRNA-transfected cells divided by that in the receptor siRNA-transfected cells. Tubulin was used as the internal control. (D) The effect of CD23 knockdown on AIMP1- or EMAP II-induced TNF-α secretion. PBMCs were transfected with CD23 siRNA before treatment with EMAP II (100 nM) or AIMP1 (100 nM). The effects of the CD23 siRNA on EMAP II- or AIMP1- induced TNF-α secretion were analyzed by the TNF-α ELISA kit. Data are expressed as the mean ± SEM (n = 3). * and ** denote differences from negative control (p = 0. 162 and p = 0.041, respectively). (E) The effect of CD23 knockdown on AIMP1 and

EMAP II-induced ERK1/2 phosphorylation in PBMCs. Cells were transfected with CD23 siRNA followed by treatment with AIMP1 (100 nM) for 30 minutes. Cell lysates were analyzed using anti-CD23, p-ERK1/2, and ERK1/2 antibodies. The ratio represents the level of phospho/total ERK1/2 protein expressed from the PBMCs treated with EMAP II or AIMP1 compared with that of untreated control cells.

Together, all of these data suggest that in immune cells including THP-1 and PBMCs, CD23 is a functional receptor for AIMP1 and mediates AIMP1-induced TNF-α secretion through the ERK pathway in a manner different from EMAP II (**Fig. 9**).

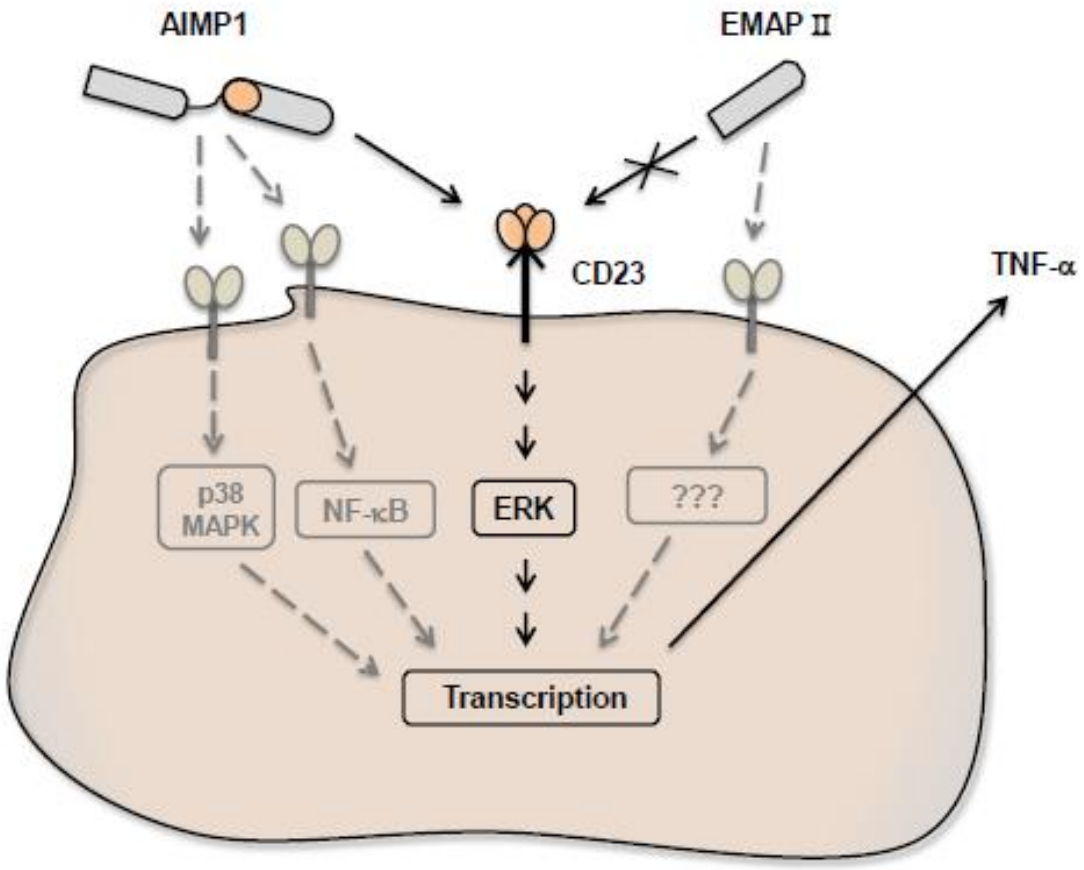


Fig. 9. Proposed model of AIMP1-induced TNF-α production through binding to CD23 on immune cells. When monocytic cells such as THP-1 cells are treated with AIMP1, it binds to cell surface membrane-bound CD23 and leads to phosphorylation and activation of the ERK pathway. The activation of the ERK pathway up-regulates the expression and secretion of TNF-α. AIMP1-induced TNF-α production is also involved in other signaling pathways through other receptors. EMAP II, which is the

C-terminal region of AIMP1, does not bind to CD23 and may produce TNF-α through another signaling pathway.

DISCUSSION

AIMP1 is a proinflammatory cytokine that works on diverse target cells such as monocytes, endothelial cells, and fibroblasts [36]. To elucidate the precise mechanisms by which it functions, there have been many efforts to identify its receptor. Whereas studies have implicated theα−subunit of ATP synthase and CXCR3 as AIMP1 receptors [19, 20] and VEGF receptors as EMAP II receptors in endothelial cells such as HUVECs [21], the precise signaling pathways that mediate the cytokine activity of AIMP1 or EMAP II in immune cells are unknown.

Here I show that CD23, a low affinity receptor for immunoglobulin E (IgE), is also a receptor for AIMP1 in immune cells. Unlike other Fc receptors for immune-globulins, CD23 is a type II integral membrane protein with a single transmembrane region, and is expressed on several cell types, including monocytes/macrophages, eosinophils, follicular dendritic cells, intestinal epithelial cells and B cells [23]. CD23 regulates monocyte activation and induces TNF-α production via the adhesion molecules CD11b-CD18 and CD11C-CD18 [37]. However, this signaling pathway is mediated by soluble CD23 cleaved from the membrane-bound form by ADAM10 [38, 39], and its function as a receptor related to TNF-α production remains unclear.

In this study, knockdown of VEGFR1 and CLEC10A significantly increased AIMP1-induced TNF-α secretion, suggesting that cross-talk exists between CD23 and VEGFR1 and/or CLEC10A. CD23 and CLEC10A contain a C-type lectin domain and showed high binding affinity for AIMP1. Moreover, VEGFR1 was identified as a receptor candidate of EMAP II, the C-terminal part of AIMP1. On explanation for my findings in that in THP-1 cells, CLEC10A and VEGFR1 bind to AIMP1 but are not functional receptors for AIMP1. Therefore the knockdown of CLEC10A and VEGFR1 may increase the AIMP1 binding affinity to CD23 and through this indirect mechanism their absence at the cell surface could increase AIMP1-induced TNF-α secretion.

Previous studies have shown that up-regulation of CD23 in primary human B cells and subsequent CD23-associated stimulation leads to the activation of ERK1/2, the tyrosine kinase Fyn, and the serine/threonine kinase Akt. However, Fyn and Akt were not shown to be activated by the cross-linking of CD23 in the monocytic cell lines U937 and THP-1 [31]. ERK1/2 and JNK are known to be involved in TNF-α production by the cross-linking of CD23 in human intestinal epithelial cells, and in these studies, p38 MAPK and NF-κB did not affect TNF-α production via CD23 [32]. Thus these earlier observations suggest that CD23 is involved in TNF-α secretion via the ERK pathway. Here I show that in THP-1 and PBMCs, AIMP1 binds to CD23 (**Fig. 3C, 8C**), induces

TNF-α secretion (**Fig. 2B, 8B**), and activates the ERK pathway (**Fig. 5C, 8E**). These results support the hypothesis that CD23 is a functional receptor for AIMP1.

My observations indicate that in THP-1 and PBMCs, the AIMP1-induced signaling pathway differs from the EMAP II-induced pathway, and that CD23 is a functional receptor for AIMP1, but not EMAP II. I identified that the central domain of AIMP1 (amino acid 101–192) is a potential CD23 binding site closely related to TNF-α production (**Fig. 6A, B**). However, EMAP II does not possess this region nor does it bind CD23. Thus, the central region of AIMP1 not containing EMAP II (amino acid 101–146) appears to mediate CD23 binding and TNF-α secretion.

AIMP1-induced TNF-α production was not completely blocked by CD23 siRNA (**Fig. 3C, 8C**) or by the MEK inhibitor, U0126 (**Fig. 5C, 7D**), suggesting that a CD23-independent signaling pathway exists for TNF-α production. TNF-α production involves other signaling pathways such as p38 MAPK and NF-κB as well as ERK1/2 [5]. Therefore it is possible that other AIMP1 receptors are linked to the p38 MAPK and NF-κB pathways. In addition, EMAP II-induced TNF-α production might be associated with receptors other than CD23. EMAP II and AIMP1 may share these receptors and/or signaling pathways.

In addition to its role as a receptor regulating IgE responses, CD23 is also cleaved

and released from the cell surface on various immune cells, a process mediated by ADAM10 metalloprotease, which yields a range of freely soluble CD23 variants that have pleiotropic cytokine-like activities [38, 39]. The identification of regions of CD23 responsible for its interaction with several known ligands has helped to elucidate structure-function relationships within the CD23 molecule. As a receptor, CD23 has multiple ligands including IgE, CD21, and αv integrins. IgE was the first defined ligand, and was found to bind to both membrane-bound and soluble forms of CD23. This interaction is carbohydrate-independent and does not require lectin-like activity of the head domain. Membrane-bound CD23 has a high binding affinity for IgE with a K_D = ~1 nM, while soluble CD23 interacts with IgE with a much lower affinity of K_D = 0.1~1 μM [40]. The second ligand for CD23 is CD21 [41], and this interaction involves both carbohydrate-dependent and -independent interactions [42]. The interaction of CD23 with CD21 is in the micromolar range ($K_D = 8.7 \times 10^{-7}$ M) [43]. In this study, I found that AIMP1 had a high binding affinity for soluble CD23 with a K_D = 4.3 nM, suggesting that AIMP1 interacts with membrane-bound CD23 with a much higher affinity than soluble form.

CD23 is a potentially useful diagnostic marker for a range of diseases and has been implicated in cellular and molecular processes associated with a variety of pathological

states. Soluble CD23 is a potent macrophage stimulator. High levels of this molecule have been reported in rheumatoid arthritis [44]. In addition, lumiliximab, an anti-CD23 monoclonal antibody, is a potential therapeutic antibody recently demonstrated to be safe in human and CD23 is important in orchestrating inflammation in allergic diseases and thus may represent an important therapeutic target [45]. In this study, a neutralizing anti-CD23 antibody (clone MHM6) which can compete with IgE for binding to a CD23 epitope suppressed AIMP1-induced TNF-α secretion (**Fig. 4C**), suggesting that the AIMP1 binding site of CD23 may overlap with the IgE binding site within its lectin-like domain and the AIMP1-CD23 interaction may be involved in pathophysiology of autoimmune disease.

I propose a model whereby AIMP1 induces TNF-α production through its binding to CD23 on THP-1 and PBMCs. When monocytic cells are treated with AIMP1, it binds to cell surface membrane-bound CD23 and leads to phosphorylation and activation of the ERK pathway. In turn, activation of the ERK pathway up-regulates the expression and secretion of TNF-α. AIMP1-induced TNF-α production then engages in other signaling pathways through other receptors. EMAP II, which is the C-terminal region of AIMP1, does not bind to CD23, but may be involved in inducing TNF-α production through another signaling pathway.

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ABSTRACT IN KOREA (국문초록)

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AIMP1/p43은 단백질합성효소 (ARS; aminoacyl-tRNA synthetase)들을 연결하는 보조인자 중 하나로 세포 밖으로 분비되어 면역반응을 조절하는 전염증성 싸이토카인으로 알려져 있다. 이러한 싸이토카인으로써의 기능에도 불구하고 아직까지 이와 관련된 AIMP1의 수용체 및 하위 신호전달체계에 대해서는 알려진 바가 없다. 본 연구에서는 대식세포로부터 종양괴사인자 알파 (TNF-α; tumor necrosis factor-alpha) 생산과 관련된 AIMP1의 수용체를 규명하고 그 수용체의 하위 신호전달체계가 어떻게 이루어 지는 지에 대하여 알아보았다. 수용성 수용체 라이브러리 검색을 통하여 AIMP1에 특이적으로 결합하는 수용체들을 선별하였으며, 이들 중 단핵구 유래 세포주인 THP-1 및 인간의 말초혈액 단핵구 (PBMCs; peripheral blood mononuclear cells)에서 AIMP1에 의한 TNF-α의 분비와 직접적으로 연관되는 수용체로 CD23/

FcγRII을 선별하였다. CD23의 발현을 조절함에 따라 THP-1 표면에 결합하는 AIMP1의 양 및 TNF-α 분비량이 조절되었으며, 이러한 현상은 CD23 하위의 ERK1/2 활성화와 관련되었다. 하지만 AIMP1의 C-말단조각으로 싸이토카인으로 알려진 EMAP II (Endothelial monocyte activating polypeptide II)는 CD23과 결합도, ERK1/2의 활성화도 일으키지 않았다. 이러한 결과들은 CD23이 THP-1 세포주와 말초혈액 단핵구와 같은 면역세포에서 AIMP1의 기능적 수용체이며, 이를 활용함으로써 AIMP1의 생체 내 싸이토카인 활성 및 면역시스템을 조절하는 새로운 치료 전략을 제시할 것으로 기대된다.

주요어: AIMP1, TNF-α, CD23, EMAP II, monocyte, cytokine

학번: 2007-30468

AKNOWLEDGEMENT (감사의 글)

여러 가지로 부족한 제가 학문적으로 한 단계 성장할 수 있었던 것은 많은 분들의 도움이 있었기에 가능한 일이었을 것입니다. 먼저 만학도인 저에게 몰입의 희열을 가르쳐 주시고 통섭적 학문으로써의 과학의 또 다른 길을 밝혀주신 김성훈 교수님께 가장 큰 감사의 말씀을 드립니다. 또한 미비한 저의 학위 논문을 하나하나 정성을 다해 지적해 주시고 바로 잡아주시며 심사해 주신 최응칠 교수님, 강창률 교수님, 고영규 교수님 그리고 박상규 교수님께 거듭 감사의 말씀 드리고 싶습니다.

저의 발전을 위해 이처럼 좋은 기회를 주시고 학위과정을 무사히 마칠 수 있도록 항상 격려와 관심으로 적극 지원해 주신 일동제약 이정치 회장님과 윤원영 회장님, 정연진 사장님, 윤웅섭 부사장님을 비롯한 여러 임원님들께 진심으로 감사 드리며 앞으로도 최선을 다해 회사의 발전에 힘쓸 것을 다짐해 봅니다. 이젠 회사엔 계시지 않지만 멀리서나마 지원을 아끼시지 않으신 김기원 박사님, 김태한 박사님, 김태용 박사님과 이금기 전회장님, 홍재선 전소장님, 강병화 전팀장님 모두 항상 건강하시길 기원합니다. 그리고

어려운 여건하에서도 끝까지 믿어주시고 많은 지원과 관심으로 살펴주신 강재훈 연구소장님께 말로는 다 표현 못하는 고마움을 전해 드립니다.

본 연구에 같이 참여해서 논문이 완성될 수 있도록 애써주시고 격려해주신 의약바이오컨버전스연구단의 한정민 박사님과 한국생명공학연구원의 박영우 박사님께도 진심으로 감사 드립니다. 또한 만학의 어려움을 함께 나누었던 존경하는 희숙 선배님을 비롯하여 부족한 선배의 졸업을 위해 물심양면으로 도와주었던 사랑스러운 후배들인 태희, 영선, 대규, 민철, 진영, 승재, 상범을 비롯한 서울대 약대 실험실 및 융합기술원 식구들에게도 고마움을 전합니다.

학위과정을 무사히 마칠 수 있도록 배려해 주시고 든든한 버팀목이 되어주신 이홍섭 이사님, 박천호 이사님, 연승우 이사님과 강대중 부장님, 이석준 부장님, 서명원 부장님, 이상영 부장님 등 일동제약 중앙연구소 팀장님 여러분들과 맡은 바 책임도 다하지 못하는 저를 항상 믿고 따라주는 고종희 과장, 김성범 대리, 권혜미 주임을 비롯한 연구원 여러분들께도 이 자리를 빌려 깊은 감사의 마음을 전합니다.

이제는 많이 쇠잔해 지셨지만 제 마음 속엔 언제나 영웅으로 남아 계시며 바르게 살아가도록 저를 일깨워 주신 아버님과 항상 따뜻한 미소로 저를 감싸 안아주시기 위해 당신의 희생을 감내하고 살아오신 어머님께 늦게나마

이 작은 결실을 보여 드릴 수 있다는 안도의 기쁨과 함께 머리 숙여 감사의 마음을 표합니다. 가까이에서 아들보다 더 소중하게 아껴주시고 챙겨주시며 때로는 저의 철없는 행동들까지도 거둬 주시는 저의 또 다른 부모님이신 장인어른과 장모님께 이루 형언할 수 없는 고마움과 함께 가슴 깊이 보은의 마음을 새겨봅니다. 그리고 바쁘다는 핑계로 가족의 일원으로써의 본분마저도 미루도록 허락해 주신 형님 내외분과 누님 내외분께도 고마움을 전해 드리며, 윗사람으로써의 도리도 잘 못하는 저를 항상 존대해 주는 처남 내외께도 감사를 표하고 싶습니다.

인생에서 즐거운 추억으로 간직해야 할 소중한 시간들을 아빠만의 욕심 때문에 함께하지 못해서 항상 미안했던 사랑하는 재연, 재민에게 항상 밝고 따뜻한 사람이 되길 바라는 마음과 함께 이 기쁨을 같이 나누고자 합니다. 무엇보다도 석사 학위논문을 준비하던 때부터 다시 박사 학위논문을 마무리하고 있는 지금까지 20 여 년간을 항상 곁에서 무한한 믿음과 힘이 되어 주었고 남은 제 삶에 늘 함께여야 할 소중한 사람인 아내 윤신선님에게 사랑하는 마음과 함께 더할 수 없는 고마움과 미안함을 전합니다.