Monocyte cell adhesion induced by a human aminoacyl-tRNA synthetase-associated factor, p43: identification of the related adhesion molecules and signal pathways

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Abstract: An aminoacyl-tRNA synthetase-associated factor, p43, was recently shown to be secreted to induce a proinflammatory response. Because a proinflammatory response involves the cell-cell adhesion between endothelial and immune cells, we first examined the mechanism of p43-induced cell-cell adhesion of myelomonocytic leukemia cells. Intercellular adhesion molecule-1 (ICAM-1) was up-regulated by p43 and mediated p43-induced cell-cell adhesion via the interaction with LFA-1 or Mac-1. We also investigated p43-stimulated signaling pathways involved in the homotypic THP-1 cell adhesion. Because the specific inhibitors for PI3-K (phosphatidylinositol 3-kinase), ERK (extracellular signal-regulating kinase), and p38 MAPK (mitogen-activated protein kinase) blocked p43-stimulated ICAM-1 expression and homotypic THP-1 cell adhesion, these kinases were responsible for p43-induced cell-cell adhesion. p43-Dependent activation of ERK was inhibited by PI3-K inhibitors, and the activation of p38 MAPK was not. Thus, the results of this work suggest that p43 should induce cell-cell adhesion via the PI3-K/ERK- and p38 MAPK-dependent up-regulation of ICAM-1. *J. Leukoc. Biol.* **71:** 223–230; 2002.

Key Words: *intercellular adhesion molecule-1 · mitogen-activated protein kinase · cell-cell adhesion · integrin β_2*

INTRODUCTION

EMAP II (endothelial monocyte-activating polypeptide II) was first isolated from the supernatant of the cultured murine fibrosarcoma [1] and functions as a novel cytokine inducing a proinflammatory response [2]. It is expressed prominently in macrophages and monocytes located in inflammatory autoimmune lesions of the rat central nervous system [3] and thus believed to activate monocytes and microglial cells in inflammatory lesion [4]. EMAP II also induces the increase in calcium concentration of the cytoplasm in mononuclear phagocytes and polymorphonuclear cells (PMNs), which activate and release myeloperoxidase [2]. In addition, it up-regulates tumor necrosis factor (TNF), TNF receptor-1, tissue factor (TF), and P-selectin and E-selectin related to the inflammation [5, 6]. These diverse functions of EMAP II imply its critical role in the inflammation processes.

EMAP II is equivalent to the C-terminal domain of p43, which is a noncatalytic protein associated with macromolecular aminoacyl-tRNA synthetase complex [7]. For this reason, EMAP II was thought to be a cleaved product of p43. However, we recently found that p43, but not EMAP II, is secreted from the intact cells to exert cytokine activities and induces various proinflammatory signaling molecules in the monocytic THP-1 cell [8]. Among p43-induced genes, expression of intercellular adhesion molecule-1 (ICAM-1) was more than tenfold increased by the treatment of p43.

ICAM-1 is a surface-adhesion molecule playing a role in lymphocyte extravasation by the mechanisms of cell-cell adhesion [9]. The homo- and heterotypic cell adhesions are involved in a variety of physiological and pathophysiological processes including inflammation and atherosclerosis [10]. ICAM-1 mediates cell-cell adhesion via its interaction with the activated lymphocyte function-associated antigen-1 (LFA-1) and/or Mac-1, called the integrin β_2 family members [11, 12]. The cell-cell adhesion is also induced by the increased level of ICAM-1 expression [13–16].

Mitogen-activated protein kinases (MAPKs) and other signaling molecules including phosphatidylinositol 3-kinase (PI3-K) are considered to play important roles in cell-cell adhesions [11, 17]. Because p43 stimulates the activity of nuclear factor-κB (NF-κB) and MAPK family members, extracellular signal-regulating kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK [8], we thought that these molecules might be involved in p43-induced expression of intercellular adhesion molecule-1 (ICAM-1) and cell adhesion. Here, we investigated whether p43-induced expression of ICAM-1 is actually responsible for homo- and heterotypic adhesion of monocytes and determined the related signal pathway.

MATERIALS AND METHODS

Cell culture and materials

THP-1 cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 50 µg/ml streptomycin and penicillin in a 5% CO_2 incubator at 37°C. Antibodies against phospho-ERK, phospho-p38, were purchased from New England Biolabs (Beverly, MA). Antiphosphotyrosine antibody was obtained from BD Transduction Laboratories (Franklin Lakes, NJ), and anti-PI3-K p85 antibody and anti-ICAM-1 antibody (sc-1511; for Western blot) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

Construction and purification of p43 deletions

The plasmids expressing p43-(1-312), p43-(1-147), and p43-(148-312) were described previously [18]. To construct p43-(1-108), pET28a (Novagen, Madison, WI) containing the full-length p43 was digested with *Asp*718 and *Sal*I. The large fragment was treated with the Klenow fragment to fill up the DNA ends, and the resulting DNAs were religated. The DNA fragments coding for p43-(1-90), p43-(91-170), p43-(91-256), p43-(91-312), p43-(218-312), and p43-(257-312) regions were synthesized by polymerase chain reaction (PCR) with specific primer sets. (The primer sequences will be available upon request.) The specific PCR products were digested with *Eco*RI and *Xho*I and ligated into pET28a cut with the same enzymes.

Each of the full-length p43 and p43-deleted constructs was expressed as His-tag fusion protein in *Escherichia coli* BL21 (DE3) and purified by nickel affinity chromatography and Mono Q or S ion-exchange chromatography as described previously [18]. To remove lipopolysaccharide (LPS), the protein solution was dialyzed in pyrogen-free buffer (10 mM potassium phosphate buffer, pH 6.0, 100 mM NaCl). After dialysis, the protein was loaded to polymyxin resin (Bio-Rad, Hercules, CA), pre-equilibrated with the same buffer, incubated for 20 min, and eluted. The concentration of the residual LPS was below 20 pg/ml when determined using the Limulus Amebocyte Lysate QCL-1000 kit (Bio-Whittaker, Walkersville, MD).

Western blots

The cells treated with p43 were harvested by centrifugation at 600 g for 5 min and lysed with 0.25 ml lysis buffer [50 mM HEPES, pH 7.4, 150 mM NaCl, 1 mM vanadate, 1 mM dithiothreitol, 1.0% Triton X-100, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF)]. For p38 MAPK assay, cells were lysed by sonication in radioimmune precipitation buffer (RIPA). The proteins in the lysates were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Milford, MA), and probed with specific antibodies. The antibodies bound to specific proteins were reacted with the secondary antibody conjugated with horseradish peroxidase and then detected with an enhanced chemiluminescence substrate kit (Amersham, Arlington Heights, IL).

PI3-K assay

After THP-1 cells were treated with p43 (100 nM), the cells were harvested at various time points. Then the cells were lysed with PI3-K lysis buffer [15 mM Tris, pH 7.50, 130 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10% glycerol, 1% Nonidet P-40 (NP-40), 5 mM sodium orthovanadate, and 0.1 mM PMSF]. The soluble lysates (500 µg) were then incubated with antibodies specific for the PI3-K subunit p85α for 3 h at 4°C, followed by additional incubation for 1 h with protein G-agarose beads (Gibco BRL-Life Technologies, Grand Island, NY). Then, the immune complex was washed four times with the PI3-K lysis buffer and resolved by 10% SDS-PAGE. Proteins on the gel were electrotransferred to a PVDF membrane (Millipore). The membranes were immunoblotted with antiphosphotyrosine antibody.

Homotypic cell-cell adhesion

THP-1 cells were inoculated at 2×10^6 cells/ml in 24-well plates. The cells were treated with each inhibitor at the indicated concentrations for 1 h. Then, each of p43 or its deletion mutants was added at 100 nM to the cells and incubated for 20 h. The homotypic adhesion of THP-1 cells was observed by microscope at 100× magnification or quantified by counting the adhesive cells in the representative aliquots from each culture on a hematocytometer as a modified method of Takeda et al. [19]. Briefly, after the THP-1 cells were

treated with none or p43, the THP-1 cells in aggregates coagulated with more than three cells were counted on the hematocytometer as the adhesive cells. We counted the adhesive cells in at least 600 cells for each execution. Degree of aggregation was calculated by the ratio of the number of the adhesive cells to the number of total cells.

For neutralization, the cells were pretreated with mouse anti-CD11a (MAB1386; Chemicon, El Segundo, CA), anti-CD11b (MAB1380; Chemicon), and anti-ICAM-1 (Chemicon) antibodies 1 h before p43 treatment. Quantification was performed as described above.

Assay of gene expression by cDNA array analysis

The Atlas Human cDNA Expression Array 1.2 (Clontech, Palo Alto, CA) was used for cDNA array analysis. Total and polyadenylated RNAs were prepared from the control or p43-treated THP-1 cells by the Atlas Pure Total RNA labeling system (Clontech) as recommended by the manufacturer. Polyadenylated RNA (1 µg) isolated from the control or p43-treated cells was converted to radioactive cDNA by reverse transcription (RT) in the presence of [α-^{32}P]-dATP. The radioactively labeled cDNA was then denatured and hybridized to the cDNA expression arrays as recommended by the manufacturer. The radioactivity on the membranes was quantified by a phosphoimager. We calculated the change in gene expression after p43 treatment as the percentage of the untreated cells, using three of the internal controls recommended by the manufacturer for normalization to ensure the comparability of the control and p43-treated samples.

Quantitative RT-PCR analysis

RNA (2 µg) was converted to cDNA by RT using Moloney murine leukemia virus (M-MLV) RT (Gibco BRL) and the anchored oligo-dT primer set. The cDNA was then amplified by PCR using the ICAM-1 primers: sense, 5′-TCACATGACTA-AGCCAAGAG-3′; antisense, 5′-CTGAGTGTCATTGTGAACAC-3′.

The reaction was run at 94°C (1 min), 58°C (40 s), and 72°C (50 s) for 35 cycles, which was within the linear reaction window. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in the same cDNA was also measured as control by RT-PCR using the GAPDH primers: sense, 5′-TCATTGACCT-CAACTACATG-3′; antisense, 5′-CCAAAGTTGTCATGGATGAC-3′. After amplification, the PCR products were resolved by agarose-gel electrophoresis.

Fluorescein-activated cell sorter (FACS) analysis of ICAM-1 surface expression on THP-1 cells

THP-1 cells (2×10^5/ml) grown in six-well plates were treated with none or 100 nM p43 for 18 h. Cells were then harvested, washed, and stained with 10 µg fluorescein isothiocyanate (FITC)-conjugated anti-CD54 antibody (Chemicon) for 3 h at 4°C. After washing, stained cells were analyzed by flow cytometry using Becton-Dickinson (San Jose, CA) FACS Caliber.

RESULTS

Monocyte cell adhesion is induced by p43

Homo- and heterotypic cell adhesion is not only involved in inflammation but also in morphogenesis, wound repair, tumor growth, and metastasis [17, 20–23]. We tested whether p43 induces hetero- and homotypic adhesion of myelomonocytic THP-1 cells. p43 stimulated the homotypic adhesion of THP-1 cells (**Fig. 1**) as well as their heterotypic adhesion with HUVECs (human umbilical vein endothelial cells; unpublished results). Here, we have just focused on the homotypic adhesion of THP-1 cells for experimental convenience because the same adhesion molecule induced by p43 is expected to be involved in homo- and heterotypic cell adhesions [24, 25]. The homotypic THP-1 cell adhesion was observed at 10–100 nM p43 (Fig. 1). Although a relatively high amount of p43 was used to induce ICAM-1 and cell aggregation (**Figs.** 1 and **2**), it appears to be a result of the low sensitivity of THP-1 cells to
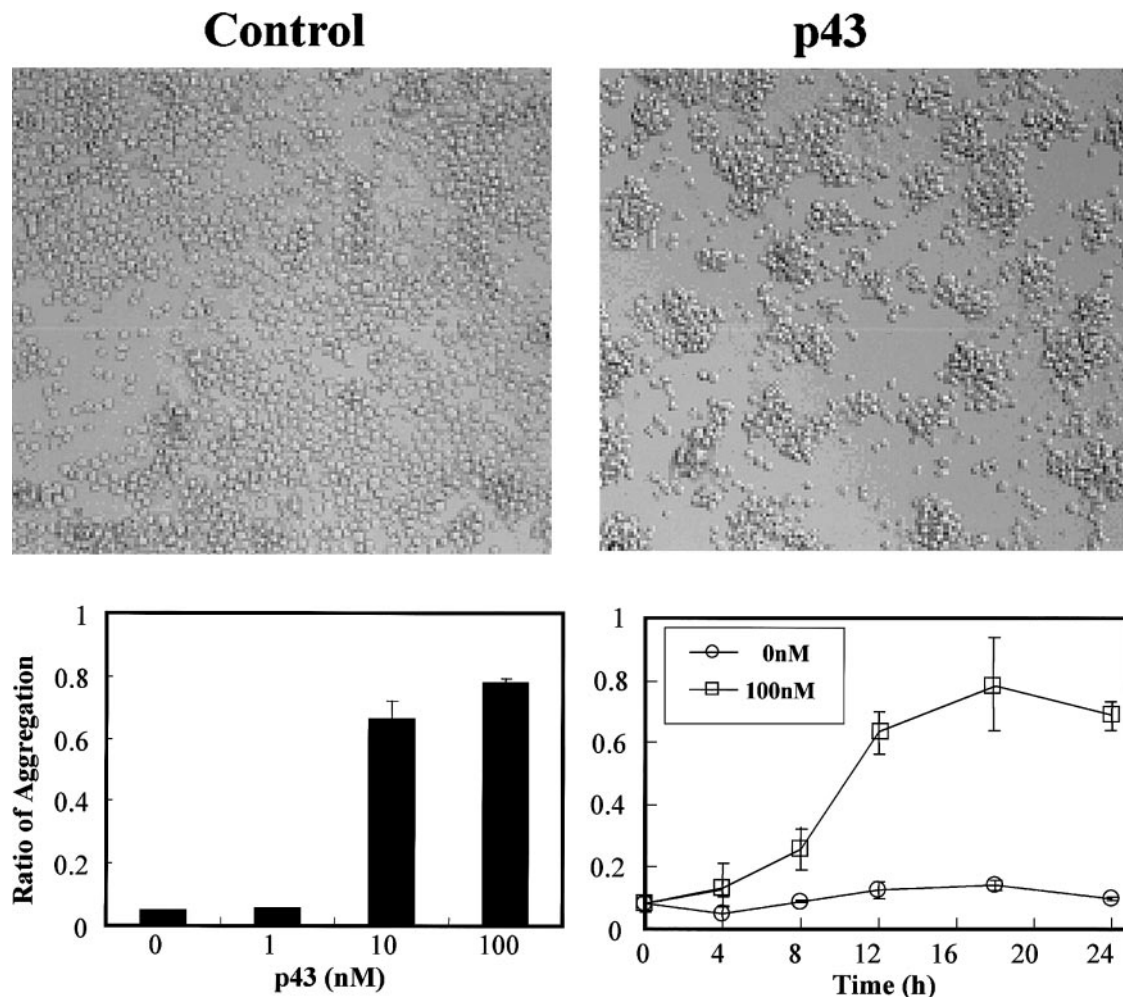
Fig. 1. Induction of homotypic adhesion of monocytic THP-1 cells by p43. THP-1 cells were untreated (Control) or treated with 100 nM p43. The cell adhesion was monitored microscopically and shown on the top panel. After quantification, the dose curve and the time course of p43-induced cell adhesion were plotted as a bar and line graph, respectively. For the dose curve, the cells were treated with p43 for 20 h. The bar and line graphs show the means ± SE obtained from four independent experiments.

p43. p43 Induced the THP-1 cell adhesion with the maximal effect about 18 h after p43 treatment (Fig. 1). The extended time course of p43-induced cell adhesion suggests that the de novo synthesis of (an) adhesion molecule(s) should be required.

p43 Induces the expression of ICAM-1 in monocytes

We have tested which adhesion molecules are induced by the treatment of p43. Among eight tested adhesion molecules, only ICAM-1 was up-regulated by p43 treatment (Fig. 2A). The mRNA level of ICAM-1 in THP-1 cells peaked 2 h after the treatment of p43. The up-regulation of ICAM-1 mRNA was also confirmed by RT-PCR analysis (Fig. 2B). Whereas GAPDH mRNA was constant at various time points of p43 treatment, the mRNA level of ICAM-1 was clearly up-regulated by p43 induction (Fig. 2B). We also monitored the protein amount of ICAM-1 by Western blot analysis and flow cytometry. The protein amount of ICAM-1 was increased gradually up to 18 h after p43 treatment (Fig. 2, C and D). All of these data suggest that p43 is capable of ICAM-1 induction, probably leading to THP-1 cell adhesion.

ICAM-1 is involved in p43-induced cell adhesion with LFA-1 and Mac-1

Because the expression level of ICAM-1 is increased robustly in THP-1 cells treated with p43, we tested whether ICAM-1 mediates p43-induced cell adhesion. When THP-1 cells were pretreated with an antibody specific for ICAM-1, p43-induced cell adhesion was prevented, indicating a critical role of ICAM-1 in cell adhesion (**Fig. 3**). Because mock immunoglobulin G (IgG) did not prevent p43-mediated cell adhesions, the prevention of cell adhesion by anti-ICAM-1 antibody was specific. Next, we tested whether ICAM-1-binding adhesion molecules, LFA-1 and Mac-1, are involved in p43-induced THP-1 cell adhesion. When THP-1 cells were preincubated with anti-LFA-1, and/or -Mac-1 antibodies before the cells were treated with p43, the adhesive effect of p43 was inhibited significantly, indicating the involvement of LFA-1 and Mac-1 as well (Fig. 3). Because LFA-1 and Mac-1 are able to associate with ICAM-1 for homotypic cell adhesion [11, 17, 26], the blocking effect of these two antibodies and p43-enhanced expression of ICAM-1 suggest that the interaction between ICAM-1 and the β2-integrin family members

Fig. 2. ICAM-1 expression was up-regulated by the treatment of THP-1 with p43. (A) THP-1 cells were treated with 100 nM p43 for the indicated time points, and the expression of the eight different cell adhesion molecules was determined by the hybridization analysis of cDNA array. Expression of each gene was quantified by a phosphoimager and normalized based on three housekeeping genes (cytoplasmic β-actin, 60S ribosomal protein L13A, and 40S ribosomal protein S9). The bar graph is representative of four independent experiments. (B) The expression of ICAM-1 mRNA was monitored by RT-PCR, after the THP-1 cells were treated with 100 nM p43 for indicated time points. GAPDH mRNA was also monitored as a control. ICAM-1 and GAPDH mRNAs were resolved by agarose gel electrophoresis. The gel picture is representative of three independent experiments. (C) The protein amounts of ICAM-1 were detected by Western blot analysis after THP-1 cells were treated with 100 nM p43 for indicated time points. Tubulin was also blotted for a control. The gel picture shown here was representative of three independent experiments. (D) ICAM-1 surface expression on THP-1 cells was measured by FACS analysis. After treatment with none or p43, cells were harvested, washed, and stained with 10 μg FITC-conjugated anti-ICAM-1 antibody for 3 h at 4°C. After washing, stained cells were analyzed by flow cytometry (Becton-Dickinson FACS Caliber). The mean fluorescence intensity (MFI) of p43-untreated cells with anti-p43 antibody to MFI of p43-treated cells with anti-p43 antibody is 1.5. The number of experiments with similar results = 3.
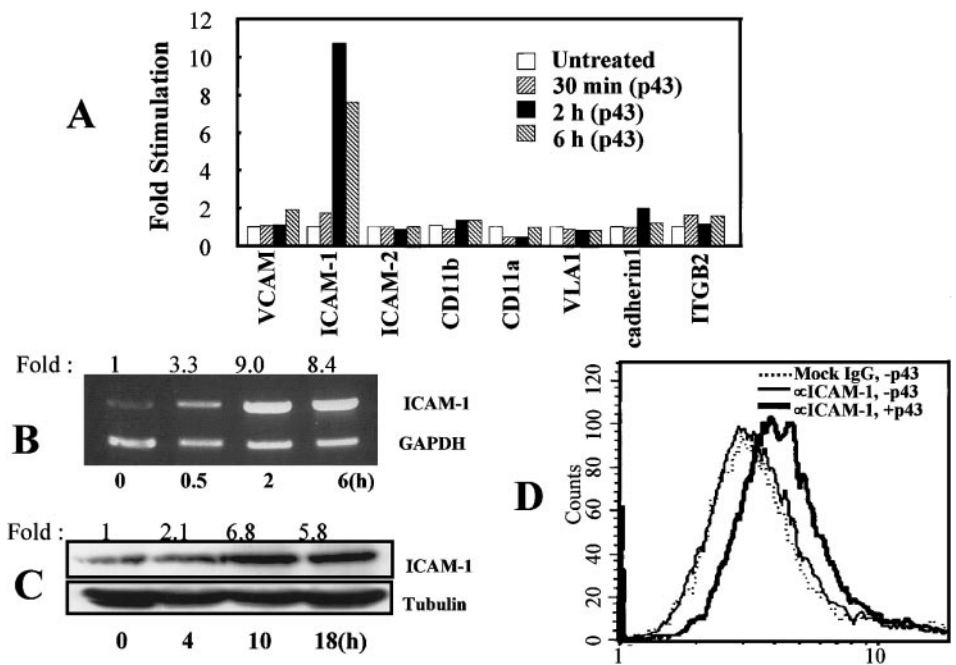
(LFA-1 and Mac-1) mainly contribute to p43-dependent intercellular adhesions.

p43-Induced expression plays a key role in p43-induced homotypic cell adhesion

To know whether up-regulation in ICAM-1 expression is essential for p43-induced cell adhesion, THP-1 cells were pretreated with cycloheximide, a translational inhibitor, before p43 incubation. Treatment with cycloheximide completely prevented p43-induced cell adhesion (**Fig. 4**). This result implies that the basal amount of ICAM-1 may not be sufficiently enough for cell adhesion, or up-regulation of other molecules induced by p43 may be important to stimulate THP-1 cell adhesion. Although we still don't know whether or how p43 increases the avidity or binding activity of β$_2$ integrins, it is concluded that p43-induced expression is required in p43-induced cell adhesion.

PI3-K and MAPKs are involved in p43-induced cell-cell adhesion

Previously, p43 has been shown to activate MAPKs and NF-κB [8]. We examined which signaling molecules among them are

Fig. 3. ICAM-1, LFA-1, and Mac-1 play a critical role in p43-induced cell adhesion. (A) Cells were preincubated with each of the indicated antibodies and then treated with 100 nM p43. The homotypic adhesion was detected under the microscope. (B) The homotypic adhesion was quantified as described in Materials and Methods. The bar graphs represent the means ± SE obtained in four independent experiments.
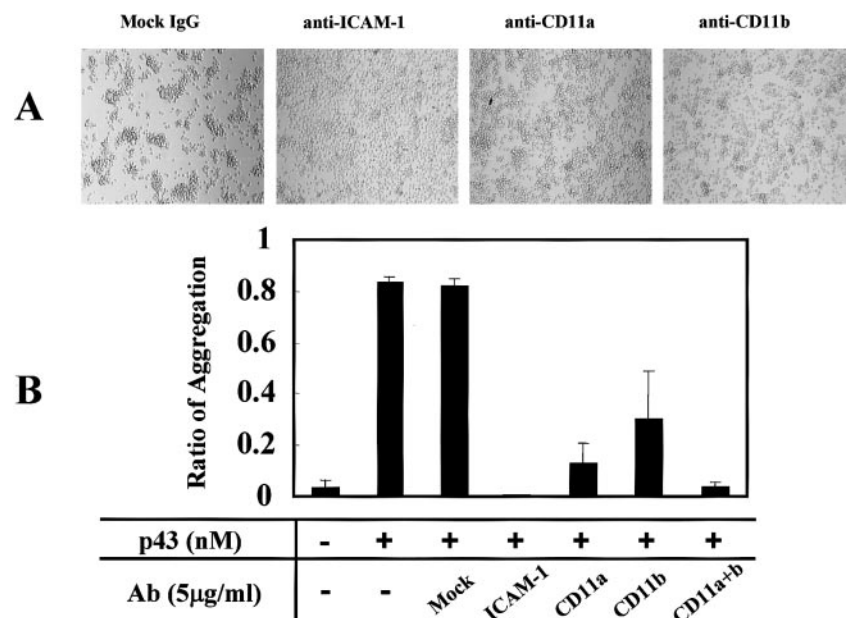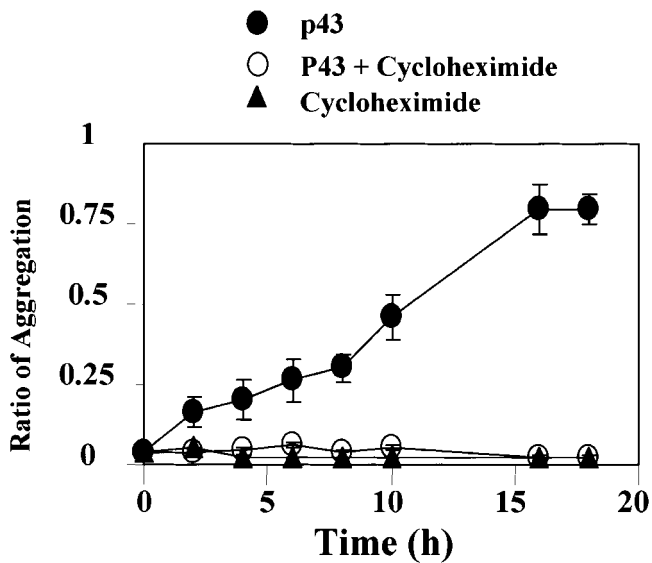
Fig. 4. THP-1 cells were pretreated with none or 5 μg/ml cycloheximide 30 min before incubation with 100 nM p43. The cells were aggregated by treatment with p43 alone (●), but the cell adhesion was not seen in cells treated with cycloheximide alone (▲) or p43 + pretreatment with cycloheximide (○).

actually involved in the up-regulation of ICAM-1 and p43-induced cell-cell adhesion. In **Figure 5A**, p43-induced cell adhesion was inhibited by pretreatment of PD98059 (PD), which specifically blocks the activation of ERK [27], or the p38 MAPK inhibitor, SB202190 (SB) [28]. To see a more specific effect of ERK, the cells were treated with U0126, a more specific inhibitor for MEK-1, and p43-induced cell-cell adhesion was inhibited by the treatment (unpublished results). Because PI3-K has been shown to mediate the adhesion of human neutrophils [17], we have also tested whether it is involved in p43-induced adhesion of THP-1 cells. The cells

were pretreated with PI3-K inhibitors, wortmannin (WT) or LY294002 (LY) [29], and subsequently with p43. p43-Induced cell adhesion was blocked by the presence of these inhibitors, indicating that PI3-K is also involved in this process (Fig. 5). We then tested whether the inhibitors block the up-regulation of ICAM-1 in response to p43. THP-1 cells were pretreated with the ERK or p38 MAPK inhibitor, and their effect on p43-induced ICAM-1 expression was monitored by the cDNA array and a Western blot (Fig. 5, B and C). p43-Induced up-regulation of ICAM-1 expression was blocked by PD98059, SB202190 [28, 30] (Fig. 5C), or U0126 (unpublished results). These results suggest that ERK and p38 MAPK are involved in ICAM-1 expression induced by p43. ICAM-1 expression in THP-1 cells was also blocked by the presence of the PI3-K inhibitor (Fig. 5, B and C), consistent with the effect of PI3-K inhibitors on THP-1 cell adhesion.
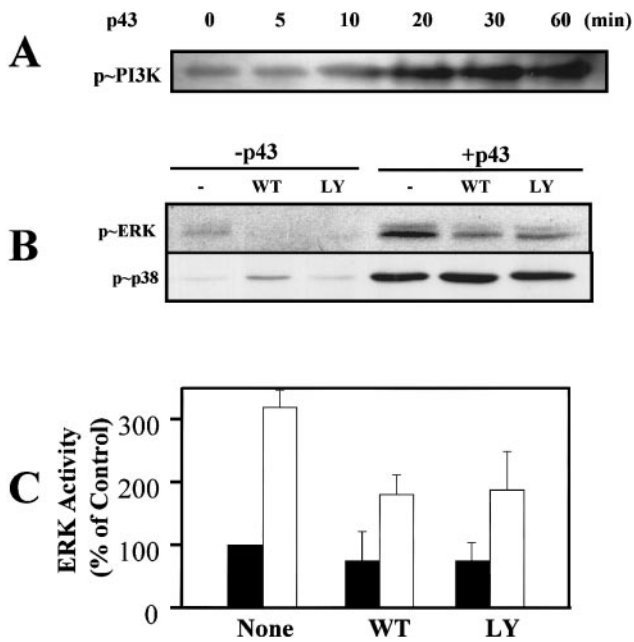
Activation of ERK is dependent on PI3-K

Because the inhibitory effect of wortmannin or LY294002 on p43-induced cell adhesion suggests that PI3-K is involved in this process, we examined whether p43 actually activates PI3-K. The time course of p43-induced PI3-K activation was monitored by tyrosine phosphorylation of p85α, which is the regulatory subunit of PI3-K [31]. p85α was precipitated with its specific antibody, and its phosphorylation was detected by Western blots using phosphotyrosine antiboby. Tyrosine phosphorylation of p85α was increased by the treatment of p43 in a time-dependent manner (**Fig. 6A**). We also observed the time course of PI3-K activation induced by p43, corresponding to the time course of p85α phosphorylation (unpublished results).

Because ERK and p38 MAPK were also activated by p43, we tested whether activation of these kinases is dependent on activation of PI3-K. THP-1 cells were pretreated with the PI3-K inhibitor, wortmannin or LY294002, and then with p43. The activation of ERK by p43 was blocked by pretreatment of
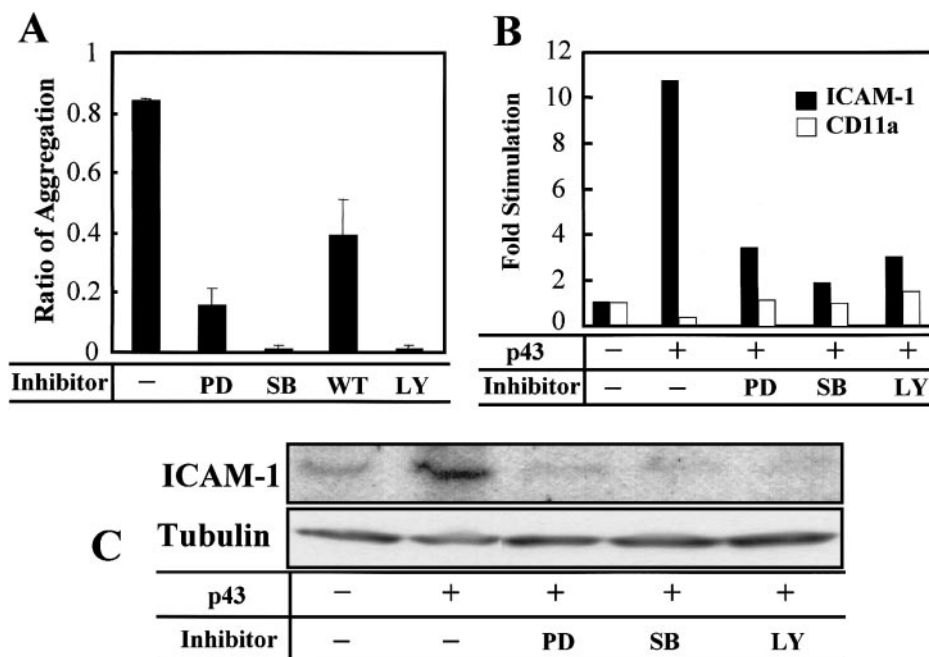
Fig. 5. PI3-K, ERK, and p38 MAPK inhibitors blocked p43-induced THP-1 cell adhesion and expression of ICAM-1. (A) THP-1 cells were preincubated with none, WT (100 nM), LY (10 μM), PD (10 μM), or SB (10 μM) and then treated with 100 nM p43 for 20 h. Homotypic cell adhesion was monitored as in Figure 1. (B) THP-1 cells were pretreated LY (10 μM), PD (10 μM), or SB (10 μM) and incubated for 2 h in the absence or presence of p43. ICAM-1 gene expression was quantified as described in Figure 2A. CD11a gene expression was seen as a control. (C) ICAM-1 expression in the THP-1 cells treated consecutively with none or each inhibitor (2 h), and p43 (20 h) was detected by Western blot analysis as in Figure 2C.

A

| p43 | 0 | 5 | 10 | 20 | 30 | 60 | (min) |

p~PI3K

B

| | -p43 | | | +p43 | | |
| | - | WT | LY | - | WT | LY |

p~ERK

p~p38

C

ERK Activity (% of Control)

None, WT, LY

Fig. 6. ERK activation is mediated by p43-induced PI3-K. (A) The THP-1 cells treated with 100 nM p43 were harvested, and the activity of PI3-K was determined as described in Experimental Procedures. PI3-K was precipitated with the antibody specific to PI3-K subunit, p85α, and its phosphorylation was determined with antiphosphotyrosine antibody. (B) The THP-1 cells were preincubated with WT (100 nM) or LY (10 μM) for 1 h and incubated for 1 h in the absence or presence of p43. The proteins extracted from the treated cells were resolved by SDS-PAGE, and the activities of ERK and p38 MAPK were determined by phosphorylation of ERK and p38 MAPK, respectively. (C) Quantification of Western blot for phosphorylation of ERK has been performed by densitometry. The bar graph shows the mean ± SE (n=3). The solid and open bars stand for the activities of ERK in the presence and absence of p43, respectively.

wortmannin or LY294002, and p38 MAPK was not (Fig. 6, B and C). This result indicates that only the activity of ERK was controlled by PI3-K, and activation of p38 MAPK by p43 is achieved in a PI3-K-independent manner.

Deletion mapping of p43

Because the full length of p43 had a stimulatory effect on cell adhesion, we investigated which region of p43 is responsible for cell adhesion. The full-length p43 and its deletion mutants
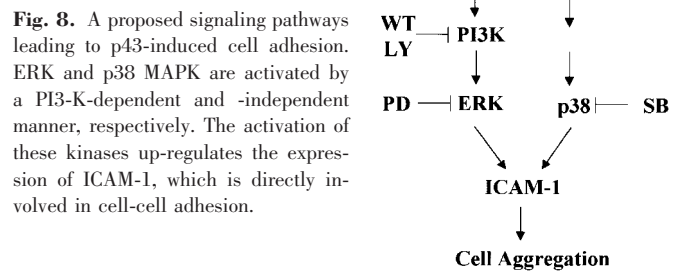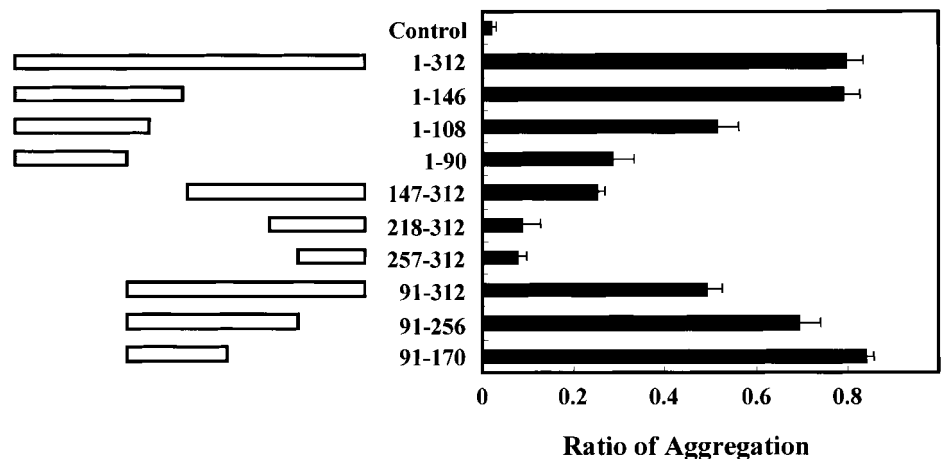
Fig. 8. A proposed signaling pathways leading to p43-induced cell adhesion. ERK and p38 MAPK are activated by a PI3-K-dependent and -independent manner, respectively. The activation of these kinases up-regulates the expression of ICAM-1, which is directly involved in cell-cell adhesion.

were expressed as recombinant proteins and purified to homogeneity. After removing trace amount of the contaminating LPS, each of the purified proteins was used for the experiments. THP-1 cells were treated with each of the purified proteins, and cell aggregates were observed 20 h after the treatment. The active domain was determined by monitoring the degree of the induced homotypic cell aggregation. Many purified p43 fragments were able to induce the homotypic cell adhesion, and the C-terminal fragments of p43 composed of residues 218–312 and 257–312 were inactive (**Fig. 7**). It is interesting that the full length or the peptides spanning outside of the EMAP II domain (residues 147–312) showed higher cell adhesion activity.

DISCUSSION

The role of EMAP II as a multifunctional cytokine has been investigated extensively [32–34]. To our surprise, p43, the precursor of EMAP II, has been found to behave like EMAP II in proinflammatory signal transduction [8]. The present work confirms more strongly our previous finding of p43 itself as a novel cytokine. Deletion mapping of p43 demonstrated that the N-terminal fragments of p43 spanning the non-EMAP II domain have the higher activity in the induction of THP-1 cell adhesion, indicating that the active domains of p43 are not restricted only in the EMAP II region (residues 147–312; Fig. 7). In addition, the p43-activated signaling pathway determined in this work further supports its specific proinflammatory cytokine activity (**Fig. 8**).

Fig. 7. Schematic diagram of p43 and its deletion mutants and their activities to induce the homotypic cell-cell adhesion. Each peptide was added at 100 nM. The bar graphs show the mean ± SE (n=3).

Control
1-312
1-146
1-108
1-90
147-312
218-312
257-312
91-312
91-256
91-170

Ratio of Aggregation

p43 induces expression of the cell-adhesion molecule, ICAM-1 (Fig. 2), which is an immunoglobulin-superfamily member that is widely expressed on the surface of vascular endothelium, monocytes, lymphocytes, and leukocytes [35–38]. ICAM-1 has been shown to interact with its primary ligand, CD11a/CD18 (LFA-1; integrin α_L/integrin β_2) and CD11b/CD18 (Mac-1; integrin α_M/integrin β_2) for cell adhesion. p43-Induced cell adhesion appears to result from up-regulation of ICAM-1 surface expression, making a firm association with LFA-1 and Mac-1, integrin β_2 family members (Fig. 3). The activity of p43 in integrin β_2-mediated homotypic cell adhesion indicates its contribution to inflammatory responses [25].

The function of p43 is not clear as to whether p43 activates integrin β_2 family members. Because p43 activates upstream signaling molecules, PI3-K and ERK, for integrin β_2 family members [17, 39], p43 is possible to enhance the avidity and/or binding activity of integrin β_2 family members. However, up-regulation of ICAM-1 induced by p43 is critical in THP-1 homotypic cell adhesion, because kinetics for cell adhesion is corresponding to the time course of ICAM-1 expression (Figs. 2 and 3), and more importantly, p43-induced cell adhesion was completely prevented by a protein synthesis inhibitor (Fig. 4). The results indicate that p43-induced cell adhesion requires up-regulation of ICAM-1 expression, but this work does not rule out a possible activity of p43 to alter the activity and/or the avidity of adhesion molecules.

We also investigated how p43 induces the expression of ICAM-1. Three MAPK family members and NF-κB were shown previously to be activated by p43 [8]. These signaling molecules are involved in intercellular adhesion of various cell types. NF-κB mediates cellular adhesion by the regulation of ICAM-1 expression in human fibroblasts and pancreatic acinar cells [13, 14]. ERK and PI3-K have been shown to be upstream regulators for integrin β_2-mediated cellular adhesion [17, 39]. Here, we dissected the p43-induced signaling pathway in a more systematic and detailed manner. PI3-K, ERK, and p38 MAPK have been found to regulate the homotypic THP-1 cell adhesion. These kinases are organized into PI3-K/ERK-dependent and p38 MAPK-dependent pathways (Fig. 8). A bifurcating point of the two parallel signaling pathways lies upstream of PI3-K. NF-κB remains to be studied as to whether it is involved in p43-dependent cell adhesion. p43-Dependent multiple signaling pathways determined here would give an important insight to understand the working mechanism of this novel cytokine. In conclusion, the results of this work identified that p43 stimulates different MAPKs via PI3-K-dependent and -independent ways, leading to cell-cell adhesion of monocytes (Fig. 8), and implicates p43 for the potent role in inflammatory and atherosclerotic processes.

ACKNOWLEDGMENTS

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