Insulin-Like Growth Factor-1 Enhances Inflammatory Responses in Endothelial Cells

Role of Gab1 and MEKK3 in TNF-α–Induced c-Jun and NF-κB Activation and Adhesion Molecule Expression

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Abstract—Insulin-like growth factor (IGF)-1 and the type I IGF-1 receptor are important regulators of vascular function that may contribute to cardiovascular disease. We hypothesized that IGF-1 causes endothelial cell dysfunction and expression of neutrophil and monocyte adhesion molecules by enhancing pro-inflammatory cytokine signal transduction. Long-term IGF-1 treatment of endothelial cells potentiated c-Jun and nuclear factor NF-κB activation by tumor necrosis factor (TNF)-α and enhanced TNF-α–mediated adhesion molecule expression. In response to IGF-1 treatment, the expression of kinases in the c-Jun/c-Jun NH₂-terminal kinase signaling pathway (MEKK1, MEK4, and JNK1/2) was unchanged, but expressions of insulin receptor substrate-1 and Grb2-associated binder-1 (Gab1) were significantly decreased. Because Gab1 is involved in both c-Jun and NF-κB activation by TNF-α, we focused on Gab1–dependent signaling. Gab1 inhibited c-Jun and NF-κB transcriptional activation by TNF-α. Interestingly, Gab1 inhibited c-Jun transcriptional activity induced by MEKK3 but not MEKK1 and MEK4. Gab1 associated with MEKK3, and a catalytically inactive form of MEKK3 inhibited TNF-α–induced c-Jun and NF-κB transcriptional activation, suggesting a critical role for Gab1 and MEKK3 in TNF-α signaling. These data demonstrate that Gab1 and MEKK3 play important roles in endothelial cell inflammation via regulating the activation of c-Jun and NF-κB. Furthermore, the IGF-1–mediated downregulation of Gab1 expression represents a novel mechanism to promote vascular inflammation and atherosclerosis. (Circ Res. 2002;90:1222-1230.)

Key Words: insulin-like growth factor-1 ■ signal transduction ■ Grb2-associated binder-1 ■ tumor necrosis factor-α ■ vascular inflammation

There is increasing support for the idea that inflammation plays a major role in the development of atherosclerosis. It is well known that inflammatory stimuli increase adhesion molecule expression at the initial sites of atherosclerosis. The adhesion of mononuclear leukocytes to the intact endothelium occurs very early in atherogenesis. In fact, it has been reported that an important feature of endothelial dysfunction, in the context of hypercholesterolemia, is the increased adhesion of mononuclear leukocytes to the endothelial surface at sites of subsequent fatty streaks and foam cell accumulation. Insulin-like growth factor (IGF)-1 is a polypeptide growth factor that binds to the type I IGF-1 receptor present on many cell types, including vascular smooth muscle cells (VSMCs) and endothelial cells. It has been reported that IGF-1 plays a role in cellular growth and survival in cardiovascular tissue, especially in pathological states. Serum and tissue concentrations of IGF-1 are highly regulated by multiple IGF-1–binding proteins. However, it is estimated that IGF-1 circulates at 50 to 200 ng/mL, making it potentially more important than insulin, which circulates at 0.5 to 6 ng/mL. Recently, Anwar et al have reported that tumor necrosis factor (TNF)-α, a cytokine that is upregulated in atherosclerotic plaques, decreases IGF-1 expression in VSMCs. Because IGF-1 is important for the growth and survival of VSMCs, these authors have suggested that IGF-1 downregulation by TNF-α plays a role in acute coronary syndromes by decreasing VSMC viability and promoting plaque instability. Conversely, in the present study, we propose that IGF-1 augments proinflammatory events that are induced by TNF-α in endothelial cells and that promote atherogenesis.

We show that long-term IGF-1 treatment enhances c-Jun and nuclear factor NF-κB activation, which is regulated by TNF-α, and enhances TNF-α–induced adhesion molecule expression in endothelial cells, leading to the increased adhesion of monocytes. IGF-1 treatment did not change the
expression of kinases involved in the c-Jun and NF-κB signaling pathway (eg, MEKK1, MEK4, and INK1/2) but decreased insulin receptor substrate (IRS)-1 and Grb2-associated binder-1 (Gab1) expression. A critical role for Gab1 has been found, inasmuch as inhibiting c-Jun and NF-κB signaling by overexpression of Gab1 decreased endothelial intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and E-selectin expression. These results provide the first evidence that long-term IGF-1 treatment leads to hypersensitivity to TNF-α-mediated signaling events via reduction in Gab1 expression.

Materials and Methods

Cell Culture and Materials
Bovine aortic endothelial cells (BAECs) were obtained from Cell Systems and maintained in culture in medium 199 (Cellgro) supplemented with 10% FBS, as described previously.6 Human umbilical vein endothelial cells (HUVECs) were isolated and maintained in 20% FBS/RPMI 1640 medium, as described previously.7 The HUVECs were plated onto gelatin-coated culture dishes or plates and were studied before cell confluence between passages 3 and 9. U937 cells were obtained from American Type Culture Collection and maintained in RPMI-1640 medium as previously described.8 Human recombinant IGF-1 was from Calbiochem. Human recombinant TNF-α was purchased from GIBCO.

Plasmids and Transfection
pDNA3 HA-Gab1 was a kind gift from Dr Hirano (Osaka University Medical School, Japan),9 and we subcloned Gab1 into pDNA3.1/His C vector (Invitrogen), which contains Xpress and His tags. pBTM-IRS-1 was a kind gift from Dr White (Harvard Medical School, Boston, Mass),10 and a constitutively active mutant form of MEK4 (pCMV CA-MEK4), in which Ser220 and Thr224 were mutated to glutamic acid and aspartic acid, was from Dr Morrison (Washington University, School of Medicine, St Louis, Mo).11 pRK5-MEKK3 (wild type), pRK5-CA-MEKK3 (constitutively active [CA] form by deletion of the first 11 amino acids), and DN-MEKK3 (dominant-negative form [DN] by changing the ATP binding site from Lys391 to Trp391) plasmids12 were subcloned into pCMV-tag2 (Stratagene), with the addition of Flag tag (Sigma Chemical Co), pFR-Luc, pFA2-c-Jun, pFC-dbdl, pNF-kB-luc, and pFC-MEK1 were from Stratagene. For transient expression experiments, BAECs and HUVECs were transfected with Lipofectamine Plus (GIBCO-BRL), as described previously.13 We determined the transfection efficiency with pcDNA3.1-LacZ transfection and by X-Gal staining. The transfection efficiency was 5% to 10% in HUVECs and 20% to 25% in BAECs.

Immunoprecipitation and Western Blot Analysis
After treatment with reagents, the cells were washed with PBS and harvested in 0.5 mL lysis buffer as described previously.13 Immunoprecipitation was performed as described previously with mouse anti-His (6x-His-Gly epitope, Invitrogen) or anti-HA (F-7) (Santa Cruz) antibody.14 Western blot analysis was performed as previously described.13 In brief, the blots were incubated for 4 hours at room temperature with the following antibodies: MEKK1, MEK4, 14-3-3, IRS-1, and IRS-2 (Santa Cruz); MEKK3 (Stressgene); Gab1 (U.B.I.); c-Jun, phospho-c-Jun, and phospho-MEK4 (Cell Signaling); Xpress (Invitrogen); or with Flag (Sigma) antibodies, followed by incubation with horseradish peroxidase–conjugated secondary

Figure 1. Long-term IGF-1 treatment enhances TNF-α–induced adhesion molecule expression and monocyte adhesion in HUVECs. HUVECs were growth-arrested for 16 hours and stimulated with or without IGF-1 (10 ng/mL) or vehicle for 16 hours. Then, cells were stimulated with TNF-α (5 ng/mL) or vehicle (A, B, and C) for 6 hours, and expression of ICAM-1 (A), VCAM-1 (B), and E-selectin (C) was measured as described in Materials and Methods. HUVECs were incubated with anti–ICAM-1 (A), anti–VCAM-1 (B), or anti–E-selectin (C) human monoclonal antibody for 1 hour and then with anti-mouse IgG horseradish peroxidase–conjugated antibody. After developing color with treatment of o-phenylenediamine dihydrochloride, the absorbance was measured by using an ELISA plate reader. Values are mean±SD (n=9). **P<0.01. An in vitro adhesion assay was performed to measure firm U937 human monocyte adhesion on TNF-α–stimulated (F and H) or vehicle-stimulated (E and G) (6 hours) HUVEC with (G and H) or without (E and F) pretreatment of IGF-1 for 16 hours. Data shown are pooled from 5 independent experiments. Fluorescent images (E and H) of U937 human monocyte adhesion on HUVECs are shown. In panel D, the attached cell numbers as seen in panels E through H were evaluated as mean±SD. **P<0.01.
antibody (Amersham). Immunoreactive bands were visualized by using enhanced chemiluminescence (ECL, Amersham).

**JNK Activity Assay**

JNK activity was measured with a commercially available kit based on phosphorylation of recombinant c-Jun (New England Biolabs), as described previously. \(^\text{13}\)

**PathDetect In Vivo Signal Transduction Pathway Reporting System**

c-Jun activity was assayed by using PathDetect Signal Transduction Pathway trans-Reporting Systems (Stratagene), as described previously. \(^\text{15}\)

NF-κB activity was measured by PathDetect Signal Transduction Pathway cis-Reporting Systems (Stratagene). Cells were cotransfected with pNF-κB-Luc reporter plasmid and pRL-PCMV and with other plasmids as indicated in the figures. After stimulation with a reagent, the cells were assessed for luciferase activity.

**Preparation of Recombinant Proteins and Coimmunoprecipitation (TNT-Coupled Reticulocyte Systems Assay)**

Recombinant His-Gab1, CA-MEK3, and DN-MEK3 were expressed by the TNT-Coupled Reticulocyte Lysate System (Promega) using pCDNA3.1 His-Gab1, pRK5 CA-MEK3, and pRK5 DN-MEK3, incorporating \(^{35}\)S]methionine, as previously described. \(^\text{16}\)

**Cell Surface Immunoassay**

To detect the expression of adhesion molecules, ELISA was performed as previously described. \(^\text{17}\) In brief, after 2% gelatin was coated in flat-bottomed 96-well plates, HUVECs were plated and incubated overnight. The cells were transfected with MEKK3 plasmid or empty vector, as described above. Forty-eight hours after transfection, HUVECs were washed with HBSS and fixed. Anti–ICAM-1, anti–VCAM, or anti–E-selectin antibody (Chemicon) was added to the wells. After horseradish peroxidase–conjugated goat anti-mouse IgG was added, the cells were incubated with o-phenylenediamine dihydrochloride in phosphate-citrate buffer. Absorbance at 490 nm was determined by a 1420 Multilabel counter (Victor2, Wallac).

**Adhesion Assay**

HUVECs were plated on 2% gelatin–coated 12-well plates and cultured to confluence in 20% FBS containing RPMI 1640 medium. The cells were incubated in 10 ng/mL IGF-1 for 16 hours and then treated with 5 ng/mL TNF-α for 8 hours. Human U937 cells were washed 3 times with serum-free RPMI 1640 medium and suspended in the serum-free RPMI 1640 medium. Approximately 1 mL of the cells (20 000 cells/mL) were put into the wells and incubated for 20 minutes. Then unfixed cells in the wells were washed out 3 times with serum-free RPMI 1640 medium. The adherent cells were counted in 5 randomly selected optical fields in each well, as previously described. \(^\text{18}\) Phase-contrast microphotographs of the cells in plates were taken under a microscope (IMT-2, Olympus) with an SC 35 Type 12 camera.

**Statistical Analysis**

Data are reported as mean±SD. Statistical analysis was performed with the StatView 4.0 package (ABACUS Concepts). Differences were analyzed by unpaired 2-tailed Student t tests or by Welch t tests, as appropriate. Values of \(P<0.05\) and \(P<0.01\) were taken as significant.

**Results**

**Effects of Long-Term IGF-1 Treatment on TNF-α–Induced Adhesion Molecule Expression and Monocyte Adhesion in Endothelial Cells**

First, we determined the effect of long-term IGF-1 treatment on TNF-α–induced ICAM-1, VCAM-1, and E-selectin expression. Because of the species specificity of the antibodies...
for adhesion molecules, we used HUVECs to determine the expression of ICAM-1, VCAM-1, and E-selectin (Figure 1). HUVECs were exposed to 10 ng/mL IGF-1 or vehicle for 16 hours. Cells were then stimulated with vehicle or TNF-α (5 ng/mL) for 6 hours. As shown in Figure 1, IGF-1 treatment (10 ng/mL for 22 hours) did not significantly increase ICAM-1, VCAM-1, or E-selectin expression. TNF-α treatment for 6 hours alone stimulated ICAM-1, VCAM-1, and E-selectin expression significantly (absorbance at 490-nm optical density was 0.37 ± 0.008, 0.41 ± 0.014, and 0.38 ± 0.01 [mean ± SD], respectively). Compared with 6 hours of TNF-α treatment alone, pretreatment with IGF-1 significantly enhanced TNF-α-mediated expression of ICAM-1, VCAM-1, and E-selectin expression (Figures 1A, 1B, and 1C).

To determine the biological significance of IGF-1 on TNF-α–induced adhesion molecule expression, we examined the ability of IGF-1 pretreatment to augment U937 cell adhesion to HUVECs. As shown in Figure 1D, treatment of HUVECs with 5 ng/mL TNF-α for 6 hours increased U937 cell adhesion to HUVECs (Figures 1E and 1F), whereas IGF-1 alone had no effect (Figure 1G). When HUVECs were pretreated with IGF-1 for 16 hours and then incubated with TNF-α for 6 hours, adhesion of U937 cells to HUVECs was significantly increased compared with TNF-α treatment alone for 6 hours (∼77% increase) (Figure 1H). In summary, these results demonstrate that long-term IGF-1 treatment enhances TNF-α–mediated adhesion molecule expression and monocyte adhesion to endothelial cells.

**Effect of Long-Term IGF-1 Treatment on TNF-α–Induced c-Jun and NF-κB Transcriptional Activation in Endothelial Cells**

It has been reported that c-Jun– and NF-κB–dependent events regulate adhesion molecule expression in endothelial cells. Therefore, we examined the effects of long-term IGF-1 treatment on c-Jun and NF-κB activation induced by TNF-α in endothelial cells. We transiently transfected BAECs with c-Jun and NF-κB reporter genes. After 24 hours of transfection, BAECs were exposed to 10 ng/mL IGF-1 or vehicle for 16 hours. Cells were then stimulated with vehicle or TNF-α (5 ng/mL) for 12 hours. As shown in Figures 2A and 2B (lanes 1 and 3), treatment with 5 ng/mL TNF-α led to a 2.8 ± 0.1- and 2.0 ± 0.2-fold stimulation of c-Jun and NF-κB activation, respectively, measured by reporter gene assay. Pretreatment with 10 ng/mL IGF-1 for 16 hours itself did not increase c-Jun and NF-κB activation (lane 2) but significantly enhanced TNF-α–induced c-Jun and NF-κB activation (3.5 ± 0.2- and 2.6 ± 0.1-fold increase, respectively; Figures 2A and 2B, lane 4). We also found a similar enhancing effect of IGF-1 on TNF-α–induced c-Jun and NF-κB activation in HUVECs (data not shown). These data suggest that long-term IGF-1 treatment potentiates c-Jun and NF-κB activation by TNF-α.
Effects of Gab1 and IRS-1 Expression on Activation of c-Jun and NF-κB in Response to TNF-α in Endothelial Cells

We first investigated whether overexpression of Gab1 inhibited TNF-α–mediated c-Jun or NF-κB activation in BAECs, which have a higher transfection efficiency than HUVECs. BAECs were transiently transfected with pBTM-IRS-1, HA-tagged Gab1 (HA-Gab1), or vector alone with c-Jun or NF-κB reporter genes. TNF-α increased c-Jun activation, as shown in Figures 4A and 4C. Overexpression of Gab1 but not IRS-1 inhibited TNF-α–induced c-Jun activation. Interestingly, overexpression of both IRS-1 and Gab1 inhibited NF-κB transcriptional activity in a dose-dependent manner (Figures 4B and 4D). We also found a similar inhibitory effect of Gab1 on TNF-α–induced c-Jun and NF-κB activation in HUVECs (data not shown).

Next, to determine the role of Gab1 on the enhancing effect of IGF-1 on TNF-α–induced signaling, we evaluated whether overexpression of Gab1 can reverse the enhancing effect of IGF-1 on TNF-α–induced c-Jun and NF-κB activation. As shown in Figure 2, 10 ng/mL IGF-1 enhanced TNF-α (5 ng/mL)–induced c-Jun and NF-κB activation (compare lanes 3 and 4). We found that overexpression of Gab1 reversed IGF-1–mediated enhancement of c-Jun and NF-κB activation (compare lanes 4 and 6). At the highest concentration of Gab1, there was inhibition of TNF-α signaling (compare lanes 3 and 7). HA-Gab1 expression was dose dependent on cDNA amount (data not shown).

Gab1 Inhibits MEKK3-Mediated, but Not MEKK1- and MEKK4-Mediated, c-Jun and NF-κB Transcriptional Activity

MEKK1 is MAPK kinase kinase-1, which has been reported to regulate ERK1/2, JNK, and NF-κB activation in several cell lines. Therefore, we evaluated the effect of Gab1 on c-Jun and NF-κB transcriptional activity induced by a CA form of MEKK1 (CA-MEKK1). As shown in Figure 5A, transient transfection of BAECs with CA-MEKK1 induced c-Jun and NF-κB transcriptional activity by 3- to 4-fold (Figures 5A and 5D). Overexpression of Gab1 did not significantly inhibit CA-MEKK1–stimulated c-Jun and NF-κB transcriptional activity (Figures 5A and 5D). MEKK3 is a MAPK kinase specific for MKK4, MKK7, and MKK6, and it regulates JNK, p38, and NF-κB activation separately from the MEKK1 pathway. Recently, Yang et al.33 reported that MEKK3 plays a critical role in TNF-α–induced NF-κB activation and directly phosphorylates IκB kinase (IKK). To examine the role of Gab1 in MEKK3-mediated c-Jun and NF-κB transcriptional activity, we transiently transfected CA-MEKK3 and determined c-Jun and NF-κB transcriptional activity by reporter gene assay. As shown in Figures 5B and 5E, CA-MEKK3 also induced a 3- to 4-fold increase in c-Jun and NF-κB transcriptional activity. Overexpression of Gab1 significantly inhibited CA-MEKK3–induced c-Jun and NF-κB transcriptional activity in a dose-dependent manner (Figures 5B and 5E). As shown in Figures 5C and 5E, a DN form of MEKK3 (DN-MEKK3) significantly inhibited TNF-α–induced c-Jun and NF-κB transcriptional activity.
Gab1 Associates With MEKK3 In Vivo and In Vitro

Because it has been reported that Gab1 associates with ERK1/2,24 we examined whether Gab1 can associate with MEKK3. We coexpressed Xpress-tagged Gab1 (Xp-Gab1) and Flag-tagged MEKK3 (Flag-MEKK3) in BAECs and performed communoprecipitation assays. Immunoprecipitation of Flag-MEKK3 with the anti-Xpress antibody brought down Xp-Gab1 (Figure 6D).

The nature of the interaction between Gab1 and MEKK3 was further studied by communoprecipitation in vitro with the use of well-characterized proteins (Figures 6E and 6F). As shown in Figure 6F, His-tagged Gab1 wild type (lanes 1 through 4) and either CA-MEKK3 (lane 2) or DN-MEKK3 (lanes 3 and 4) were expressed in vitro by using the rabbit reticulocyte lysate system and communoprecipitated by using anti-His mouse monoclonal IgG2a (lanes 1 through 3) or anti-Flag mouse monoclonal IgG2a antibody as a control (lane 4) (Figure 6F).

Effects of Gab1 on CA-MEKK1- or CA-MEKK3-induced c-Jun and NF-κB transcription activity and the effects of DN-MEKK3 on TNF-α-mediated c-Jun and NF-κB transcription activity in BAECs. BAECs were transfected with pFR-Luc plasmid and pFA2-c-Jun plasmid (A through C) or pNF-κB-luc-plasmid (D through F) with pcDNA3.1 HA-Gab1 in the indicated amounts. To control transfection efficiency, pRL-CMV was cotransfected with a luciferase control reporter vector (A through F), CA-MEKK1 (A and D), CA-MEKK3 (B and E), or DN-MEKK3 (C and F) was transfected in the indicated amounts, and pcDNA3.1 vector was used to provide equal amounts of transfected DNA (A through F). After 24 hours of transfection, growth-arrested BAECs were stimulated by TNF-α (20 ng/mL) (C and F). After 16 hours of stimulation (C and F) or 48 hours after transfection (A, B, D, and E), luciferase c-Jun transcriptional activity (A through C) or NF-κB transcriptional activity (D through F) was assayed by using a dual-luciferase reporter assay system, and luciferase luminescence was counted with a luminometer and then normalized to cotransfected luciferase activity, as described in Materials and Methods. Results are the mean±SD of 3 to 6 independent experiments. *P<0.01.

Expression of Gab1 Regulates
CA-MEKK3–Induced ICAM-1, VCAM-1, and E-Selectin Expression in HUVECs

Both c-Jun and NF-κB are important mediators of adhesion molecule gene expression in HUVECs.19,25 Because we found that Gab1 plays a critical role in TNF-α-induced and MEKK3-induced c-Jun and NF-κB transcriptional activity, we determined the effects of overexpression of Gab1 on CA-MEKK3–induced ICAM-1, VCAM-1, and E-selectin expression in HUVECs. As shown in Figures 7A through 7C, Gab1 significantly inhibited CA-MEKK3–induced ICAM-1, VCAM-1, and E-selectin expression (measured by ELISA) in a dose-dependent manner. These data suggest that Gab1 inhibits c-Jun and NF-κB transcriptional activity and decreases MEKK3–induced adhesion molecule expression.

Discussion

Inflammatory stimuli, including TNF-α, increase adhesion molecule expression and contribute to the initial phase of atherogenesis.1 In the present study, the increased response to inflammatory stimuli demonstrated with IGF-1 treatment can
be extrapolated to states of hyperinsulinemia and obesity. Specifically, reduction of Gab1 expression by long-term IGF-1 treatment in endothelial cells may contribute to the pathogenesis of atherosclerosis in hyperinsulinemia and obesity by promoting inflammation. The mechanism by which IGF-1 reduces IRS-1 family member expression is unclear. However, deVente et al.28 have reported that protein kinase C decreases IRS-1 expression via a transcriptional

Figure 6. Effect of Gab1 on CA-MEK3-mediated MEK4 and IKK activation (A) and CA-MEK4-mediated c-Jun activation and interaction of Gab1 with MEK3 in vivo (D) and in vitro (E). A and B, BAECs were cotransfected with or without CA-MEK3 and with or without HA-Gab1 constructs as indicated. After 24 hours, the medium was changed to serum-free F-12 for an additional 24 hours. Then the cells were harvested in lysis buffer, and MEK4 and IKK activity was measured by Western blot analysis with phosphospecific MEK4 (A) and IKK (B) antibody. No significant difference in the amount of MEK4 and IKK was observed in samples by Western blot analysis with anti-MEK4 and anti-IKK (data not shown). C, BAECs were transfected with pFR-Luc plasmid and pFA2-c-Jun plasmid with pcDNA3.1 HA-Gab1 in the indicated amounts. To control transfection efficiency, pRL-CMV was cotransfected with a luciferase control reporter vector. CA-MEK4 was transfected in the indicated amount, and pcDNA3.1 vector was used to provide equal

Figure 7. Effects of Gab1 on MEK3-mediated ICAM-1, VCAM-1, and E-selectin expression in HUVECs. After transfection of CA-MEK3 and Gab1 (A through C) construct and pcDNA3.1 vector at the indicated amounts in 96-well plates, HUVECs were incubated in full serum-containing medium for 32 hours, and then the cells were incubated with anti-ICAM-1 (A), anti-VCAM-1 (B), or anti-E-selectin (C) human monoclonal antibody for 1 hour and then with anti-mouse IgG horseradish peroxidase (HRP)-conjugated antibody. After developing color with treatment of o-phenylenediamine dihydrochloride, the absorbance was measured by using an ELISA plate reader. *P<0.05, **P<0.01.

Gab1

A. ICAM-1

B. VCAM-1

C. E-selectin
mechanism. These data plus our observation of Gab1-induced and IRS-1–induced decreases after IGF-1 treatment suggest that this is an important regulatory pathway.

It has been reported that IGF-1 is growth promoting for VSMCs and plays a pathological role in cardiovascular diseases. Until now, the primary role of IGF-1 in endothelial cells has been to stimulate angiogenesis and tube formation. In the present study, we found that a long-term low dose of IGF-1 (10 ng/mL) significantly enhances TNF-α–induced adhesion molecule expression in endothelial cells. These novel findings suggest a role for IGF-1 during the initial phase of atherosclerosis, related to vascular inflammation. Of interest, Balaram and colleagues have reported that a high concentration of IGF-1 (150 ng/mL) increases ICAM-1 expression but that the effect of IGF-1 on ICAM-1 expression is much weaker than that of TNF-α.

There is increasing evidence to indicate that IGF-1 may have a critical role in plaque stability via regulating VSMC survival. Patel et al have reported that human plaque–derived VSMCs show an intrinsic sensitivity to apoptosis, which is caused in part by defective expression of the IGF-1 receptor, and impaired IGF-1–mediated survival signaling. Interestingly, Anwar et al have reported that TNF-α reduces IGF-1 in VSMCs, and this downregulation by TNF-α may play a role in acute coronary syndrome by decreasing VSMC viability and promoting plaque instability. On the basis of our findings, we propose that IGF-1 is an enhancing factor for cytokine-induced endothelial cell inflammation and may play a role in the early phase of atherogenesis. Further investigation will be needed to clarify the role of IGF-1 in early atherosclerosis formation.

In the present study, we found that Gab1 associates directly with MEKK3, and that Gab1 then inhibits MEKK3-mediated c-Jun and NF-κB transcription activity and adhesion molecule expression (Figure 8). Members of the mammalian MEKK family of serine/threonine kinases have been demonstrated to stimulate MEKK4 and JNK signaling. Of note, it has been reported that MEKK3 plays a major role in TNF-α–induced NF-κB activation and that MEKK3 directly phosphorylates IKK. Because MEKK3 is able to regulate both c-Jun and NF-κB pathways, MEKK3 activity may have a greater effect on the expression of adhesion molecules than other MAPK kinase kinases, such as ASK1 or TAK1. Recently, Roshan et al and Gual et al have reported that Gab1 associates directly with phosphorylated ERK2 and protein kinase C and that Gab1 acts as a substrate for these kinases. The mechanism of interaction between Gab1 and these serine/threonine kinases (including the role of serine/threonine phosphorylation of Gab1) remains unclear. We also determined whether Gab1 can coimmunoprecipitate with MEKK1, but we could not detect any association of Gab1 with MEKK1 (data not shown). However, we could not conclude that there is no association of Gab1 with MEKK1, because the detection of interaction by using coimmunoprecipitation is heavily dependent on the antibodies used.

In contrast to our results, Garcia-Guzman et al have reported that the complex formation of tyrosine-phosphorylated Gab1 and Crk is correlated with stimulation of the JNK pathway. One explanation for this difference is that Garcia-Guzman studied hepatocyte growth factor, which stimulated IGF1 tyrosine phosphorylation by ~5- to 10-fold, whereas tyrosine phosphorylation of Gab1 by TNF-α was increased only ~1.5-fold (data not shown). These results suggest that Gab1-MEKK3 interaction and MEKK3 activation may be regulated by Gab1 phosphorylation, an important area for future investigation.

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References


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