Glucose Regulates Interleukin-8 Production in Aortic Endothelial Cells through Activation of the p38 Mitogen-activated Protein Kinase Pathway in Diabetes*

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From the Départment of Diabetes, Beckman Research Institute, City of Hope National Medical Center, Duarte, California, the Ðvision of Cardiology, UCLA, Los Angeles, California, and the Ñivision of Endocrinology and Metabolism, Cardiovascular Research Center and Ðivision of Pharmacology, University of Virginia, Charlottesville, Virginia 22908

We have shown that chronic elevated glucose (25 mM) increases monocyte adhesion to human aortic endothelial cells (EC). This increased adhesion is mediated primarily through induction of interleukin (IL)-8 via activation of the transcription factor AP-1 (Srinivasan, S., Yeh, M., Danziger, E. C., Hatley, M. E., Riggan, A. E., Leitinger, N., Berliner, J. A., and Hedrick, C. C. (2003) Circ. Res. 92, 371–377). In the current study, we identified the elements in the AP-1 transcriptional complex that are activated by glucose. These elements include c-Jun, c-Fos, and Fra-1. AP-1 is activated by cellular oxidative stress, and we have reported significant production of ROS by high glucose-cultured cells. We examined signaling pathways upstream of AP-1 in EC that lead to AP-1 activation by HG. EC cultured in 25 mM glucose had a 2-fold increase in p38 phosphorylation compared with control normal glucose-cultured EC. Inhibition of the p38 pathway using 5 μM SB203580 significantly reduced glucose-mediated IL-8 mRNA production by 60%. Furthermore, blocking p38 pathway activation using a dominant-negative p38 construct significantly reduced glucose-mediated monocyte adhesion by 50%. Thus, glucose-stimulated monocyte adhesion is primarily regulated through phosphorylation of p38 with subsequent activation of AP-1, leading to IL-8 production. To study this pathway in the setting of diabetes, we used the db/db mouse. P38 phosphorylation was increased in diabetic db/db mice compared with control mice. We found a dramatic elevation in plasma levels of KC, the mouse ortholog of IL-8 in diabetic db/db mice (1800 ± 100 pg/ml KC in db/db versus 300 ± 75 pg/ml in C57BL/6j control mice, p < 0.0001). Inhibition of the p38 pathway in diabetic db/db mice significantly reduced monocyte adhesion by 50%. Taken together, these data indicate that chronic elevated glucose in diabetes activates the p38 MAP kinase pathway to increase inflammatory IL-8 gene induction and monocyte/endothelial adhesion.

Atherosclerosis is increased severalfold in patients with diabetes. In fact, atherosclerosis remains the primary complication of patients with type 2 diabetes (1–7). A key early event in atherosclerosis development is increased interaction of monocytes and endothelial cells in the vessel wall (8, 9). Monocytes are the primary inflammatory cells that are localized to human atherosclerotic plaques (10, 11). In the monocyte recruitment cascade, monocytes are recruited to sites of endothelial cell activation and roll along the vascular endothelium, where they become activated by chemokines, including monocyte chemotactic protein-1 and interleukin-8 (IL-8). The monocytes slow down and arrest firmly to the endothelium and then transmigrate through the EC monolayer (12–14). Monocyte chemotactic protein-1 is secreted by activated endothelial cells, and IL-8 is secreted by activated endothelial cells and monocytes (15). Monocyte chemotactic protein-1 is highly expressed in atherosclerotic lesions (16). We have recently shown that IL-8 production is increased in human EC cultured chronically in elevated glucose and that this IL-8 is a potent mediator of monocyte adhesion to human EC (17). IL-8 is also a potent mediator of monocyte rolling (18, 19). Mice do not express IL-8, but KC is considered to be the chemokine that is most closely related to human IL-8. KC is a primary mediator of monocyte arrest on atherosclerotic endothelium in mice (20, 21). KC regulates macrophage localization to atherosclerotic lesions in mice (21).

p38 is a member of the mitogen-activated serine/threonine protein kinase family. p38 is activated by dual phosphorylation on Thr180 and Tyr182 by an upstream MAP kinase kinase. The p38 MAP kinase pathway in endothelial cells is activated by stress-inducing stimuli, including reactive oxygen species (ROS), hyperglycemia, and proinflammatory cytokines, such as TNFα (22). Activation of p38 regulates induction of inflammatory genes and activation of other inflammatory signaling pathways (including NF-κB and arachidonate-signaling pathways) (23). Data demonstrate that p38 phosphorylation and activation are involved in endothelial actin cytoskeletal reorganization and induction of adhesion molecule expression, leading to leukocyte adhesion and migration (24, 25). Thus, activation of

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the p38 pathway in vascular endothelium by chronic elevated glucose could contribute significantly to the development of vascular complications of diabetes.

In this study, we further examined the regulation of endothelial production of IL-8 by chronic elevated glucose and examined p38 pathway activation in diabetic db/db mice, a model of Type 2 diabetes. We have recently shown increased monocyte adhesion to aortic EC isolated from diabetic db/db mice compared with control mice (26). We show that inhibition of the p38 pathway in endothelial cells blocks endothelial production of IL-8 and also blocks monocyte adhesion to diabetic db/db mouse endothelial cells. These studies indicate that activation of the p38 MAP kinase pathway in endothelial cells is a major trigger of proinflammatory signaling events that lead to glucose-mediated monocyte recruitment and adhesion in the vessel wall in diabetes.

**EXPERIMENTAL PROCEDURES**

Reagents—SB203580 and SB202474 were purchased from Calbiochem. Phosphorylated p38 antibody (T180/Y182; catalog no. AF869), phosphorylated Erk1/Erk2 antibody (T202/Y204; catalog no. AF1018), recombinant murine TNFβ, recombinant human IL-8, and ELISA kits for mouse KC were obtained from R&D Systems. Total Erk1 antibody (K-23; catalog no. sc-6254), and total c-Jun N-terminal kinase antibody (catalog no. sc-474) were purchased from Santa Cruz Biotechnology, Inc. Total c-Jun antibody (catalog no. sc-6254), and total c-Jun N-terminal kinase antibody (catalog no. sc-474) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Calcein-AM was purchased from Molecular Probes, and anti-c-Jun (50,000/well) were added to EC monolayers and incubated for 30 min at 37°C. Unbound monocytes were rinsed, cells were fixed in 1% glutaraldehyde, and bound labeled monocytes were counted within a 10 × 10 grid using epifluorescence microscopy. As a positive control for monocyte adhesion, EC were incubated with 10 units/ml recombinant human TNFα for 4h. In subsets of studies using HAEC, recombinant human IL-8 (10 ng/ml), the p38 inhibitor SB203580 (5 μM), or control SB202474 (5 μM) were incubated with the cells for 4h prior to performing a monocyte adhesion assay. Cells were rinsed with media and incubated with labeled monocytes as described above for 30 min at 37°C.

In subsets of studies using HUVEC, EC were cultured in NG- and HG-containing medium in 48-well plates as described above. HUVEC were transfected with the dominant-negative p38α plasmid (DNP38α) (27) or a wild type p38α plasmid (WTP38α) plasmid using Lipofectin according to the manufacturer's instructions. At 48 h post-transfection, HUVEC were used in a monocyte adhesion assay as described above.

**IL-8 Promoter Study**—The human IL-8 promoter-reporter construct contained −1481 to +44 bp of the human IL-8 promoter as described previously (17, 33). HUVEC were cultured in NG and HG media as described above. HUVEC were utilized in these transfection studies because transient transfection of primary HAEC is quite difficult. Transfection rates of HUVEC were 70–80% of cells (data not shown). HUVEC were transfected in 6-well plates using 2 μg of plasmid DNA per well and incubated with the cells for 4 h prior to harvest as a positive control for IL-8 activation. Cells were harvested for luciferase activity using a reporter lysis kit (Promega) at 24 h post-transfection. Luminescence was analyzed on a Turner Designs, Inc. luminometer. Luminescence was normalized to total cell protein as we have described previously (17).

**Quantitative PCR for Human IL-8**—HAEC were cultured in NG or HG in the absence or presence of 5 μM SB203580 or 5 μM SB202474 for 4 h at 37°C. Total cellular RNA was obtained from HAEC using Trizol. Reverse transcription of 2 μg of total RNA was performed as described previously (17). For quantitative measurements of IL-8 mRNA, 2 μl of cDNA from each experimental group were utilized. The quantitative PCR was performed in a Bio-Rad iCycler iQ real-time PCR detection system. Data were analyzed as described by our group previously (17). Nanograms of IL-8 mRNA were calculated by the standard curve method using a pool of HAEC cDNA and normalizing to β-actin levels obtained for each sample. The above procedures were repeated on each of the different samples.

**Mouse Monocyte Adhesion Assay**—Our laboratory has recently developed a monocyte adhesion assay that utilizes primary MAEC and WEHI78/24 cells. WEHI78/24 cells are a mouse monocyte cell line that has been characterized by McEvoy and colleagues (29, 30). WEHI were cultured in DMEM plus 10% heat-inactivated FBS. WEHI cells were labeled with calcine-AM using standard methods described by the manufacturer. For the adhesion assay, MAEC were cultured to confluence in a 48-well plate and incubated with 35,000 calcine-labeled WEHI cells/well for 30 min at 37°C. Nonadherent cells were rinsed, and adherent cells were fixed with 1% glutaraldehyde. The number of adherent monocytes within a 10 × 10 eyepiece grid at 40× magnification was counted using epifluorescence microscopy. As a positive control for monocyte adhesion, MAEC were incubated with 10 units/ml recombinant murine TNFα for 4h. In subsets of studies, the p38 inhibitor SB203580 (5 μM) or its control SB202474 (5 μM) were incubated with the cells for 4 h prior to performing a monocyte adhesion assay.

**Human EC Cell Culture**—Primary human EC (HAEC) and HUVEC were cultured for 7 days in Medium 199 containing 20% heat-inactivated FBS, 20 μg/ml endothelial cell growth supplement, and 90 μg/ml heparin in the presence of 5.5 mM glucose (NG) or 25 mM glucose (HG) for 7 days. The 7-day, 25 mM HG incubation condition was chosen because this is the maximum concentration of glucose and time of incubation (31). The experimental use of HAEC, HUVEC, and human monocytes was approved by the University of Virginia Institutional Review Board, and all procedures were performed in accordance with university guidelines.

**Human Monocyte Adhesion Assay**—HAEC and HUVEC were cultured to confluence in NG- and HG-containing media as described above in 48-well plates. Prior to the assay, EC were rinsed with 1% M109. Human primary monocytes were isolated from healthy normal volunteers using a modification of the Recalde method (32). After isolation, monocytes were labeled with Calcien AM for 10 min at 37°C according to manufacturer's instructions. Labeled human primary monocytes (25,000/μl) were added to EC monolayers and incubated for 30 min at 37°C. Unbound monocytes were rinsed, cells were fixed in 1% glutaraldehyde, and bound labeled monocytes were counted within a 10 × 10 grid using epifluorescence microscopy. As a positive control for monocyte adhesion, EC were incubated with 10 units/ml recombinant human TNFα for 4h. In subsets of studies using HAEC, recombinant human IL-8 (10 ng/ml), the p38 inhibitor SB203580 (5 μM), or control SB202474 (5 μM) were incubated with the cells for 4 h prior to performing a monocyte adhesion assay. Cells were rinsed with media and incubated with labeled monocytes as described above for 30 min at 37°C.

In subsets of studies using HUVEC, EC were cultured in NG- and HG-containing medium in 48-well plates as described above. HUVEC were transfected with the dominant-negative p38α plasmid (DNP38α) (27) or a wild type p38α plasmid (WTP38α) plasmid using Lipofectin according to the manufacturer's instructions. At 48 h post-transfection, HUVEC were used in a monocyte adhesion assay as described above.
Nuclear Protein Extraction and Electrophoretic Mobility Shift Assay—HAEC were cultured in NG and HG as described above. Cells were harvested by scraping with a cell scraper. Nuclear proteins were prepared using the CelLytic NuCLEAR extraction kit from Sigma. Nuclear proteins were quantitated using a Bio-Rad DC protein assay kit.

Electrophoretic mobility shift assays for AP-1 were performed as described using 5 μg of nuclear extract (17, 33). The sense strand of the double-stranded AP-1 oligonucleotide probe is 5′-CGCTTGATGAGT-CAGCCGGAA-3′. Bands were supershifted using antibodies to c-Jun, c-Fos, and Fra-1 by incubating nuclear extracts for 15 min at room temperature with 2 μl of each antibody.

Immunoblotting for Phosphorylated p38 Protein—MAEC from B6 control and db/db mice or NG-cultured and HG-cultured HAEC were harvested in 1× cell lysis buffer (Cell Signaling, Inc.) in the presence of a protease inhibitor mixture (Sigma). 50 μg of total EC protein was analyzed by 4–12% SDS-PAGE in MOPS running buffer and transferred to nitrocellulose. Pierce Blocker BLOTTO in Tris-buffered saline was used as a blocking agent. Membranes were probed with a 1:2000 dilution of affinity-purified rabbit anti-phospho-p38 MAP kinase antibody. Phospho-p38 protein was detected using a 1:1000 dilution of anti-rabbit IgG-horseradish peroxidase and chemiluminescence. Bands were normalized to p38 (1:2000 dilution), and quantitated using densitometry.

Immunoblotting for Phosphorylated Erk1/Erk2 and Jnk1 Proteins—NG-cultured and HG-cultured HAEC were harvested in 1× cell lysis buffer (Cell Signaling, Inc.) in the presence of the protease inhibitor mixture (Sigma). 50 μg of total EC protein was analyzed by 4–12% SDS-PAGE in MOPS running buffer and transferred to nitrocellulose. Pierce Blocker BLOTTO in Tris-buffered saline was used as a blocking agent. Membranes were probed with 1:2000 dilutions of polyclonal anti-phospho-Erk or monoclonal phospho-Jnk antibodies. Phosphoproteins were detected using a 1:2000 dilution of anti-mouse or anti-rabbit IgG-horseradish peroxidase and chemiluminescence. Bands were normalized to total Erk or total Jnk (1:2000 dilution of primary antibodies) and quantitated using densitometry.

Statistical Analyses—Comparisons between groups were performed using ANOVA. Data are represented as the mean ± S.E. of six experiments performed in quadruplicate (unless otherwise noted in the figure legends). Mouse analyses were performed using six mice per group. All comparisons were made using Fisher's LSD procedure, so that multiple comparisons were made at the 0.05 level only if the overall F-test from the ANOVA was significant at p < 0.05.

RESULTS

Glucose Activates the p38 MAP Kinase Pathway in Aortic Endothelial Cells—We have previously reported increased production of IL-8 by human aortic endothelial cells cultured in glucose through activation of the transcription factor AP-1 (17). Components of the AP-1 transcriptional complex that are activated by chronic elevated glucose include c-Jun and c-Fos, with a small contribution by Fra-1 (Fig. 1). AP-1 binding is increased in HG cells. The HG-mediated AP-1 binding was blocked by supershifting using antibodies to Fra-1, c-Fos, and c-Jun. To examine signaling pathways upstream of AP-1 that could mediate IL-8 production, we examined activation of MAP kinase pathways, since these pathways can be activated by oxidative stress in EC. We measured phosphorylation of p38 by glucose in HAEC and found a significant 2-fold increase in phosphorylation as shown in Fig. 2. We found no increase in phosphorylation of either Erk1/Erk2 or Jnk1 by chronic elevated glucose (data not shown).

p38 Pathway Mediates Glucose-induced Monocyte/Endothelial Adhesion and IL-8 Production—We next wanted to test the hypothesis that inhibition of the p38 pathway reduced glucose-mediated endothelial activation and monocyte adhesion. HG-cultured HAEC have a significant 2.5-fold increase in IL-8 protein production (Fig. 3). We used 1-glucose (25 mM, 7 days) as a control and found no effect of 1-glucose on monocyte adhesion to HAEC. As shown in Fig. 4, inhibition of the p38 pathway in HAEC using the pharmacological inhibitor SB203580 (at 5 μM; A) or inhibition of the p38 pathway in HUVEC using a dominant-negative construct for p38α (B) significantly reduced the glucose-mediated increase in IL-8 protein production.

Interestingly, overexpression of p38α using a wild type p38α construct significantly increased IL-8 production by HG-cultured HUVEC (Fig. 4). This wild type p38α construct caused a 3-fold increase in p38α protein expression (data not shown). To test whether the observed changes in IL-8 production were caused by changes in IL-8 mRNA transcription, we quantified IL-8 mRNA levels in HG-HAEC using real time quantitative PCR. HG-HAEC have a significant 2-fold increase in IL-8 mRNA production (Fig. 5). A known pharmacological inhibitor of p38, SB203580 (5 μM), significantly reduced glucose-mediated endothelial production of IL-8 mRNA to levels similar to
those observed for NG-cultured HAEC (Fig. 5), suggesting direct action of p38 on IL-8 mRNA levels. We next examined the ability of SB203580 to block glucose-mediated activation of the human IL-8 promoter. We have previously reported induction of human IL-8 promoter activity in response to chronic elevated glucose (17). For this set of experiments, we used a luciferase expression vector, pGL2Basic (Promega) that contained −1481 to +44 bp of the human IL-8 promoter. We transfected the IL-8 promoter-reporter construct as described under “Experimental Procedures.” At 48 h post-transfection, EC were treated for 4 h with 5 μM SB203580 (+SB203580) or SB202474 (+SB202474). Cells were harvested, and luciferase was measured as described under “Experimental Procedures.” Luciferase values were normalized to mg of total cell protein. *, significantly higher than NG, p < 0.0001; **, significantly lower than HG, p < 0.001 by ANOVA.

![Fig. 3](image3.png)

**Fig. 3.** Chronic exposure of HAEC to elevated glucose increases endothelial IL-8 production. Human aortic endothelial cells were cultured for 7 days in either 5.5 mM glucose (NG), 25 mM r-glucose (HG), or 25 mM glucose. Human IL-8 secreted into medium was measured using ELISA as described under “Experimental Procedures.” *, significantly higher than NG, p < 0.0001 by ANOVA. Data represent the mean ± S.E. of 10 experiments.

![Fig. 4](image4.png)

**Fig. 4.** Inhibition of the p38 pathway blocks glucose-mediated IL-8 production. HAEC or HUVEC were cultured in NG (NG) or HG-containing medium (HG) for 7 days. A, HAEC were treated for 4 h with 5 μM SB203580 (+SB203580) or SB202474 (+SB202474). Medium was collected and analyzed for IL-8 using ELISA as described under “Experimental Procedures.” *, significantly higher than NG, p < 0.0001; **, significantly lower than HG, p < 0.001 by ANOVA. B, HUVEC were transfected with a control pcDNA plasmid (+CTRVEC), a dominant-negative p38α plasmid (+DNp38), or a wild-type p38α plasmid (+WTp38) as described under “Experimental Procedures.” At 48 h post-transfection, medium was collected for IL-8 measurement by ELISA. *, significantly higher than NG, p < 0.0001; **, significantly lower than HG, p < 0.003.

![Fig. 5](image5.png)

**Fig. 5.** Inhibition of the p38 pathway reduces glucose-mediated expression of IL-8 mRNA in endothelial cells. Human aortic endothelial cells were cultured for 7 days in either 5.5 mM glucose (NG) or 25 mM glucose (HG). HAEC were incubated with 5 μM SB203580 or SB202474 for 4 h at 37 °C. Total cellular RNA was isolated as described under “Experimental Procedures.” Real time quantitative PCR for human IL-8 was performed as described under “Experimental Procedures.” Samples were normalized to β-actin as a control. *, significantly higher than NG by ANOVA, p < 0.009; **, significantly lower than HG by ANOVA, p < 0.01.

![Fig. 6](image6.png)

**Fig. 6.** A p38 inhibitor blocks glucose-mediated activation of the human IL-8 promoter. HUVEC were transfected with a human IL-8 promoter-reporter construct as described under “Experimental Procedures.” At 48 h post-transfection, EC were treated for 4 h with 5 μM SB203580 (+SB203580) or SB202474 (+SB202474). Cells were harvested, and luciferase was measured as described under “Experimental Procedures.” Luciferase values were normalized to mg of total cell protein. *, significantly higher than NG, p < 0.0001; **, significantly lower than HG, p < 0.0001.
construct also reduced monocyte adhesion by 50%, thus confirming our data using SB203580 (Fig. 8). Taken together, these data indicate that glucose regulates production of IL-8 and enhances monocyte/endothelial interactions through activation of the p38 MAP kinase pathway.

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**p38 Pathway Is Induced in Diabetic db/db Mice**

We have recently shown that diabetic db/db mice have increased monocyte adhesion to endothelium compared with control mice (26). Based upon our earlier data in human EC, in which glucose increases endothelial IL-8 production, we measured levels of the mouse ortholog of IL-8, KC, in control C57BL/6J and diabetic db/db mice. We found that diabetic db/db mice have dramatic elevations in levels of plasma KC, the mouse ortholog of human IL-8 (1800 pg/ml for db/db mice versus 300 pg/ml for control mice, p < 0.0001) (Fig. 9). We next wanted to determine whether the p38 pathway was activated in the diabetic db/db mice and test the role of the p38 pathway in mediating monocyte adhesion in diabetic db/db mice. We first measured KC production in EC of diabetic db/db mice and found both KC mRNA (Fig. 10A) and protein levels (Fig. 10B) to be significantly elevated by 2.5-fold. We then measured p38 phosphorylation in control and diabetic db/db EC. We found a significant
increase in phosphorylated p38 expression in diabetic db/db EC compared with C57BL/6J control EC (Fig. 11). Normal EC do not bind monocytes unless they are activated. We found that db/db endothelial cells are "preactivated" to bind monocytes (Fig. 12). Furthermore, inhibition of the p38 pathway in diabetic db/db EC using the compound SB203580 significantly reduced monocyte adhesion by 50% to levels observed for C57BL/6J control mice (Fig. 12), suggesting that the p38 pathway directly mediates monocyte/endothelial interactions in diabetic db/db mice.

**DISCUSSION**

This study demonstrates, for the first time, the crucial role of the p38 MAP kinase pathway in regulation of glucose-mediated monocyte adhesion to aortic endothelial cells. The study also demonstrates that the p38 pathway is activated in endothelial cells of diabetic db/db mice, suggesting that chronic hyperglycemia in diabetes leads to endothelial p38 activation and contributes to the increased monocyte/endothelial interactions observed in diabetes. This study documents that the increased production of IL-8 in response to chronic elevated glucose is caused by activation of p38. We have previously shown increased production of ROS and regulation of IL-8 production by the transcription factor AP-1 in glucose-cultured endothelial cells (17). We hypothesize that in the setting of diabetes, glucose activates EC to produce ROS. This increased oxidative stress activates p38, which in turn increases production of IL-8 through activation of AP-1. Our proposed model of this signaling event is shown in Fig. 13. The p38 pathway can also activate other proinflammatory signaling pathways (23, 36) that contribute to increased monocyte/EC interactions in diabetes.

We have previously shown that db/db mice have increased production of 12/15 lipoxygenase products, including (12S)-hydroxyeicosatetraenoic acid and (13S)-hydroxyoctadecadienoic acid. These eicosanoids mediate monocyte adhesion to EC in db/db mice (26). The p38 pathway activates eicosanoid pathways (23), so we questioned whether the regulation of IL-8 production by the p38 pathway was directly due to activation by (12S)-hydroxyeicosatetraenoic acid or (13S)-hydroxyoctadecadienoic acid. Although we found that the p38 inhibitor SB203580 blocked (12S)-hydroxyeicosatetraenoic acid production in HAEC, there was no effect of either eicosanoid on endothelial IL-8 production (data not shown). Thus, these eicosanoids must mediate monocyte/endothelial interactions in diabetes through other mechanisms. These other mechanisms may include activation of chemokines other than IL-8, induction of IL-8 by EC mediates monocyte recruitment and adhesion to endothelial cells in diabetes.

**FIG. 11.** Phosphorylation of p38 MAP kinase is increased in endothelial cells of diabetic db/db mice. Mouse aortic endothelial cells from diabetic db/db (Dbldb EC) and C57BL/6J control (B6 CTR EC) mice were harvested and cultured as described under "Experimental Procedures." Phosphorylation of p38 was analyzed using immunoblotting as described under "Experimental Procedures." *, significantly higher than B6 by Student’s t test, p < 0.01.

**FIG. 12.** Inhibition of the p38 pathway in diabetic db/db mice reduces monocyte adhesion. Mouse aortic endothelial cells from diabetic db/db (Dbldb EC) and C57BL/6J control (B6 CTR EC) mice were harvested and cultured as described under "Experimental Procedures." The p38 inhibitor SB203580 (+SB203580) and control compound SB202474 (+SB202474) were added to the cells for 4 h prior to performing a monocyte adhesion assay. *, significantly higher than B6 control by ANOVA, p < 0.0001; #, significantly higher than B6 control, p < 0.01; †, significantly lower than db/db, p < 0.001.

**FIG. 13.** Model of glucose-mediated endothelial activation contributing to monocyte adhesion. In diabetes, EC are activated by glucose to produce ROS. ROS stimulate the p38 MAP kinase pathway, which in turn, activates the transcription factor AP-1. Activation of AP-1 leads to transcription of inflammatory genes, resulting in increased endothelial production of IL-8. The release of the chemokine IL-8 by EC mediates monocyte recruitment and adhesion to endothelium in inflammation and vascular complications of diabetes.

We definitively show in the current study that inhibition of the p38 pathway blocks glucose-mediated induction of IL-8 mRNA in human EC. These data indicate that p38 activation leads to endothelial IL-8 production in both aortic EC and umbilical vein EC. Our data also indicate that much of glucose-mediated monocyte adhesion is due to IL-8 production by EC. The p38 inhibitor SB203580 dramatically reduces glucose-mediated monocyte adhesion, but this effect can be reversed by the...
exogenous addition of IL-8 (Fig. 6). SB203580 primarily inhibits the p38α isoform at 5 μM concentration. At higher concentrations (greater than 10 μM), SB203580 can have nonspecific effects on other MAP kinase pathways as well as on nitric-oxide synthase enzyme activity in EC (37). The control compound SB202474 does not inactivate any p38 isoform (38); thus, it is used as a negative control in our study. We also utilized a dominant-negative p38α construct to reduce IL-8 production and monocyte adhesion in HUVEC. Transfection of HUVEC with a wild-type p38α construct produced a significant 3-fold overexpression of p38 protein that resulted in a significant 2.5-fold increase in endothelial IL-8 production. Finally, our data in the diabetic db/db mouse show a significant increase in endothelial production of KC, the mouse ortholog of human IL-8. The aortic endothelial cells from these diabetic db/db mice overexpress of p38 protein that resulted in a significant 3.5-fold increase in endothelial IL-8 production. Endothelial IL-8 production, in turn, mediates much of the monocyte/endothelial adhesion observed in diabetes.

We find increased AP-1 activation in human EC cultured chronically in elevated glucose. AP-1 consists of a dimer composed of Fos and Jun proteins. The Fos-Jun family members can form up to 18 different homodimers and heterodimers in cells, and those expressed depend somewhat on the extracellular stimuli provided to cells (39). We found that c-Jun, c-Fos, and Fra-1 were part of the AP-1 complex that is activated by chronic elevated glucose. We found no increase in Fra-2, Jun-b, or Jun-d (data not shown). We have not yet examined the regulation of AP-1 in diabetic db/db mice.

In conclusion, we have demonstrated that chronic elevated glucose causes phosphorylation of p38 MAP kinase. Inhibition of p38 MAP kinase blocks IL-8 production and monocyte adhesion to endothelial cells. Probably, these processes are mediated through generation of ROS, resulting in p38 phosphorylation, which leads to activation of the AP-1 transcription factor complex and induction of IL-8 gene expression. Future therapies to reduce p38 activation will probably be important for reduction of vascular complications in diabetes.

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