Tumor Necrosis Factor–Mediated E2F1 Suppression in Endothelial Cells
Differential Requirement of c-Jun N-Terminal Kinase and p38 Mitogen-Activated Protein Kinase Signal Transduction Pathways
Raj Kishore, Corinne Luedemann, Evelyn Bord, David Goukassian, Douglas W. Losordo

Abstract—After balloon angioplasty, locally expressed tumor necrosis factor (TNF)-α disrupts endothelial cell (EC) proliferation and reendothelialization of the injured vessel. We have previously reported that TNF inhibits the EC cycle and downregulates the transcription factor E2F1. Ectopic expression of E2F1 at the site of injury improves reendothelialization of the injured vessel. In this study, we report that c-Jun N-terminal kinase (JNK) 1 and p38 mitogen-activated protein kinases (MAPKs) are differentially required for E2F1 expression and activity in ECs. Overexpression of constitutively active JNK1 mimicked TNF-mediated inhibitory events, whereas dominant-negative JNK1 prevented these effects. E2F cis elements in the promoter of E2F1 gene mediate suppressive actions of TNF, because removal of these sites rendered E2F1 promoter activity insensitive to TNF. JNK1 physically interacted with E2F1 and inactivated it via direct phosphorylation. Additionally, TNF inhibited Rb phosphorylation and dissociation from E2F1. Overexpression of constitutively active p38 MAPK facilitated Rb-E2F1 dissociation, whereas that of dominant-negative p38 MAPK did not. Taken together, these data suggest a differential requirement of JNK1 and p38 MAPK in TNF regulation of E2F1. Targeted inactivation of JNK1 at arterial injury sites may represent a potential therapeutic intervention for ameliorating TNF-mediated EC dysfunction. (Circ Res. 2003;93:932-940.)

Key Words: angioplasty ■ endothelium ■ mitogen-activated protein kinases ■ signal transduction

Restenosis in stented coronary arteries remains a significant clinical problem that undermines the benefits of balloon angioplasty. Arterial abrasion during balloon angioplasty, which triggers the proliferation and migration of normally quiescent endothelial cells (ECs), leads to reendothelialization at sites of vascular injury.1,2 Recent studies have demonstrated that tumor necrosis factor (TNF), expressed locally at the sites of arterial injury, delays reendothelialization by inhibiting the EC cell cycle and enhancing apoptosis.3–5 Recently we reported that in vivo blockade of TNF accelerates functional endothelial recovery and inhibits neointimal lesion formation after balloon angioplasty.6 We also showed that TNF induces EC cell-cycle arrest, at least in part via mechanisms involving the repression of the transcription factor E2F1. Specifically, in vitro and in a rat carotid injury model, adenovirus-mediated restoration of E2F1 activity prevented TNF-α–induced cell-cycle arrest and apoptosis in ECs.7,8 These findings imply that repression of E2F1 activity is crucial to TNF-induced inhibition of endothelial recovery. However, the signaling mechanisms by which TNF mediates E2F1 repression in vascular ECs remain unclear.

The E2F family of transcription factors are important mediators of cell proliferation, differentiation, and apoptosis.9–12 E2F1 has been shown to be a major downstream target of the retinoblastoma (Rb) family of proteins and is necessary for the transcription of many components of the cell-cycle machinery.13,14 In nonproliferating cells, Rb is bound to E2F1 and suppresses its transcriptional activity. Disruption of this association by hyperphosphorylation of Rb releases free, transcriptionally active E2F1, thus permitting cell proliferation.15,16

As vital regulators of the transition between the G and S phases of the cell cycle, E2F1 and Rb are likely components of several signaling cascades. Although TNF has been shown to inhibit E2F1 by preventing Rb phosphorylation,17 it is not clear which of the many TNF-induced signaling pathways affect Rb-mediated E2F1 repression, especially in vascular endothelial cells. Moreover, little is known about additional, non-Rb–mediated signaling events that may be involved in TNF-induced E2F1 inhibition. In this study, we examined whether kinases involved in specific nonproliferative pathways affect E2F1 expression and function. We focused our attention on the effects of c-Jun N-terminal kinase (JNK) 1 and p38, members of the mitogen-activated protein kinase (MAPK) family that are rapidly induced in response to various stimuli, including TNF. Although both kinases are
known to modulate the transcriptional activity of the transcription factors AP1 and ATF2, information about the effects of these kinases on the component of the cell-cycle machinery is scarce.

We provide evidence that JNK1 and p38 MAPKs differentially affect TNF-mediated suppression of E2F1 in ECs via two distinct and opposing mechanisms. In response to TNF, JNK1 physically associates with E2F1 and inactivates the transcription factor via direct phosphorylation. In contrast, insufficient activation of p38 in TNF-exposed ECs results in reduced Rb hyperphosphorylation and continued inactivation of E2F1 by its continued association with Rb. Data from our study may ultimately provide novel options for therapeutic interventions to improve endothelial function.

Materials and Methods

Cell Culture
Bovine arterial endothelial cells (BAECs) were isolated as described previously. For synchronization to the G0 phase, cells were serum starved for 48 to 72 hours.

Antibodies, Expression Vectors, Recombinant Proteins
Anti-phospho JNK1, anti-phospho p38, total JNK1, and total p38 MAPK antibodies were purchased from New England Biolabs (Beverly, Mass). Anti-E2F1, -E2F2, -E2F4, and -Rb and phospho-Rb and anti-Flag antibodies were obtained from Santa Cruz Biotech (Santa Cruz, Calif). E2F1 promoter-reporter constructs (E2F-CAT and E2Fmut-CAT) and fusion proteins glutathione S-transferase (GST)-E2F1 and GST-DP1 were provided by Dr S. Chellappan (University of South Florida, Tampa, Fla) and have been described previously. Constitutively active and dominant-negative MAPK constructs, each containing a Flag-tag, were provided by Dr L. Nagy (Case Western Reserve University, Cleveland, Ohio) and have been described previously. Recombinant c-jun fusion proteins, GST-E2F1, and GST-DP1 as substrates, as described previously.

Transient Transfections
BAECs were transiently transfected with various expression vectors using Transfast (Promega) following the manufacturer’s instructions. A portion of the cells was treated with 40 ng/mL recombinant human TNF for indicated durations before protein and RNA preparations. As a control for transfection efficiency, total proteins from transfected cells were assayed for alkaline phosphatase activity from a cotransfected pSVAPAP plasmid and for tagged protein expression (flag) in the case of transfections with MAPK constructs in Western blots and by immunochemical staining.

Thymidine Uptake Assay
Cell proliferation was assayed by thymidine uptake assay as described previously.

RNA Isolation and Ribonuclease Protection Assay
Total cellular RNA was isolated using Trizol reagent (Life Technologies Inc). Human E2F multiprobe DNA, CAT, and β-actin DNA templates (Ambion and Pharmingen) and [α32P]UTP (New England Nuclear) were used to synthesize in vitro transcribed antisense riboprobes. Ribonuclease protection assay (RPA) was carried out using RPA III kit (Ambion) following the manufacturer’s instructions and as described previously.

CAT Assay
E2F1 promoter analysis was done by analyzing the CAT mRNA expression driven by E2F1 promoter in ECs transfected with E2F-CAT or E2Fmut-CAT constructs. pTri-CAT template DNA from Ambion Inc was in vitro transcribed and used as antisense CAT riboprobe in RPA assays.

Oligonucleotides and Electrophoretic Mobility Shift Assays
Electrophoretic mobility shift assays (EMSA) using consensus E2F oligonucleotides and 5 µg of nuclear proteins from variously treated cells were carried out as described previously. Reactions were electrophoresed on 5% nondenaturing polyacrylamide gels, dried, and processed for autoradiography. For competition experiments, 25- to 100-fold excess of unlabelled DNA was added to the reaction 20 minutes before the addition of the radiolabeled probe.

Immunoprecipitation and Western Blots
Total cellular proteins were immunoprecipitated with the desired antibodies using immunoprecipitation kits from Promega. Immunoabsorbent beads were thoroughly washed, and proteins were eluted by boiling for 10 minutes in 2X SDS loading buffer. Eluted proteins were size-fractionated on 10% SDS-PAGE gels, transferred to PVDF membranes, and probed for specific proteins in Western blots using specific antibodies.

Kinase Assays
For JNK1 kinase assays, proteins from JNK-flag or p38-flag transfected cells, treated with or without TNF, were immunoprecipitated with an anti-flag antibody in a buffer containing 20 mmol/L Tris, 1% Triton X-100, 0.5% deoxycholate, and 0.5 mol/L LiCl. The beads were washed extensively, and kinase assays were performed using c-jun fusion protein, GST-E2F1, and GST-DP1 as substrates, as described previously.

Results

TNF Suppresses E2F1 Expression and DNA-Binding Activity
We have previously shown that TNF inhibits E2F1 activity in ECs. To determine whether TNF might suppress members of the E2F family in addition to E2F1, RNA isolated from BAECs that were exposed to TNF or control medium for 16 hours was analyzed by RPA for mRNA expression of E2F1, E2F2, E2F4, and their dimerization partner DP1. As shown in Figures 1A and 1B, TNF significantly reduced E2F1 mRNA expression. The effect of TNF on E2F1 seemed to be specific, because TNF treatment had no significant effect on other E2Fs or DP1 mRNA expression in the same RNA samples. Furthermore, TNF suppressed E2F1 DNA-binding activity as measured by the ability of proteins from TNF-treated cells to bind E2F oligonucleotides in EMSA (Figure 1C).

TNF-Induced Transcriptional Repression of E2F1 Is Mediated by cis Elements in the E2F1 Promoter
To investigate whether TNF-mediated inhibition of E2F1 mRNA expression and activity might reflect transcriptional repression, we analyzed the activity of the E2F1 promoter. BAECs were transiently transfected with E2F1 promoter linked to the CAT reporter gene (E2F-CAT) or its mutant variant (E2Fmut-CAT), which contains mutated E2F binding sites. Total RNA from transfected cells treated or not with TNF was analyzed for expression of the CAT transgene. E2F1 promoter activity was significantly reduced in cells transfected with E2F-CAT in the presence of TNF, as evident from the greatly reduced CAT mRNA expression in these cells compared with cells cultured in serum alone (Figure 2). As expected, removal of E2F sites from the E2F1 promoter substantially reduced promoter activity. However, there was no additional reduction in reporter CAT mRNA expression in
the presence of TNF. Taken together, these results demonstrate the following two important observations: that inhibition of E2F1 mRNA by TNF is caused by reduced transcriptional activation and that E2F binding sites in the promoter region of the E2F1 gene are the target of TNF suppressive activity.

**TNF-Induced JNK1 and p38 MAPK Activation Display Differential Kinetics in ECs**

We investigated signaling mechanisms involved in TNF-mediated E2F1 suppression. Because both JNK and p38 MAPKs are strongly activated by TNF and have previously been shown to modulate cell-cycle gene expression, we evaluated the activation kinetics of these two kinases. Total cellular proteins from control and TNF-treated cells were analyzed for phospho-JNK1 and phospho-p38 MAPK in Western blots. As shown in Figures 3A and 3B and quantified in Figure 3C, TNF-induced phosphorylation showed differential kinetics for these two kinases. In response to TNF, JNK1 phosphorylation was observed as early as 15 minutes and was sustained for up to 4 hours after TNF treatment. In contrast, p38 was transiently activated at 30 minutes after TNF exposure and then was quickly dephosphorylated at subsequent time points. Because TNF has been shown to activate p38 MAPK in other contexts, we examined whether this quick inactivation of p38 might reflect TNF-mediated induction of p38-specific MAPK phosphatase-1 (MKP-1). Interestingly, as shown in Figure 3D, TNF treatment did induce MKP-1 expression, which was inversely correlated with p38 activation at all time points.

**Constitutively Active JNK1 Mimics TNF Suppressive Action on EC Proliferation**

Because differential activation of JNK1 and p38 MAPKs in response to TNF may modulate E2F1 expression and lead to EC growth arrest, we investigated the effect of constitutively active JNK and p38 MAPKs on EC proliferation. BAECs were transiently transfected with constitutively active JNK1 (CAJNK1), dominant-negative JNK1 (dnJNK1), constitutively active wild-type p38 (wtp38), and dominant-negative p38 (dnp38). All of the expression vectors were Flag-tagged to permit identification of transfectants. Transfection efficiency was monitored by analyzing the expression of tag protein by immunocytochemistry in separate transfections.
and was found to be comparable between transfections, with a transfection efficiency of ≈50% (Figure 4A) (data not shown). Transfected cells were treated with or without TNF for 24 hours in the presence of 5 μCi/mL 3H-thymidine. Incorporation of thymidine uptake by proliferating cells was then quantified. As shown in Figure 4B, overexpression of constitutively active CAJNK1 inhibited BAEC proliferation by almost 50% (P<0.01) compared with BAECs transfected with empty vector alone, resulting in a decrease in EC proliferation that was similar to that resulting from TNF exposure. These results suggest that TNF-mediated inhibition of BAEC proliferation may involve upregulation of active JNK1.

This hypothesis was corroborated by the ability of dnJNK1 transfection of BAECs to prevent TNF-induced suppression of proliferation (P<0.01, dominant-negative versus control). Overexpression of p38 MAPK had an opposite effect on BAEC proliferation. Proliferation of BAECs was significantly increased in wtp38-transfected, TNF-treated cells compared with TNF-treated cells transfected with empty vector (P<0.05). In contrast, overexpression of dnp38 suppressed BAEC proliferation by 30% (P<0.05) in control cells and additionally reduced EC proliferation in the presence of TNF. Taken together, these data indicate a differential requirement of JNK and p38 MAPK for optimal EC proliferation.

### JNK1 Inhibits E2F1-Promoter and DNA-Binding Activity and Expression

To investigate whether JNK-induced inhibition of EC proliferation was in part mediated by suppression of E2F1 expression and activity, we examined E2F1 promoter activity, DNA-binding activity, and mRNA expression in BAECs transfected with JNK expression vectors. CAT reporter mRNA was significantly reduced in cells cotransfected with CAJNK1 and E2F-CAT (Figures 5A and 5B). This inhibition in promoter activity was reversed when cells were cotransfected with dnJNK1, even in the presence of TNF treatment. As observed with TNF treatment (Figure 2), inhibition of E2F1 promoter activity by JNK-1 was mediated via E2F binding sites, because E2F1 promoter activity was not influ-
enced by overexpressed CAJNK1 in cells cotransfected with E2Fmut-CAT (Figures 5A and 5B). Similarly, transfection with CAJNK1 completely abolished E2F DNA-binding activity, whereas transfection with dnJNK1 rescued TNF-mediated inhibition of E2F-binding activity (Figure 5C). In contrast, overexpression of wtp38 reversed TNF-induced inhibition of E2F-binding activity, additionally confirming the differential requirement of these two MAPK members for optimum EC growth.

Finally, JNK1-mediated modulations in E2F1 activity translated into E2F1 mRNA expression. TNF-induced repression of E2F1 mRNA was mimicked by overexpressed CAJNK1, whereas overexpression of dnJNK1 rescued TNF-induced inhibition of E2F1 mRNA (Figures 6A and 6B). Taken together, these data strongly suggest that TNF-induced inhibition of E2F1 in ECs is mediated via a differential activation of JNK and p38 MAPK pathways in response to TNF.

JNK1 Physically Associates and Phosphorylates E2F1

We next attempted to elucidate the mechanisms by which JNK1 regulates E2F1 activity and expression. First, we examined whether JNK1 associates with E2F1 in vivo. Whole-cell extracts from TNF-treated BAECs were immunoprecipitated with antibodies to E2F1, E2F2, E2F4, and control IgG. A Western blot analysis of immunoprecipitates containing anti-JNK1 antibodies detected JNK1 only in the E2F1 immunoprecipitate (Figure 7A). These data demonstrate the specificity of JNK1 for physically associating with E2F1 in vivo and show that this interaction can be detected without overexpression of any component. Because JNK1 was found to bind to E2F1, we examined the ability of JNK1 to phosphorylate E2F1 or its dimerization partner DP1 in vitro. Lysates from BAECs transfected with JNK1-flag expression vectors were immunoprecipitated with an anti-flag antibody, and in vitro kinase reactions were performed using GST-E2F1, GST-DP1, or GST-c-jun as substrates. As shown in Figure 7B, CAJNK1 efficiently phosphorylated E2F1 but not DP1. Additionally, wtp38 did not phosphorylate E2F1. These data additionally corroborate the existence of a specific JNK1-E2F1 association. That JNK1 phosphorylates E2F1 was additionally evident from the experiments in which E2F1 was found to be serine-threonine phosphorylated both in response to TNF treatment and in cells transfected with constitutively active CAJNK1, whereas no E2F1 phosphorylation was observed in control cells or in cells overexpressing dominant-negative JNK1.
TNF-Induced Suppression of E2F1-Rb Dissociation Is Partly Mediated by p38 MAPK

Next we investigated a potential mechanism for the partial reversal of TNF-mediated inhibition of EC proliferation by overexpression of constitutively active wtp38 (Figure 4). Because p38 does not associate with or phosphorylate E2F1 or DP1 (Figure 7), we evaluated the effect of p38 on E2F1-Rb association. Because hyperphosphorylation of Rb and the consequent dissociation with E2F1 is a prerequisite for E2F1 activation and because previous studies have shown a role for p38 MAPK in Rb-mediated E2F1 inhibition, we first examined the effect of TNF on Rb phosphorylation.

Phosphorylation of Rb was inhibited by TNF at all time points studied, whereas TNF did not affect total Rb protein expression (Figures 8A and 8B). Indeed, immunoprecipitation with anti-Rb antibody followed by Western blot analysis with anti-E2F1 antibody revealed that more Rb was associated with E2F1 in the lysates of TNF-treated cells than control cells (Figure 8C). To examine whether TNF-induced JNK or p38 MAPK plays a role in preventing E2F1-Rb dissociation, similar immunoprecipitation Western blot experiments were performed on lysates from TNF-treated cells transfected with JNK or p38 expression vectors. As shown in Figure 8D, CAJNK, dnJNK, and dnp38 did not influence TNF-mediated E2F1-Rb association. On the contrary, overexpression of wtp38 partially reduced the E2F1-Rb association. This finding may explain the enhanced proliferation of wtp38-transfected ECs in the presence of TNF.

Taken together, our data not only provide insight into the mechanisms underlying TNF-mediated E2F1 repression but also provide direct evidence of a differential requirement of JNK and p38 MAPKs in E2F1-mediated EC proliferation.

Discussion

Prior investigation has suggested that TNF contributes to restenosis in the stented coronary vessel by delaying reendothelialization and functional endothelial recovery at sites of arterial injury.2–5 The clinical relevance of TNF expressed at sites of balloon injury was suggested in our earlier studies, showing that reendothelialization in injured vessels was accelerated by the blockade of locally expressed TNF by systemic administration of a TNF-soluble receptor.6 We have also previously shown that TNF inhibits EC proliferation in vitro via cell-cycle arrest and enhanced apoptosis through mechanisms involving TNF-mediated repression of cell-cycle regulatory proteins, including E2F1.7 Overexpression of E2F1 restored the TNF-induced inhibitory effects on EC proliferation and cell-cycle arrest in vitro.7 Furthermore, adenoviral-mediated transfer of E2F1 to sites of arterial injury resulted in increased reendothelialization,8 demonstrating a causative link between TNF, E2F1 inhibition, and resultant EC dysfunction. Although other studies have demonstrated TNF-mediated EC cell-cycle arrest,17,23 the underlying molecular mechanisms and signaling pathways involved in these events are incompletely defined and poorly understood. In the present study we provide evidence that JNK1 and p38 MAPKs transduce signals in response to TNF that have distinct and opposing effects on E2F1 expression and EC proliferation.

Our finding that JNK1 can specifically target E2F1 may be related to a shift in the proliferative status of the cells because of the stress situations that typically exist when JNK is expressed. JNK-mediated inhibition of E2F1 and EC cell cycle may therefore have significant bearing on endothelial recovery after balloon injury. Indeed, several recent studies have reported significant activation of vascular JNK after balloon injury.24–27 Furthermore, a recent study has shown that balloon injury activation JNK expression leads to neointima formation at sites of injury.24 In fact, viral transfer of dominant-negative JNK attenuated neointimal thickening by inhibiting vascular smooth muscle cell (VSMC) proliferation.
However, none of these studies documented the effect of JNK on endothelial cell proliferation either in vitro or on reendothelialization of injured vessels in vivo. When evaluated in light of the results of Izumi et al., our data suggest that activation of JNK expression after arterial injury may influence neointimal thickening by transducing signals that have a differential effect on EC and VSMC proliferation. This diversity in JNK function has been previously reported for cell types in addition to ECs. For example, the activation of the JNK pathway plays a positive role in the growth of fibroblasts, T lymphocytes, and cardiac myocytes, whereas other studies have shown the negative role of JNK in hippocampal neurons as well as neuronal PC12 cells. The differential and apparently opposing effects of JNK expression on ECs and VSMCs suggests that JNK may provide a unique therapeutic approach for inhibiting restenosis after balloon angioplasty. However, additional study is required to validate this hypothesis.

Our results indicate that JNK1 is capable of phosphorylating E2F1 directly. Previous studies have demonstrated that phosphorylation events mediated by cyclin-dependent kinases regulate E2F1 activity. These studies indicated that phosphorylation modulates the ability of E2F1 to bind Rb and to induce transcription. These findings were additionally supported by the observation that phosphatase PP2A dephosphorylates and activates E2F1 in adipogenic cells. In this system, an increase in E2F1 phosphorylation correlated with reduced DNA-binding activity, similar to our findings on TNF and JNK1 overexpression. Wang et al. have shown a similar role for JNK1 in Saos-2 osteosarcoma cells. Given these findings, we believe it is plausible that kinases responding to stress signals modulate E2F1 function directly by modifying its DNA binding properties. Wang et al. also reported that JNK1 phosphorylated E2F1 by physically interacting with DP1. We found no such interaction between JNK1 and DP1 in our system.

On the contrary, our data show that JNK1 physically and specifically associates with E2F1 and directly phosphorylates and inactivates the transcription factor. This conclusion was additionally supported by the observation that TNF or JNK overexpression had no effect on mRNA expression of DP1 or E2F transcript factors other than E2F1 (Figure 1). Because DP1 also dimerizes with E2F2 and E2F4, a physical association between JNK1 and DP1 would result in the phosphorylation and suppression of E2F2 and E2F4; this is not evident in our system. However, additional study is required to determine the basis of JNK1 specificity to E2F1 and to identify potential JNK1 phosphorylation sites on E2F1.

Although it is known that Rb can be phosphorylated in vitro by several kinases, we observed that p38 MAPK is required for E2F1 activation via Rb phosphorylation in vivo. Rb has been shown to be involved in cell-cycle modulation in response to nonmitogenic signals; p38 MAPK seems to target Rb in response to such signals. Rb regulation via p38 MAPK has previously been described by others. However, our data demonstrate TNF-related dampening of optimal p38 MAPK activation in ECs. It is well-known that TNF is a strong inducer of p38 MAPK. Yet in our experiments, the kinetics of TNF-induced p38 MAPK activation seems to be weak and TNF-induced phospho-p38 MAPK was quickly inactivated in ECs treated with TNF (Figure 3).

Although additional study to understand this phenomenon is underway, we now propose a hypothesis explaining this observation. We believe that TNF directly induces MAPK phosphatase (MKP)-1, which in turn inactivates p38 MAPK. MAPK activation occurs through the dual phosphorylation of a threonine and an adjacent tyrosine residue (TXY). At least nine individual dual-specificity MKPs have been shown to specifically recognize this unique phosphorylation motif in MAPK factors, inducing their dephosphorylation. Each of these MKPs differs in substrate specificity, with MKP-1 being most selective for p38 MAPK. Additionally, TNF may indirectly induce MKP-1 activation via calcineurin (PPA2B) phosphatase. TNF has previously been shown to induce calcineurin in cardiac myocytes, leading to cardiac hypertrophy by inhibiting p38 MAPK activation. This
hypothesis, however, has not yet been validated in our system.

Radiation therapy is presently available for inhibition of restenosis in stented coronary arteries. Although effective in reducing the incidence of recurrent neointimal proliferation, this intervention is compromised by delayed reendothelialization, which is associated with a significant incidence of late-stent thrombosis. More recently, reports indicating that rapamycin and other cell-cycle inhibitors are capable of eliminating restenosis have generated a great deal of interest. Because both chemotherapy and radiation therapy share an approach that has complete disregard for endothelial functional recovery, it is not surprising that both have been associated with late events attributable to endothelial dysfunction. In contrast to these strategies, results from an approach that has complete disregard for endothelial functional recovery, it is not surprising that both have been associated with late events attributable to endothelial dysfunction. In contrast to these strategies, results from an approach that has complete disregard for endothelial functional recovery, it is not surprising that both have been associated with late events attributable to endothelial dysfunction. In contrast to these strategies, results from an approach that has complete disregard for endothelial functional recovery, it is not surprising that both have been associated with late events attributable to endothelial dysfunction.

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References

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