Cyclic Stretch Controls the Expression of CD40 in Endothelial Cells by Changing Their Transforming Growth Factor–β1 Response

Thomas Korff, PhD; Karin Aufgebauer, MD; Markus Hecker, PhD

Background—CD40 is a costimulatory molecule that acts as a central mediator of various immune responses, including those involved in the progression of atherosclerosis. Correspondent to its function, CD40 is present not only on many immune cells, such as antigen-presenting cells and T cells, but also on nonimmune cells, such as endothelial cells.

Methods and Results—Ex vivo analyses in mice revealed that CD40 is strongly expressed in distinct venous and capillary but not arterial endothelial cell populations. Therefore, we analyzed to what extent determinants of an arterial environment control CD40 expression in these cells. In vitro studies indicated that the presence of smooth muscle cells or exposure to cyclic stretch significantly downregulates CD40 expression in human endothelial cells. Interestingly, endothelial cells cocultured with smooth muscle cells upregulated CD40 expression in response to cyclic stretch through a transforming growth factor–β1/activin-receptor–like kinase-1 (Alk-1)–dependent mechanism. To corroborate that this mechanism also operates in arteries in vivo, we analyzed the expression of Alk-1 and CD40 at atherosclerosis-prone sites of the mouse aorta that also appear to be exposed to increased stretch. In wild-type mice, both Alk-1 and CD40 revealed a comparably heterogeneous expression pattern along the aortic arch that matched those sites in low-density lipoprotein–receptor–deficient mice where atherosclerotic lesions develop.

Conclusions—Cyclic stretch thus increases the abundance of CD40 in endothelial cells through transforming growth factor–β1/Alk-1 signaling. This mechanism in turn may be responsible for the heterogeneous expression of CD40 at arterial bifurcations or curvatures and would support a site-specific proinflammatory response that is typical for the early phase of atherosclerosis. (Circulation. 2007;116:2288-2297.)

Key Words: endothelial cells • CD40 antigens • TGF-beta-1 • smooth muscle cells

The costimulatory molecule CD40 plays a central role in controlling inflammatory responses, including autoimmune syndromes, allograft rejection, and atherosclerosis. It is expressed by numerous cell types dependent on their activation. Although CD40 appears crucial for the proinflammatory phenotype of genuine immune-competent cells such as B cells, this costimulatory molecule is also expressed in vascular cells such as endothelial and smooth muscle cells.1 Experiments in low-density lipoprotein (LDL) receptor–deficient mice have shown that neutralization of the CD40 ligand CD154 significantly reduces the size and the lipid, macrophage, T-cell, and vascular cell adhesion molecule-1 (VCAM-1) content of atherosclerotic lesions,2–4 hence pointing toward a role for CD40 in atherogenesis. Likewise, analyses of human cultured endothelial cells revealed that CD40 expression is upregulated by interleukin-1β, tumor necrosis factor-α, and interferon-γ, proinflammatory cytokines that abound in atherosclerotic plaques.1 Moreover, ligation of CD40 in these cells enhances the expression of intercellular adhesion molecule-1, VCAM-1, and E-selectin.5 Taken together, these findings have prompted the hypothesis that CD40 signaling in endothelial cells contributes to the recruitment and activation of circulating T cells and monocytes, thus amplifying the chronic inflammatory response in the vessel wall that leads to atherosclerosis.6

Clinical Perspective p 2297

Although CD40 expression in plaque-associated vascular cells has been verified,7 it is not known whether biomechanical forces that are implicated not only in the early but also in the progression phase of atherosclerosis also affect CD40 expression in these cells. Two biomechanical forces strongly influence the functional and structural phenotype of vascular cells. Laminar shear stress, which mainly acts on endothelial...
cells, is generally viewed as an atheroprotective force that upregulates both the activity and expression of endothelial cell nitric oxide synthase, with the resulting enhanced production of nitric oxide in turn blocking proatherosclerotic gene expression in these cells. In contrast, cyclic stretch, which affects endothelial and vascular smooth muscle cells alike, is thought to promote atherosclerosis through upregulation of endothelial cell reactive oxygen species formation and hence proatherosclerotic gene expression.

Here, we report that in vascular homeostasis, CD40 is preferentially expressed in venous and capillary endothelial cells that are typically involved in the recruitment and activation of leukocytes during inflammatory processes. Although generally not detectable in arterial endothelial cells, CD40 is also expressed at atherosclerosis-prone sites along the mouse aortic arch. This surprising finding prompted us to analyze biomechanical and cellular determinants that may be responsible for this nonuniform expression pattern of CD40.

**Methods**

**Materials**

Transforming growth factor–β1 (TGF-β1), activin-receptor–like kinase-1 (Alk-1) chimeric protein, and anti-human TGF-β1 and anti-human Alk-1 antibodies were purchased from R&D Systems GmbH (Wiesbaden, Germany). Anti-phospho-Smad 1/5 and anti-phospho-Smad 1/5/8 antibodies were from Cell Signaling (Danvers, Mass).

**Animal Studies**

All animal studies were performed with permission of the Regional Council of Karlsruhe and conformed to the “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health (National Institutes of Health publication No. 85-23,
revised 1996). Adult (>6 months old) C57/BL6 or LDL-receptor-deficient mice were euthanized, and the aortic arch was perfused with zinc fixative, excised, and processed for histological examination.

Cell Culture

Human umbilical vein endothelial cells (HUVECs) were isolated from freshly collected umbilical cords and cultured in M199 medium (Invitrogen, Karlsruhe, Germany) containing 20% fetal bovine serum (Life Technologies, Karlsruhe, Germany), 50 U/mL penicillin, 50 μg/mL streptomycin, and 5 mmol/L HEPES, supplemented with endothelial growth supplement (PromoCell, Heidelberg, Germany). Human smooth muscle cells were isolated from human thymus glands after thyrectomy and cultured in DMEM (Invitrogen) supplemented with 50 U/mL penicillin, 50 μg/mL streptomycin, and 10% fetal bovine serum. The phenotype of these cells was confirmed by α-smooth muscle actin and desmin-specific immunofluorescence analysis. Human aortic and saphenous vein endothelial cells were purchased from PromoCell and cultured according to the manufacturer’s instructions. Cells were cultured at 37°C, 5% CO2, and 100% humidity. Only cells cultured up to passage 4 were used for the experiments. All types of cells were cultured on plastic dishes or BioFlex collagen type I 6-well plates (Flexcell, Hillsborough, NC) coated with gelatin (2 mg/mL gelatin in 0.1 mol/L HCl for 30 minutes at ambient temperature). Stretched was performed with a FX-3000 FlexerCell stretch unit with 15% cyclic elongation at 0.5 Hz, which corresponds to the physiological maximum deformation in the human carotid artery.9

Coculture of Endothelial and Smooth Muscle Cells

Before cells were seeded onto a BioFlex collagen type I 6-well plate, a silicon wall (self-made with biologically inert medical silicone from Wacker, Munich, Germany) was inserted into each well, equally dividing it into 2 areas. If HUVECs were cultured alone, they were seeded into one half of the well only to ensure that they were grown to confluence at the beginning of each experiment. For coculture conditions, equal numbers of HUVECs and human smooth muscle cells were simultaneously seeded in individual halves of 1 well. Both cell types were cultured in M199 medium containing 20% fetal bovine serum, 50 U/mL penicillin, 50 μg/mL streptomycin, and 5 mmol/L HEPES. The silicon wall was removed after cellular adhesion, followed by changing of the medium. Thereafter, cells were grown overnight before experiments were started. In all experimental setups, the same number of endothelial cells was used.

Reverse-Transcription Polymerase Chain Reaction

Total RNA was isolated from the cultured cells by solid-phase extraction with an RNeasy kit (Qiagen, Düsseldorf, Germany) according to the manufacturer’s instructions. Reverse transcription and polymerase chain reaction for human TGF-β1 and 60S ribosomal protein L32 (RPL32) cDNA as an internal standard was performed as described previously.12 The following primers were used for amplification: human TGF-β1 forward, 5’-GCCCTGGACACCAACTTATT-3’; human TGF-β1 reverse, 5’- TCAACTGGACTTTCAGAGG-3’; human RPL32 forward, 5’- GTTCATCCGGCACAGTCA-3’; human RPL32 reverse, 5’- AC GTCACATGAGCTGCCTAC-3’; human CD40 forward, 5’- CAGAGTTCACACTGAAACGGAT-GCC-3’; human CD40 reverse, 5’-TGCCGTCCCTGTTCGACCAAC-3’; human VCAM-1 forward, 5’- CATGACCTGGTTCACCAGG-3’; human VCAM-1 reverse, 5’- CATTCCAGGACCAC-CACTC-3’; human Alk-1 forward, 5’- CGAGCCGGAGCCAGAAGCAG-3’; human Alk-1 reverse: 5’- TGAACTCGCCGCTGGCAATGG-3’.

Western Blotting

Cells cultured as spheroids or a monolayer were lysed with sample buffer containing 1% Triton X-100. Samples were resolved on a 10% SDS-PAGE gel and blotted. The blots were probed with a polyclonal rabbit anti-human CD40 antibody (1:2000 dilution, Research Diagnostics, Concord, Mass) or rabbit anti-human VCAM-1 antibody

| Table. Distribution of CD40 Expression as Judged by Immunohistochemistry in Endothelial Cells of Different Mouse Organs |
|------------------------|--------|-------|
| Arteries | Veins | Capillaries |
| Main blood vessels | – | + |
| Skeletal muscle | – | + |
| Heart | – | – |
| Lung | – | – |
| Kidney | – | +/– |
| Ileum | – | – |
| Liver | – | – |
| Pancreas | – | +/– |
| Spleen | – | – |
| Lymph node | – | – |

+ Indicates strong expression; +/-, partial expression; and –, no expression detected.

(1:2000 dilution, Santa Cruz Biotechnology, Heidelberg, Germany) and reprobed for CD31 (goat polyclonal, sc-1506, Santa Cruz Biotechnology) and β-actin (1:3000 dilution, Sigma-Aldrich, Munich, Germany) after stripping.

Morphological, Immunocytochemical, and Immunohistochemical Analyses

Cells were fixed with buffered 4% paraformaldehyde, blocked with 1% BSA/PBS (albumin bovine fraction V, Sigma-Aldrich), and incubated with a monoclonal mouse anti-human CD40 antibody (Dianova, Hamburg, Germany), a polyclonal goat anti-mouse Alk-1 antibody, or a monoclonal mouse anti-human CD31 antibody (Dako, Glostrup, Denmark). Binding of primary antibodies was detected by an anti-mouse or anti-goat–specific antibody conjugated to Cy3 (Invitrogen). Nuclei were visualized by Hoechst dye 33258.

Mouse tissue specimens were fixed for 24 hours in zinc fixative, dehydrated, and embedded in paraffin. Immunohistochemical staining for CD40 or Alk-1 and phosphorylated Smad 1/5/8 was performed on 4-μm sections with a polyclonal rabbit anti-mouse CD40 antibody (Dianova) or a polyclonal anti-mouse Alk-1 antibody in combination with a 3,3-diaminobenzidine (DAB)–based enhanced detection method (EnVision, Dako) according to the manufacturers’ instructions. Localization of atherosclerotic lesions in the aortic arch of LDL receptor–deficient mice was macroscopically and microscopically determined by the white appearance of these plaques, which could be differentiated easily from healthy vessel segments.

Statistical Analysis

All results are expressed as mean±SD. Differences between experimental groups were analyzed by unpaired Student t test with P<0.05 considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Venous and Capillary but Not Arterial Endothelial Cells Express CD40 During Vascular Homeostasis

CD40 has been shown to be expressed in vascular cells under proinflammatory conditions.5,13 To get an idea of its distribution in native nonactive blood vessels, we analyzed CD40 expression by immunohistochemistry in different organs of
NMRI mice. Surprisingly, CD40 was strongly expressed in distinct venous and capillary but not arterial endothelial cells (Figure 1; Table).

**Endothelial Cell CD40 Abundance Is Downregulated by Interaction With Smooth Muscle Cells or Exposure to Cyclic Stretch**

To test whether CD40 expression is affected by environmental parameters, we cultured arterial and venous endothelial cells and exposed them to cyclic stretch. Although CD40 protein was readily detected in all types of cells, its abundance decreased markedly on prolonged (18 hours to 3 days) exposure to cyclic stretch (Figure 2A through 2C) but returned to baseline within 3 hours after cessation of stretch (Data Supplement Figure I). In contrast to cyclic stretch, exposure to arterial shear stress (30 dyne/cm²) did not affect endothelial cell CD40 expression (data not shown). Further analyses of HUVECs revealed that these usually CD40-negative cells (Figure 2D and 2E) rapidly upregulate CD40 in culture (Figure 2F) if the influence of smooth muscle cells is lacking. When both cell types were cocultured, however, endothelial cell CD40 protein abundance declined significantly (Figure 2G).

**Cyclic Stretch Upregulates CD40 Expression in Endothelial Cells Cocultured With Smooth Muscle Cells**

Next, we investigated whether cyclic stretch and the presence of smooth muscle cells complement each other in terms of their effect on endothelial cell CD40 expression. To this end, a coculture system was set up (Figure 3A and 3B) in which...
HUVECs and human smooth muscle cells were simultaneously exposed to cyclic stretch followed by analysis of CD40 protein and mRNA expression (Figure 3E and 3F). In contrast to the stretch-dependent downregulation of CD40 protein in solo endothelial cell cultures (Figure 3E), this was surprisingly upregulated under conditions in which both cell types had been cultured in close proximity (Figure 3C through 3E). Distant coculture of both cell types (separated by a defined space), conversely, fell short of upregulating endothelial cell CD40 abundance but prevented its downregulation (Data Supplement Figure III). Even though Western blot analyses have shown that human cultured smooth muscle cells do not express CD40 in vitro (data not shown), we verified by immunohistochemistry that the observed upregulation of CD40 abundance was limited to the endothelial cells (Figure 3C and 3D). RNA expression analyses further revealed that downregulation of endothelial cell CD40 protein is not accompanied by significant changes in CD40 mRNA (Figure 3F), and only on interaction with the smooth muscle cells was upregulation of endothelial cell CD40 protein accompanied by a significant rise in mRNA abundance (Figure 3F).

**Upregulation of CD40 Expression in Endothelial Cells Cocultured With Smooth Muscle Cells Is Controlled by TGF-β1**

On the basis of the fact that TGF-β1 is a key regulator of endothelial cell/smooth muscle cell communication in vivo and in vitro, we analyzed to what extent this cytokine might be involved in the control of CD40 expression in the coculture setup. To this end, TGF-β1 activity was blocked with a neutralizing antibody, and this prevented the stretch-induced increase in CD40 mRNA and protein outright (Figure 4A and 4B; Data Supplement Figure III). The stretch-induced downregulation of CD40 expression in solo endothelial cell cultures, on the other hand, was not affected by the neutralizing anti-TGF-β1 antibody. Interestingly, additional polymerase chain reaction and cytokine array analyses revealed only a modest increase in TGF-β1 expression in stretch-stimulated cocultures of endothelial and smooth muscle cells (Data Supplement Figure II).

**Stretch-Induced Upregulation of Endothelial Cell CD40 Expression Is Mediated by Alk-1**

Assuming that the observed stretch-induced increase in endothelial cell CD40 expression cannot be based solely on an...
increase in biologically active TGF-β1 but may also be due to a heightened endothelial cell responsiveness to TGF-β1, we analyzed whether TGF-β receptor expression was changed under the chosen experimental conditions. In contrast to the TGF-β type I receptor Alk-5 and the TGF-β type II receptor (data not shown), endothelial cell expression of Alk-1, another TGF-β type I receptor, was strongly upregulated in the coculture setup after exposure to cyclic stretch (Figure 5A through 5C). Again, close proximity between the endothelial and smooth muscle cells appeared to be mandatory for this effect to occur (Data Supplement Figure IV). Furthermore, the disturbance of Alk-1 function by the employment of soluble Alk-1 receptor molecules blocked the increase in CD40 protein under these conditions (Figure 5D). In addition to cyclic stretch, exposure to TGF-β1 per se resulted in an upregulation of endothelial cell Alk-1 expression (data not shown).

Heterogeneous Expression of CD40 in Endothelial Cells at Distinct Sites of the Aortic Arch Coincides With the Distribution of Alk-1

On the basis of the aforementioned findings, we hypothesized that arterial segments that are continuously exposed to cyclic stretch upregulate CD40 in endothelial cells as the result of an Alk-1–dependent alteration of their TGF-β1 responsiveness. To corroborate this notion, we analyzed CD40 and Alk-1 protein abundance at sites of the mouse aortic arch that are supposed to be exposed to an increased level of cyclic stretch.9 In contrast to the heterogeneous expression of the 2 proteins among the endothelial cells lining the aortic arch (Figure 6B through 6D), straight segments of the abdominal aorta that are thought to be exposed to a comparatively smaller biomechanical strain were devoid of any CD40 or Alk-1 staining (Figure 6F through 6H). By semiquantitatively mapping the expression pattern of both proteins on an anatomic scheme of the aortic arch, endothelial cell CD40 and Alk-1 expression appeared to colocalize (Figure 6A and 6E). A corresponding staining pattern was also found for phosphorylated Smad 1 and 5 in endothelial cell nuclei (Figure 6I through 6M), indicative of increased TGF-β1 activity at these sites. Furthermore, the expression pattern of CD40, Alk-1, and phosphorylated Smads 1/5 in wild-type mice largely coincided with sites along the aortic arch of low-density–lipoprotein–receptor–deficient mice at which atherosclerotic plaques develop (cf Figure 6A, 6E, 6I, and 6N).

Discussion

The surface receptor CD40 is an important costimulatory molecule for both the humoral and cellular immune response that can be expressed in leukocytes and in vascular cells.14 Gene-targeting studies support the pivotal role of CD40 for T-cell–dependent immunoglobulin class switching and germinal center formation.15 Furthermore, CD40 signaling appears critical for T-cell–dependent activation of macrophages16 and endothelial cell stimulation of extravasating monocytes.17 Likewise, small interfering RNA silencing of endothelial cell CD40 expression has been shown to prevent CD154-activated leukocyte adhesion.18 These results underline the importance of CD40 expression in endothelial cells for proinflammatory responses.

Despite this, little is known about CD40 distribution and the molecular mechanisms that control its expression in vascular homeostasis. Here, we report that CD40 is expressed in distinct venous and capillary endothelial cell populations but usually not in arterial endothelial cells, which suggests that it may be affected by microenvironmental conditions. Artery-specific determinants may prevent CD40 expression, whereas CD40 expression is promoted by venous- or capillary-specific determinants, the latter representing a vascular entity that is typically involved in the recruitment and activation of leukocytes during inflammatory responses. Expression of specific molecules on distinct endothelial cell
subpopulations is well established throughout the literature. Similar to the present findings that CD40, contrary to the situation in the native vasculature, is expressed in all cultured endothelial cells irrespective of their origin, most endothelial cell–specific molecules are not part of an imprinted phenotype but are regulated by microenvironmental parameters such as the local hemodynamics or communication with other cell types. The present results suggest that endothelial cell CD40 protein abundance is affected by at least 3 determinants, ie, cyclic stretch, smooth muscle cells, and TGF-β1.

First, we identified cyclic stretch as a biomechanical force that downregulates endothelial cell CD40 protein abundance. This effect was critically dependent on the microenvironment. For instance, if the cells were cultured on top of a Matrigel-coated surface, CD40 expression was increased on exposure to cyclic stretch (data not shown). Similarly, coculture with smooth muscle cells transformed the observed downregulation of CD40 abundance into an increase. In line with these results, additional in vivo analyses indicated that baseline CD40 expression in arteries or veins is not affected by acute alterations of the main biomechanical forces to which the endothelial cells are exposed (data not shown). Likewise, varying the frequency and/or intensity of cyclic stretch did not affect CD40 downregulation in human cultured endothelial cells. Moreover, the decline in CD40 protein content was not preceded or accompanied by a significant change in CD40 mRNA abundance, which suggests that it may be caused by a posttranslational mechanism such as degradation of the protein, a hypothesis that is currently under investigation (A.H. Wagner, PhD, oral communication, March 2007). On the basis of these results, cyclic stretch can be viewed as an important modulator of baseline CD40 expression in endothelial cells.

Second, we demonstrated that smooth muscle cells affect CD40 expression in endothelial cells. The influence of smooth muscle cells on the proinflammatory and arteriovenous differentiation of endothelial cells has been demonstrated in various experimental setups and may also be responsible for the observed decline in CD40 protein content. However, the complex nature of this cellular interplay is still obscure, and the present protein array studies did not identify a particular cytokine that may be responsible for the smooth muscle cell–dependent decrease in endothelial cell CD40 abundance. Coculture of both cell types at close proximity appears to be essential for intercellular communication, and this was the case under the present experimental conditions. In contrast, a coculture setup in which both cell types were separated by a defined space did not induce downregulation of endothelial cell CD40 expression.

Third, we identified the endothelial cell response to TGF-β1 as a third factor that determines CD40 expression that may be due to a smooth muscle cell–mediated sensitization to or an increase in the local concentration of biologically

Figure 5. Expression of Alk-1 in stretch-stimulated HUVECs (EC) cocultured with human smooth muscle cells (SMC). A, Semiquantitative reverse-transcription polymerase chain reaction analyses with the Alk-1/RPL32 ratio under control conditions set to 100% revealed that after exposure to cyclic stretch for 18 hours, Alk-1 mRNA is upregulated only in stretch-stimulated EC/SMC cocultures (P<0.05; n.s. indicates not significant). B, A weak Alk-1–specific fluorescence signal was detected in the EC islands under control conditions, which was increased after 18 hours of exposure to cyclic stretch (C; scale bar=50 μm). D, Treatment with chimeric Alk-1 protein (2 μg/mL) did not affect the stretch-induced downregulation of CD40 protein in EC but abolished its upregulation in EC/SMC cocultures exposed to cyclic stretch for 18 hours (***P<0.001; n.s. indicates not significant).
active TGF-β1. Typically, CD40 expression is elevated if endothelial cells are exposed to proinflammatory cytokines that are abundantly expressed in atherogenesis.1,25 The present data show that TGF-β1 is capable of upregulating CD40 expression in stretch-stimulated endothelial cells and thus may act as an immunomodulatory molecule.

TGF-β1 is a well-known vasculotropic cytokine, present at high levels in the vessel wall,26 which has been shown to inhibit proliferation and migration of smooth muscle cells.27,28 Moreover, TGF-β1 inhibits proinflammatory responses both in leukocytes and endothelial cells29,30 and stabilizes atherosclerotic plaques.31,32 Conversely, TGF-β1 has been shown to stimulate vascular remodeling processes such as arteriogenesis,33 adhesion molecule expression in and adhesion of leukocytes to endothelial cells,34,35 and leukocyte chemotaxis.36 These somewhat contradictory findings may be explained at least in part by the fact that TGF-β1–mediated inhibitory or stimulatory effects are strongly dependent on the level of the biologically active cytokine and on the biological context.37

We noted that only exposure to cyclic stretch unmasks the stimulatory effect of TGF-β1 on endothelial cell CD40 expression and that this was mediated through an Alk-1–dependent signaling pathway, notwithstanding the fact that additional stretch-dependent TGF-β1–sensitizing mechanisms may exist (cf Data Supplement Figure V). Alk-1 appears to be critically involved in balancing the activation state of endothelial cells,38–40 whereby its activation stimulates expression of Id-1 (inhibitor of differentiation/DNA synthesis-1), which in turn promotes endothelial cell proliferation and migration.41 Moreover, the level of expression of TGF-β1 and its receptors in atherosclerotic lesions may play an important role in controlling the proinflammatory and profibrotic responses to this cytokine.32,40,42 Thus, our observation in the present study of increased Alk-1 expression and the phosphorylation of its downstream transcription factors...
Smad 1 and 5 at atherosclerosis-prone sites in the aortic arch of the mouse makes sense. It is broadly accepted that atherosclerosis preferentially develops at specific sites along the arterial tree comprising branches, bifurcations, and curvatures.43,44 Here, the usually laminar blood flow becomes turbulent, which results in a major change of the biomechanical forces acting on the vessel wall. Shear stress changes from laminar to oscillatory while pulsatile alterations in wall strain are accentuated, which results in increased cyclic stretch.45 In particular, cyclic stretch is thought to promote atherogenesis by stimulating the production of reactive oxygen species, which in turn activate transcription factors that upregulate the expression of proatherosclerotic gene products, especially in endothelial cells.10,11,16,47 Compatible with this, we continuously observed an expression of CD40 at atherosclerosis-prone sites of the aortic arch that was not detectable in other arterial endothelial cells of the mouse but that coincided with expression of the TGF-β receptor Alk-1. We conclude, therefore, that the increased level of cyclic stretch at these sites changes the endothelial cell response to TGF-β1 by inducing Alk-1 expression, which in turn enables the upregulation of CD40 expression. However, whether the heterogeneous expression pattern of the 2 molecules is in fact also indicative of endothelial cell dysfunction that precedes atherosclerotic plaque formation remains to be established. In this context, it is important to note that attributing the observed changes in gene expression solely to changes in cyclic stretch would oversimplify the complex local hemodynamics along the aortic arch and its tributaries, with changes in shear stress (from laminar to oscillatory) and blood flow per se accompanying those in cyclic stretch. Moreover, the development of atherosclerotic lesions along the aortic arch of LDL receptor–deficient mice, although it too presumably has a hemodynamic basis, most likely will be governed by the excessive dyslipidemia in this animal experimental model. Taken together, the present findings suggest the following: (1) the expression of CD40 in endothelial cells is highly dependent on the local environment; (2) cyclic stretch up-regulates CD40 abundance in endothelial cells under the influence of smooth muscle cells; (3) this is dependent on TGF-β1; and (4) this is mediated though endothelial cell Alk-1. This sequence of events may be responsible for the expression of CD40 at atherosclerosis-prone sites of the arterial tree and thus may act as an early site-specific indicator of endothelial cell dysfunction.

Acknowledgments

The authors would like to acknowledge Professor Katrin Schäfer (University of Göttingen) for providing the LDL receptor–deficient mice and the excellent technical assistance of Odran Scheib, Lorena Urda, Sabine Krull, and Kathrin Schreiber.

Sources of Funding

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB TR23, project C5).

Disclosures

None.

References

Atherosclerosis is a multistep process thought to be due to a local destabilization of vascular homeostasis that leads to endothelial cell dysfunction. This process preferentially occurs at arterial bifurcations, branches, or curvatures, sites limited, these observations suggest that the altered hemodynamics at atherosclerosis-prone sites have an impact not only on the activation state of infiltrating T cells and/or monocyte/macrophages but also on the responsiveness of the resident cells of the vessel wall to cytokines such as transforming growth factor– beta. In addition to interventional strategies such as angioplasty, passivation or stabilization of atherosclerotic lesions is regarded as an important strategy to prevent plaque rupture and hence the risk of an acute and potentially life-threatening ischemia. Another promising approach, therefore, may be to identify agonists that compensate for the local disturbances in vascular homeostasis, thereby delaying the development of vulnerable plaques. Such a strategy might comprise stabilization of Alk-5 expression in vascular cells, as angioplasty, passivation or stabilization of atherosclerotic lesions is regarded as an important strategy to prevent plaque rupture and hence the risk of an acute and potentially life-threatening ischemia. Another promising approach, therefore, may be to identify agonists that compensate for the local disturbances in vascular homeostasis, thereby delaying the development of vulnerable plaques. Such a strategy might comprise stabilization of Alk-5 expression in vascular cells, which has been shown to inhibit endothelial cell and smooth muscle cell migration and proliferation and to trigger collagen synthesis in vascular smooth muscle cells.

CLINICAL PERSPECTIVE

Atherosclerosis is a multistep process thought to be due to a local destabilization of vascular homeostasis that leads to endothelial cell dysfunction. This process preferentially occurs at arterial bifurcations, branches, or curvatures, sites limited, these observations suggest that the altered hemodynamics at atherosclerosis-prone sites have an impact not only on the activation state of infiltrating T cells and/or monocyte/macrophages but also on the responsiveness of the resident cells of the vessel wall to cytokines such as transforming growth factor–beta. In addition to interventional strategies such as angioplasty, passivation or stabilization of atherosclerotic lesions is regarded as an important strategy to prevent plaque rupture and hence the risk of an acute and potentially life-threatening ischemia. Another promising approach, therefore, may be to identify agonists that compensate for the local disturbances in vascular homeostasis, thereby delaying the development of vulnerable plaques. Such a strategy might comprise stabilization of Alk-5 expression in vascular cells, which has been shown to inhibit endothelial cell and smooth muscle cell migration and proliferation and to trigger collagen synthesis in vascular smooth muscle cells.
Cyclic Stretch Controls the Expression of CD40 in Endothelial Cells by Changing Their Transforming Growth Factor–β1 Response
Thomas Korff, Karin Aufgebauer and Markus Hecker

Circulation. 2007;116:2288-2297; originally published online October 29, 2007; doi: 10.1161/CIRCULATIONAHA.107.730309
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/116/20/2288

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2007/11/06/CIRCULATIONAHA.107.730309.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/