Bone Morphogenic Protein 4 Produced in Endothelial Cells by Oscillatory Shear Stress Stimulates an Inflammatory Response*

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Atherosclerosis is now viewed as an inflammatory disease occurring preferentially in arterial regions exposed to disturbed flow conditions, including oscillatory shear stress (OS), in branched arteries. In contrast, the arterial regions exposed to laminar shear (LS) are relatively lesion-free. The mechanisms underlying the opposite effects of OS and LS on the inflammatory and atherogenic processes are not clearly understood. Here, through DNA microarrays, protein expression, and functional studies, we identify bone morphogenetic protein 4 (BMP4) as a mechanosensitive and pro-inflammatory gene product. Exposing endothelial cells to OS increased BMP4 protein expression, whereas LS decreased it. In addition, we found BMP4 expression only in the selective patches of endothelial cells overlying foam cell lesions in human coronary arteries. The same endothelial patches also expressed higher levels of intercellular cell adhesion molecule-1 (ICAM-1) protein compared with those of non-diseased areas. Functionally, we show that OS and BMP4 induced ICAM-1 expression and monocyte adhesion by a NFκB-dependent mechanism. We suggest that BMP4 is a mechanosensitive, inflammatory factor playing a critical role in early steps of atherogenesis in the lesion-prone areas.

Endothelial cells are constantly exposed to shear stress (a dragging force generated by blood flow), which controls cellular structure and function such as regulation of vascular tone and diameter, vessel wall remodeling, hemostasis, and inflammatory responses (1). The importance of various types of shear stress is highlighted by the focal development of atherosclerosis (2). Atherosclerosis preferentially occurs in the arterial regions exposed to unstable shear stress conditions in branched or curved arteries, whereas straight arteries exposed to unidirectional laminar shear (LS) are relatively lesion-free (1–3). Atherosclerosis is now known as an inflammatory disease caused by endothelial dysfunction (3, 4). One of the first visible markers of endothelial dysfunction in the lesion-prone areas is up-regulation of inflammatory adhesion molecules such as E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and ICAM-1 (3–6). These endothelial adhesion molecules play essential roles in adhesion and recruitment of monocytes to the subendothelial layer (3, 4).

How do unstable shear conditions such as low and oscillating shear stress (OS) cause inflammation in those lesion-prone areas, whereas LS exerts anti-inflammatory effects? The opposite effects of LS and OS may be determined by differential expression of genes and proteins, ultimately inducing anti- and pro-inflammatory and atherogenic responses. Recently, several studies (7–10) have begun to address the initial question to determine the expression profiles of mechanosensitive genes. However, the functional importance of those genes has not been clearly established.

Here, we report identification of a mechanosensitive gene, BMP4, by DNA microarray analyses and subsequent verification by a variety of additional approaches in both cultured endothelial cells and human coronary arteries. More importantly, we discovered a novel role of BMP4 as an inflammatory cytokine, providing a potential mechanistic link from shear forces to inflammatory responses and atherogenesis.

**MATERIALS AND METHODS**

Endothelial Cells—Mouse aortic endothelial cells (MAEC) were cultured and used at passages 4–8 as described by us (11). Human aortic endothelial cells (HAEC) purchased from Clonetics were cultured using the EGM-2 bullet kit (Clonetics) and used at passages 4–8.

Shear Stress Studies—Confluent endothelial monolayers grown in 100-mm tissue culture dishes were exposed to an arterial level of unidirectional LS (15 dyn/cm²) in the growth medium by rotating a Teflon cone (0.5° cone angle) as described previously by us (12). To mimic unstable shear conditions in vivo, endothelial cells were exposed to OS with directional changes of flow at 1 Hz cycle (±5 dyn/cm²) by rotating the cone back and forth using a stepping motor (Servo Motor) and a computer program (DC Motor Company, Atlanta, GA). In some studies, 5 dyn/cm² unidirectional LS was used for comparison to OS (±5 dyn/cm²).

Preparation of Cell Lysates and Immunoblotting—Following experimental treatments, endothelial cell lysates were prepared and analyzed by Western blot analysis as described by us (13, 14). Briefly, cells were washed in ice-cold phosphate-buffered saline and lysed in 0.1 ml of boiling lysis buffer A (10 mM Tris-HCl, pH 7.6, 1 mM sodium vanadate, and 1% SDS). The lysate was further homogenized by repeated aspiration through a 25-gauge needle. Protein content of each sample was measured by using a Bio-Rad DC assay (15). To detect secreted BMP4 in conditioned media, endothelial monolayers were first washed in serum-free Dulbecco’s modified Eagle’s medium supplemented with minimum non-essential amino acids and pyruvic acid and exposed to OS, LS, or static conditions for 1 day. The conditioned media were then centrifuged at 1,000 × g for 10 min. Aliquots (2 ml) of the supernatant were subjected to 12.5% SDS-PAGE and the gel was stained with silver.
were collected and placed on ice with 10 ml of ice-cold acetone to precipitate protein for 30 min. Samples were pelleted by centrifugation (15,000 × g for 10 min) and resuspended in 100 µl of sample buffer for SDS-PAGE (13, 14). Aliquots of cell lysates (20 µg of protein each) were resolved on a 10% SDS-PAGE gel and transferred to a polyvinylidene difluoride membrane (Millipore). The membrane was incubated with a primary antibody overnight at 4 °C and then with a secondary antibody conjugated to alkaline phosphatase (1 h at room temperature), which were detected by a chemiluminescence method (15). The intensities of immunoreactive bands in Western blots were analyzed by using the NIH Image program. The following primary antibodies were used: a monoclonal BMP4 antibody, rabbit ICAM1 antibody, goat VCAM1 antibody, and goat actin antibody (Santa Cruz Biotechnology).

Fluorescence-activated Cytometry Sorting (FACS) Analysis—Treated cells were dissociated into single-cell suspensions using 0.25% trypsin-EDTA and resuspended in a FACS buffer (Hank’s buffered solution containing 5% fetal bovine serum). Aliquots of cell suspensions were incubated with ICAM1 antibody (R&D Systems) for 20 min on ice, washed twice with FACS buffer, and incubated with secondary antibody (fluorescein-5-isothiocyanate- or phycoerytherin-conjugated; Becton-Dickinson) using CellQuest software. The fluorescence intensity of cell lysates by fluorescence spectrophotometry using a plate reader. Both assays showed similar results. Some studies were performed with a non-transfected with 0.5 µg/ml mouse ICAM1 antibody (Y11; Southern Biotechnology) (22).

RESULTS

Differential Regulation of the BMP4 Gene by LS and OS in Endothelial Cells—To identify the genes that may be responsible for the athero-protective and pro-atherogenic effects of LS and OS, respectively, we performed DNA microarray studies using cultured MAEC. Exposing MAEC to LS, but not OS, for 1 day using the modified “cone-and-plate” device (12) induced a cell shape alignment to the direction of the flow from a typical polygonal “cobblestone shape” found in static cultured cells (Fig. 1).

The total RNAs prepared from these cells were used to determine mRNA expression profiles by using Affymetrix and/or Motorola DNA chips according to the manufacturers’ protocols. The analyses of these studies showed that LS exposure signif-

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BMP4 expression (*, p < 0.05) significantly decreased BMP4 protein expression (*, a loading control). The band intensities were quantified and expressed as % of static controls as shown in the bar graphs (mean ± S.E.). Western blot (IB). Cell lysates obtained from cells exposed to LS, OS, or St were analyzed by Western blot with a BMP4 antibody or an actin antibody (used as a loading control). The band intensities were quantified and expressed as % of static controls as determined by Western blot analysis using a monoclonal antibody (data not shown), providing further confidence in our results.

BMPs play an important role in bone formation, embryonic development, and differentiation (23, 24). Although BMP4 protein has been found previously in calcified atherosclerotic plaques (25), its expression and functional importance in endothelial cells have not been determined. Therefore, we decided to verify the microarray results by independent methods at the levels of mRNA and protein as well as the functional roles of BMP4 in endothelial biology and pathobiology.

First, we verified the BMP4 mRNA data by using a quantitative real-time PCR method. Exposure of endothelial cells to LS almost eliminated the BMP4 mRNA level (n = 6, p < 0.001) (Fig. 2B). In contrast, OS marginally, statistically not significant, increased BMP4 mRNA level compared with that of static control (n = 3). These results confirmed the DNA microarray results.

Next, BMP4 protein expression was determined by immunoblot studies. BMP4 protein is synthesized as an inactive precursor (48–55 kDa) that is proteolytically cleaved by proprotein convertases, and the active ~23-kDa protein is secreted (23, 24). In endothelial cell lysates, the BMP4 precursor was detected as a 54-kDa protein, and the mature form (p23) was detected in the conditioned media collected from static or shear-exposed cells (Fig. 2C). Exposure of cells to LS significantly down-regulated expression of BMP4 precursor in a time-dependent manner (Fig. 2C). After 16–24 h of LS exposure, BMP4 precursor expression was virtually undetectable (Fig. 2C, left panel, p < 0.05). In contrast, exposure of MAEC to OS significantly increased BMP4 precursor protein level by 2-fold above control (Fig. 2C, middle panel, p < 0.05). Consistent with the cell lysate result, the conditioned media of MAEC exposed to LS (15 dyn/cm²) showed a barely detectable amount of secreted form of BMP4 (p23) (Fig 2C, right panel). In contrast, OS exposure did not significantly change the p23 BMP4 level in the conditioned medium (Fig. 2C, right panel, p < 0.05). Because the cells were exposed to LS (15 dyn/cm²) and OS (~5 dyn/cm²), we next determined whether it was the shear magnitude difference that accounted for our results observed so far. To address this question, we compared the effects of LS and OS using the same magnitudes (5 dyn/cm² LS versus ~5 dyn/cm² OS). As shown as Fig. 2C, right panel, at the same shear magnitude, OS-exposed cells had more than 3-fold BMP4 protein than that of LS. However, the higher LS magnitude (15 dyn/cm²) showed a much lower amount of BMP4 than that of lower LS (5 dyn/cm²). These results show that LS exposure inhibits BMP4 expression in a force-dependent manner, whereas OS maintains high BMP4 expression.

BMP4 Expression in the Selective Patches of Endothelial Cells over Foam Cell Lesions in Human Coronary Arteries—Next, using the human coronary arteries we determined whether BMP4 protein is expressed in endothelial cells of human atherosclerotic lesions. The coronary arteries exhibiting a
consistent with the finding reported by Endress in the adjacent serial sections (data not shown). This result is

select areas of endothelium. Furthermore, immunostaining for BMP4 and ICAM-1 in overlapping patches (D and F, arrowheads) in the serial sections. B and E, endothelial cells are marked with arrowheads.

spectrum of atherosclerotic lesion complexity were obtained from patients undergoing heart transplants and examined by immunohistochemical staining (17). BMP4 protein expression was not apparent in the intimal endothelial cells in relatively normal, "minimally diseased" human coronary arteries (Fig. 3A) or in advanced lesions (data not shown). As shown in Fig. 3D, one exception was found in the endothelial cells (arrow) overlying foam cell lesions that were stained strongly against the BMP4 antibody. As shown in Fig. 3C, isotype-matched nonspecific mouse IgG used as a negative control further supported the specificity of BMP4 staining. In contrast, the medial smooth muscle cells and macrophages (Fig. 3, A and D) were most intensely stained against a monoclonal BMP4 antibody (smooth muscle cells and macrophages identified by α-actin and CD-68 staining; data not shown). To verify the identity of endothelial cells, the serial sections were stained with a von Willebrand factor antibody (endothelial marker, Fig. 3, B and E), demonstrating the location of BMP4 staining in select areas of endothelium. Furthermore, immunostaining with an ICAM1 staining showed that the expression of this pro-inflammatory adhesion molecule was selectively increased in the similar endothelial areas expressing BMP4 (Fig. 3, D and F, arrowheads). On the other hand, we failed to detect VCAM-1 in the adjacent serial sections (data not shown). This result is consistent with the finding reported by Endress et al. (7).

BMP4 Produced in Endothelial Cells by OS Stimulates Monocyte Adhesion—The selective expression of BMP4 protein in endothelial cells above foam cell lesions (an early form of atherosclerotic lesions) prompted a speculation that BMP4 may be involved in the inflammatory responses observed in lesion-prone areas (3, 4). To begin to test this hypothesis, MAEC were treated with increasing amounts of BMP4 for 24 h and then monocyte adhesion to endothelium was determined. As a positive control, some cells were treated with a well known inflammatory cytokine, TNFα (100 units/ml). BMP4 stimulated monocyte binding in a concentration-dependent manner with a maximum activation of 4–7-fold over control (Fig. 4A, p < 0.05). As low as 0.1 ng/ml BMP4 induced a statistically significant increase, whereas 50 ng/ml BMP4 induced a maximum effect. A similar effect of BMP4 on monocyte adhesion was also observed by transfecting MAEC or HAEC with a vector expressing mouse BMP4 (data not shown).

OS has been shown to induce monocyte adhesion both in vivo and in cultured endothelial cells by increasing surface expression of adhesion molecules (21). Therefore, we used a BMP4 inhibitor, noggin (18, 20), to examine whether OS induces monocyte adhesion in endothelial cells in a BMP4-dependent manner. Exposure of endothelial cells to OS for 24 h significantly increased monocyte adhesion (Fig. 4B, p < 0.05). Treatment of MAEC with noggin (50 ng/ml) inhibited OS-induced monocyte adhesion (Fig. 4B).

In contrast, exposure of MAEC to LS for 24 h inhibited monocyte adhesion by ∼50% of static control level (Fig. 4C, p < 0.05) as expected (26). Because LS exposure significantly inhibited BMP4 expression in endothelial cells (Fig. 2), we next examined whether the inhibitory effect of LS on monocyte adhesion could be reversed by BMP4 addition. For this study, we exposed MAEC in the presence of BMP4 during shear or static control for 24 h, followed by monocyte adhesion assay. The inhibitory effect of LS on monocyte adhesion was lost when MAEC were sheared in medium supplemented with BMP4 (Fig. 4C, p < 0.05). Taken together, these results suggest that BMP4 produced from endothelial cells by OS exposure leads to monocyte adhesion.

BMP4 Stimulates Monocyte Adhesion by Inducing ICAM-1 Expression in an NFκB-dependent Manner—Next, we examined the mechanism by which BMP4 increases monocyte adhesion to endothelial cells. Adhesion of monocytes to endothelial cells is mediated by sequential coordinated molecular interactions between the integrins expressed on monocyte surface and several adhesion molecules expressed on the endothelial surface, including ICAM-1, VCAM-1, and E-selectin (3). Moreover, it has been shown previously that expression of ICAM-1, VCAM-1, and E-selectin on endothelial cell surface is increased in atherosclerosis-prone areas (3). Therefore, we first determined whether the endothelial expression of ICAM-1, VCAM-1, and E-selectin was modified in response to OS by FACS analysis.

Exposure of HAEC to OS (1 day) increased ICAM-1 expression by 2.8-fold above control (Fig. 5A, p < 0.05). For compar-
Fig. 4. BMP4 stimulates monocyte adhesion to endothelial cells. A, to determine monocyte adhesion, BCECF-labeled THP-1 monocytes were added to MAEC that were treated with increasing concentrations of BMP4 overnight. Bar graph represents means numbers of bound monocytes per ×10 objective field (6–12 different fields per dish) mean ± S.E. (n = 4–6). As a positive control, monocyte binding was determined using MAEC treated with TNFα (100 units/ml for 2 h). B, MAEC were exposed to OS or static condition in the presence or absence of noggin or vehicle (Veh), and monocyte adhesion was determined. Bar graph represents the numbers of bound monocytes expressed as % of static control (mean ± S.E., n = 4) (*, p < 0.05). C, MAEC were exposed to LS in the presence of recombinant BMP4 or vehicle control, followed by monocyte binding assay. Data are expressed as in panel B (mean ± S.E., n = 4) (*, p < 0.05).
Fig. 5. BMP4 produced by OS selectively increases surface expression of ICAM-1, but not VCAM-1 and E-selectin, in an NFκB-dependent manner. A, after exposing HAEC to OS in the presence or absence of noggin or vehicle control, expression of ICAM-1 was determined by FACS analysis. The mean of log fluorescence values was obtained and expressed as % of static control values. Bar graph represents mean ± S.E., n = 4 (*, p < 0.05). As a positive control, HAEC were treated with TNFα (100 units/ml, for 2 h). B, HAEC were treated with TNFα (100 units/ml for 6 h) in the presence or absence of noggin (50 ng/ml), followed by FACS analysis to determine ICAM-1 expression as in panel A (mean ± S.E., n = 3, *, p < 0.05). C and D, HAEC treated with OS, BMP4, or static condition were analyzed by FACS using antibodies specific to VCAM-1 (C) and E-selectin (D) as described in panel A, using TNFα (100 units/ml, for 2 h) as a positive control. E, HAEC exposed to LS (15 dynes/cm²) in the presence or absence of BMP4 for 1 day were lysed and analyzed by Western blot using specific antibodies to ICAM-1, VCAM-1, and actin (as a loading control). TNFα-treated HAEC were used as a positive control. LS completely blocked VCAM-1 expression, whereas BMP4 did not induce statistically significant effects on VCAM-1 and ICAM-1 expressions (n = 3 to 4, p < 0.05). F, HAEC were transfected with BMP4 cDNA (pAdTrack-CMV vector) or empty vector control with or without LipofectAMINE2000 (Lipo). Transfected cells were then incubated for 10 h in the presence or absence of NFκB inhibitors, SN50 (50 μg/ml) or the inactive peptide SN50 M (50 μg/ml) or MG132 (6 μM). TNFα (100 units/ml for 3 h) was used as a positive control. Surface expression of ICAM-1 expression was then determined by FACS. SN50 or MG132 completely prevented ICAM-1 expression induced by BMP4 transfection, whereas SN50 M did not (n = 3–6, *, p < 0.05). G, BMP4 expression in 20 μg of cell lysates (a p54 precursor form) and equal amount of conditioned media (precipitated from 2 ml each) (a p23-secreted form) obtained from samples described in panel F was determined by Western blot analysis and showed specific overexpression of BMP4 by transfection with the BMP4 vector. Western blot analysis using an actin antibody was used as a loading control for cell lysates. H, an NFκB reporter construct (1 μg, NFκB-SEAP vector) expressing a secreted form of placental alkaline phosphatase was co-transfected with 0.5 μg of either BMP4 vector or empty vector control using LipofectAMINE 2000. Six hours post-transfection, heat-resistant alkaline phosphatase activity secreted into the media was determined by chemiluminescence assay. Bar graph represents mean ± S.E. (n = 3, *, p < 0.05).
conditions may represent low or no-shear conditions and may not represent true “control” conditions. Many in vivo studies examine the differences between the normal regions exposed to LS in straight arteries and the lesion-prone areas exposed to disturbed and low shear conditions in branched or curved arteries.

If we compare the OS effects on expression of BMP4 and ICAM-1 as well as monocyte adhesion to those of LS instead of static conditions, the effects of the pro-atherogenic force become more pronounced than what we have shown under “Results.” For example, endothelial cells exposed to LS express BMP4 mRNA and protein at almost undetectable levels. Exposure to OS, however, dramatically up-regulated expression of BMP4 mRNA and protein (Fig. 2). This is consistent with our human coronary artery data showing that BMP4 expression is undetectable in normal (minimally diseased) arteries, whereas it is strongly expressed in endothelial patches overlying foam cell lesions. In typical studies, OS increases monocyte binding 8–10-fold above that of LS (data not shown).

BMPs are members of the transforming growth factor-β superfamily and play important roles in bone formation, embryonic development, and differentiation (23, 24). Although BMP4 protein has been found previously in calcified atherosclerotic plaques (25), its expression and functional importance in endothelial cells have not been determined. There are two types of signaling receptors specific for BMPs: BMPR-I and BMPR-II. It appears they are both required for signaling (20). Three BMP type I receptors, BMPR-IA (also known as ALK3, Actiniv-Like Kinase-3), BMPR-IB (ALK6), and ALK2 and one BMP type II receptor have been identified (33). Although somewhat variable depending upon species and vascular bed origins, endothelial cells from mouse arteries as well as cultured murine and bovine aortic endothelial cells have been shown to express both type I (ALK2, 3, and 6) and type II BMPRs (34). Unlike their well-known effects in bone formation and embryonic development, the functional importance of BMPRs in vascular wall is not clear. One notable exception is the link in vascular smooth muscle cells as demonstrated by the loss-of-function mutations of the type II BMPR in familial primary pulmonary hypertension and sporadic primary pulmonary hypertension (35). In endothelial cells, transfection with constitutively active mutants of ALK2, ALK3, and ALK6 has been shown to stimulate expression of id gene and angiogenic responses (34).

As far as we are aware, BMP4 has not been shown to induce inflammatory responses previously, especially in endothelial cells. Our finding that BMP4 stimulates monocyte adhesion by increasing surface expression of ICAM-1 in endothelial cells seems to be the most interesting and novel aspect of the current study.

Atherosclerosis is a focal and inflammatory disease preferentially occurring at the lesion-prone areas exposed to unstable and low shear stress (1–4). The branched, bifurcated, and curved arteries such as the lesser curvature of the ascending aorta, the outer wall across the apex of the carotid sinus, and the left descending coronary arteries are the preferential sites of atherosclerotic development (1–4). Endothelial cells in the lesion prone areas become dysfunctional and have been shown to express adhesion molecules including ICAM-1, VCAM-1, and E-selectin (3). Circulating blood monocytes and lymphocytes then bind to these adhesion molecules, migrate beneath endothelium, engulf lipids, and transform into macrophage foam cells, eventually becoming the site of advanced atherosclerotic plaques (3, 4).

Unexpectedly, the current study showed that OS and BMP4 selectively regulate expression of ICAM-1 without significantly affecting VCAM-1 and E-selectin. These seemingly conflicting results on ICAM-1 and VCAM-1 have been reported in mouse atherosclerosis models as well. Cybulsky et al. (5) showed that VCAM-1, but not ICAM-1, expression was up-regulated by a high fat diet in low density lipoprotein receptor−/− mice. In contrast, Nakashima et al. (36) reported that only ICAM-1, but not VCAM-1, expression was up-regulated in a disturbed flow-dependent manner in the lesion-prone areas such as the aortic sinus, whereas VCAM-1 expression was robustly increased by high fat diet feeding in ApoE−/− mouse. Evidence from other mouse atherosclerosis models lacking expression of ICAM-1 or VCAM-1 has shown the importance of both adhesion molecules (5, 27). In addition, this concept is consistent with data from Nagel et al. (37), who used cultured endothelial cells to show that ICAM-1 expression, but not VCAM-1 and E-selectin, was up-regulated by shear stress. Moreover, Endres et al. (6) reported that the early atherosclerotic lesions found in the outer wall of human carotid artery bifurcations showed increased expression of ICAM-1, but not VCAM-1 and E-selectin. However, expressions of VCAM-1 and E-Selectin did increase in advanced atherosclerotic plaques (7). These findings are con-
sistent with our hypothesis that oscillatory shear stress selectively up-regulates ICAM-1 expression. Therefore, although both VCAM-1 and ICAM-1 are important in the pathogenesis of atherosclerosis, ICAM-1 expression in the lesion-prone areas seems to be regulated mainly by oscillatory shear stress, whereas VCAM-1 seems to be more responsive to high cholesterol conditions.

Our findings on the selective effect of OS on ICAM-1 expression are consistent with previous reports in cultured endothelial cells using a similar cone-and-plate shear system (37). However, using a parallel plate shear device and human umbilical endothelial cells, OS has been shown to stimulate VCAM-1 and E-selectin in addition to ICAM-1 (21). These discrepancies may be due in part to subtle differences in flow profiles generated in the two different shear devices. Most importantly, however, BMP4 increased expression of ICAM-1, but not VCAM-1 and E-selectin, demonstrating its selective effect on ICAM-1.

VCAM-1 expression and monocyte adhesion are kept very low in most healthy arteries by unknown mechanisms (7, 36). In our current study, we found that chronic exposure of endothelial cells to LS (a physiological condition expected for healthy straight arteries in vitro) virtually eliminated VCAM-1 expression. This may be an important mechanism by which LS acts as a potent anti-inflammatory and anti-atherogenic force. Our results suggest that LS inhibits monocyte adhesion by multiple mechanisms, including the direct inhibition of VCAM-1 expression as well as down-regulation of BMP4 protein, diminishing its effect on ICAM-1 expression. The inhibitory effect of LS on VCAM-1 has been shown to be regulated by the NO-dependent mechanisms (26).

Based on the current data and literature, we propose that BMP4 plays a critical role as a mechanosensitive and pro-inflammatory cytokine mediating the opposite effects of OS and LS. Our working hypothesis is shown in Fig. 7: The endothelial cells in lesion-prone areas (Fig. 7, dark line) experience low and disturbed shear stress, including OS, which induces endothelial BMP4 expression. BMP4 then initiates inflammatory cascades in an NFκB-dependent manner by stimulating ICAM-1 surface expression in those activated endothelial cells. ICAM-1 expression is consistent with previous reports in cultured endothelial cells using a similar cone-and-plate shear system (37). In contrast, LS acts as a potent anti-inflammatory and anti-atherogenic force by not only inhibiting BMP4 expression but also directly down-regulating VCAM-1 expression. Interestingly, it has been shown (38) that LS induces expression of two signaling molecules, SMAD 6 and 7, which are known to inhibit BMP4 action, providing an additional mechanism by which LS prevents BMP4-dependent responses. The identification of BMP4 as an inflammatory cytokine may provide not only opportunities for better understanding of vascular biology and atherogenesis but also novel diagnostic and therapeutic approaches in atherosclerosis.

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REFERENCES